2-Deoxy-D-glucose reverses the Indian red scorpion venom-induced cardiopulmonary abnormalities in anesthetized rats

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Role of 2-Deoxy-D-glucose (2-DG) in reversing the Indian red scorpion (Mesobuthus tamulus concanesis Pocock, MBT) venom-induced toxicity was examined. Femoral arterial pressure, ECG and respiratory movements were recorded in urethane anesthetized rats. Plasma glucose and serum insulin levels were also estimated. Intravenous injection of 5 mg/kg MBT venom produced immediate decrease in mean arterial pressure, heart rate and respiratory frequency followed by an increase and subsequent progressive decrease. ECG pattern exhibited ischaemic changes. There was hyperinsulinemia after venom without corresponding decrease in plasma glucose. The animals died within 37±9 min and demonstrated significant increase in pulmonary water content. 2-DG pretreatment (0.5 g/kg, iv) improved the cardiopulmonary abnormalities induced by venom and the animals survived for nearly 120 min. There was no hyperinsulinemia and increased pulmonary water content in these animals. In insulin (2 IU/kg) treated rats, the MBT venom-induced cardiopulmonary abnormalities were attenuated and ECG abnormalities were reversed. The pulmonary water content in these animals exhibited a decreasing trend and the animals survived for 120 min. Repaglinide (10 µg/kg, iv) pretreatment failed to reverse the venom-induced cardiopulmonary changes including the increased pulmonary water content. The survival time was similar to venom only group. The present results reveal that 2-DG reverses the venom-induced cardiopulmonary toxicity probably by restoring insulin sensitivity.

Keywords: Insulin, Insulin sensitivity, Mesobuthus tamulus venom, Pulmonary edema, Repaglinide

The Indian red scorpion (Mesobuthus tamulus concanesis Pocock, MBT) sting produces fatal toxicity resulting from multi-system failure. The toxicity manifests as cardiopulmonary abnormalities such as dysrrhythmia, acute myocarditis, ischemia-like changes, pulmonary edema etc. In addition, severe metabolic abnormalities such as hyperglycemia, hyperlipidemia and hyperinsulinemia are also reported. A number of treatment strategies have been advocated for scorpion envenomation. They are antivenin, α-adrenoceptor antagonist (prazosin), antihistamine, kinin synthesis inhibitor (aprotinin) and insulin. Insulin is an anabolic hormone that regulates the cellular utilization of glucose and energy production. Hence, insulin has been advocated effectively in the treatment of scorpion envenomation syndrome in man or experimental animals. Instances of hyperglycemia along with either hypo-insulinemia or hyperinsulinemia are reported in scorpion envenomed animals. Hyperglycemia in presence of hyperinsulinemia indicates decreased insulin sensitivity or insulin resistance. Number of factors can be responsible for insulin resistance after envenomation. They are massive release of catecholamines, increased renin-angiotensin activity, and increased levels of anti-insulin hormones (glucagon, cortisol and thyroid hormones). Deficiency or failure of insulin action (due to decreased sensitivity) decreases the glucose metabolism in vital organs (such as liver, lungs and heart) and further enhances the toxicity. 2-Deoxy-D-glucose (2-DG), an analogue of glucose, is shown to increase the insulin sensitivity in rats. It is hypothesized that 2-DG may protect the animals against venom-induced toxicity by restoring the insulin sensitivity. Therefore, the present study has been undertaken to examine the role of 2-DG in reversing the scorpion venom-induced toxicity and compared it with the exogenous insulin or insulinotropic agent.

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**Materials and Methods**

*Animals and anesthesia*—All the experiments were performed according to the guidelines of the ethical committee of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India for conducting animal experimentation. Healthy albino rats of either sex (Charles-Foster strain; 180-220 g) were used in this study. The animals were exposed to 12:12 h light/dark cycle. The animals (after fasting overnight) were anesthetized with an ip injection of urethane (1.5 g/kg). A maintenance dose (50-100 mg) of anesthesia was given as required.

*Dissection and recording*—Under the anesthetic effect, tracheal cannulation was performed to keep the respiratory tract patent. The femoral artery was dissected and cannulated and the cannula was connected to a Statham Strain Gage pressure transducer (Bio-Devices, Ambala, India) to record the blood pressure. Right jugular vein was cannulated to inject venom or drugs. ECG recordings were made by needle electrodes connected in limb lead II configuration. The skin over the xiphisternum was secured to a force-displacement transducer via a thread to record the respiratory excursions. ECG, BP and respiratory excursions were taken on a chart recorder (Bio-devices). The mean arterial pressure (MAP), respiratory frequency (RF) and heart rate (HR) were computed manually.

*Drugs and chemicals*—Crude MBT venom was obtained from Haffkine Institute, Mumbai, India; heparin was from Biological Evans Ltd, Hyderabad, India; insulin (recombinant DNA human) was from M.J. Pharmaceutical, Gujarat, India; 2-DG was from Sisco Research Laboratories Pvt. Ltd. Mumbai, India and repaglinide was from Torrent Pharmaceutical Co. Ltd., Ahmedabad, India. The stock solution of venom (5 mg/ml) was prepared in distilled water. The working solution of MBT venom and other drugs was made in saline at the time of experimentation.

*Experimental protocol*—Animals were allowed to stabilize for 30 min after dissection. The animals were divided into five groups including control (saline) group. In group I (n = 10), after initial recordings of BP, ECG and respiratory excursions, crude MBT venom (5.0 mg/kg in 0.1) was injected in jugular vein and the cardiorespiratory parameters were recorded continuously for the initial 5 min and then at every 15 min up to 120 min (observation period).

In group II, (n = 10), after the initial recordings, crude MBT venom was injected and 2 min later, a total dose of 2 IU/kg of insulin was administered (0.4 IU/kg, iv + 1.6 IU/kg, sc). The cardiorespiratory parameters were recorded continuously for the initial 5 min and subsequently at 15 min interval up to 120 min as mentioned above.

In groups III (n = 12) and IV (n = 8), the rats were pretreated with 2-DG (0.5 g/kg, iv) or repaglinide, an insulinotropic agent (10 µg/kg, iv) and 25 min after venom (5.0 mg/kg, iv) was injected and the cardiorespiratory parameters were recorded for 120 min as mentioned for group II.

*Estimation of serum insulin and plasma glucose*—In group I and group III, besides the recording of the cardio-pulmonary parameters, additional experiments (n = 4-6) were performed to estimate glucose/insulin. In these experiments, 0.5 ml of femoral arterial blood was collected and 20 µl of this was used to estimate plasma glucose by glucose-oxidase method. The remaining amount was used for the estimation of serum insulin using rat insulin ELISA kit. In venom only group (Gr. I), the blood samples were collected before and 15 min after the administration of venom. In 2-DG pretreated group (Gr. III), three blood samples were collected, first before 2-DG, then 25 min after 2-DG and finally 15 min after venom administration. In these animals, blood was drawn slowly to minimize the sudden changes in cardiopulmonary parameters. After drawing the blood, 1 ml of normal saline was injected through jugular vein. No significant differences were observed in the cardiopulmonary parameters in these animals after collecting blood.

*Determination of pulmonary water content*—Pulmonary water content was determined by the method described earlier. Briefly, lungs were dissected out, weighed and dried in an oven to a constant weight to know the percentage of pulmonary water content.

*Statistical analysis*—The pooled data showing mean ± SE values are presented. The effects after exposure to venom/drugs were compared for the significance using one-way or two-way ANOVA followed by Student’s Neuman Keul’s test for multiple comparisons. Student’s *t* test was also used for paired or unpaired observations as required. A *P* <0.05 was considered significant.

**Results**

*Venom produced cardiopulmonary abnormalities*—Injection of 5.0 mg/kg of venom produced immediate
decrease in blood pressure (about 55%) within 20 s followed by an increase above the initial level (by 68%) within 1 min. The MAP subsequently exhibited time-dependent decrease till death (Fig. 1A and E).

Respiratory changes were manifested as immediate apnea after venom administration (Fig. 1A and C). The respiration subsequently resumed but at a slower rate (only 50% of the initial at 5 min). Subsequently, it declined in a time-dependent manner and ultimately stopped around 45 min (Fig. 1C).

The heart rate changes demonstrated a profound decrease (about 80%) within 20 s after venom administration. At 5 min, the heart rate (HR) recovered to 85% of the initial and thereafter HR decreased progressively in a time-dependent manner (Fig. 1A and D). The mean survival time of these animals after venom was 37±9 min.

2-DG reversed the venom-induced cardiopulmonary abnormalities—2-DG pretreatment per se did not alter the MAP (Fig. 1B and E). In these animals, there was immediate (within 40 s) decrease in MAP (about 68%) after BT venom. Subsequently, MAP increased to initial level around 2 min. Later on, it increased further to 71% of initial by 5 min and then began to decrease and reached the initial level around 15 min. After 15 min, the MAP decreased slightly up to 120 min (Fig. 1B and E). The MAP changes in 2-DG pretreated group (Gr.III) were different from Gr.I (Fig. 1B and E).

A decrease in respiratory frequency of about 28% was observed after 2-DG pretreatment. In these animals, MBT venom decreased the RF by about 84% immediately (1 min). The RF recovered to 38% of 2-DG pretreated level at 5 min which remained at that level throughout the experiment as compared to

![Diagram](image-url)

Fig. 1—Effect of 2-DG pretreatment on MBT venom induced alterations in mean arterial pressure (MAP), heart rate (HR) and respiratory rate (RF). Original tracings show respiratory movements (Resp), ECG and blood pressure (BP) in venom only (A) and ‘2-DG+Venom’ groups (B). Note the progressive deterioration of all parameters in venom only group and protection by 2-DG (0.5 g/kg). Vertical dotted lines indicate the point of venom administration. Horizontal line in ‘A’ corresponds to 5 s for respiration and 50 s for MAP. The mean±SE values of responses are presented in panels C-E. The responses in 2-DG pretreated rats (n = 12) are significantly different from venom only group (n = 10; P <0.05, Two way ANOVA). P <0.05, * as compared to the corresponding points in venom only group (Student-Newman-Keuls test).
Gr. I (Fig. 1A and C). The RF responses between 5-45 min were significantly different from Gr. I ($P<0.05$; 2-way ANOVA; Fig. 1).

2-DG pretreatment per se did not alter the heart rate. Injection of MBT venom in these animals, produced immediate decrease in heart rate (about 89%) within 40 s but by 2 min the HR recovered to about 46% of the 2-DG pretreated level and by 15 min, the HR returned back to the initial level. Thereafter, the HR was maintained at that level up to 120 min (Fig. 1B and D). In this group, 10 of 12 rats survived throughout the period of observation. Even in remaining 2 rats, the survival time was > 90 min as one died at 110 min and the other at 90 min.

**Insulin attenuated the venom-induced cardiopulmonary abnormalities**—Insulin was administered immediately after venom injection. These animals exhibited an initial fall of MAP as seen in Gr.I but was significantly lesser (22%, Fig. 2A and D) followed by an increase above the initial level (50%) up to 5 min which returned to the initial level within 15 min. Thereafter, MAP remained at that level throughout the experiment (Fig. 2A and D).

There was immediate decrease in respiratory frequency (about 81%) after venom injection in this group (Gr. II). After insulin administration in these animals, the RF returned back to the initial level within 5 min. Subsequently, RF decreased by 50% of initial at 15 min. This level was maintained with slight fluctuations up to 105 min in contrast to the progressive decrease seen in Gr.I (Fig. 2A and B). The RF values between 5-45 min were significantly greater than Gr.I (Fig. 2A and B).

There was immediate decrease in the heart rate (by 71%) but after insulin administration, HR returned back to the initial level within 15 min and was maintained at that level up to 105 min. At 120 min, the HR was about 50% of the initial value (Fig. 2A and C). HR values between 15-45 min were significantly different from Gr.I. All the animals in Gr.III survived throughout the period of observation (120 min).

**Repaglinide failed to block the cardiopulmonary abnormalities induced by venom**—Repaglinide (an insulinotropic agent) pretreatment failed to reverse the cardiopulmonary changes produced by the venom (Fig. 3). Repaglinide per se did not alter MAP. In the presence of repaglinide, venom increased the MAP up to 5 min and the increase was about three times the initial level. Subsequently, MAP decreased in a time-dependent manner. The decrease between 15-60 min was similar to Gr.I ($P>0.05$ Student’s $t$ test, 2-way ANOVA, Fig. 3A and D).

Repaglinide per se did not alter RF. In repaglinide pretreated rats (Gr.IV), a prolonged apnea was observed after venom. Subsequently the respiration resumed and RF was 70% of the initial level by 15 min. Thereafter, RF decreased in a time-dependent
manner and stopped at 60 min. The RF changes between 30-60 min were similar to Gr.I (Fig. 3A and B).

Repaglinide per se did not alter the HR. In Gr.IV, there was immediate decrease in HR (by 76%) which subsequently recovered to 68% of the initial value by 15 min. Thereafter, there was time-dependent decrease in HR. The HR changes between 15-45 min were similar to venom only group (Fig. 3A and C). The mean survival time of repaglinide pretreated animals after venom was 35.5±3.5 min.

Venom-induced ischemia-like changes in ECG were not seen in 2-DG pretreated or insulin treated group—The typical changes in wave pattern of ECG after BT venom (5.0 mg/kg) are presented in Fig. 4A. Ischaemia-like wave pattern along with different types of heart block and dysrhythmia were noted in venom only group. In 2-DG pretreated rats, the venom-induced severe ECG abnormalities were not observed (Fig. 4B). Insulin administration following venom injection restored the ECG alterations within 15 min (Fig. 4C). Repaglinide pretreatment failed to reverse the ECG abnormalities induced by venom (Fig. 4D).

2-DG prevented the venom-induced pulmonary edema—The pulmonary water content of the lungs in saline treated rats was 76.0±1.25%. In Gr.I animals, it was 79.6±0.87 and was significantly greater than the saline treated group (P<0.05; Student’s t test for unpaired observations). The pulmonary water content in 2-DG pre-treated group was 77.8±0.3% and was significantly lesser than Gr.I (P<0.05; Student’s t test for unpaired observations). Eventhough, insulin treated group exhibited decrease in pulmonary water content (78.86±0.6%) as compared to Gr.I, the decrease was not significant. In repaglinide treated rats, pulmonary water content was 80.18±0.49%; significantly greater than saline group (P<0.05; Student’s t test for unpaired observations) and was similar to Gr.I.

2-DG prevented the venom-induced hyperglycemia and hyperinsulinemia—There was more than 4 fold increase in serum insulin concentration after venom administration but the plasma glucose did not decrease and was 121% of saline group (Fig. 5). The insulin concentration in 2-DG pretreated rats was not increased after venom administration as compared to venom only group (P<0.05; Student’s t test for unpaired observations; Fig. 5). However, 2-DG alone increased the plasma glucose and insulin by 30%.

Discussion
The observations of the present study reveal that scorpion envenomation produces lethal cardiopulmonary abnormalities associated with decreased insulin sensitivity. 2-Deoxy-D glucose (2-DG) prevented the venom-induced toxic manifestations and restored the insulin sensitivity. Exogenous insulin protected the animals from toxicity and there was a decreasing trend in pulmonary edema. Since, insulin was given as a therapy after the venom
injection, it takes time to reverse the already established edema. This is substantiated by the fact that insulin reversed the haemodynamic changes and pulmonary edema after a time lag (>2 h) of treatment in scorpion stung children or adult. Repaglinide, an insulinotropic agent, failed to prevent the toxicity. These observations indicate that 2-DG reversed the scorpion venom induced cardiopulmonary abnormalities.

2-DG, a non-metabolizable glucose analogoue, competes with glucose for cellular transport. Intracellularly, 2-DG is phosphorylated to 2-DG-6-phosphate which is not metabolized any further. The accumulated 2-DG-6-phosphate inhibits the conversion of glucose-6-phosphate to fructose-6-phosphate. Thus a condition of cellular energy deprivation was produced in spite of the increased plasma glucose level. Further, 2-DG increases the plasma insulin levels in rats and the present results support this phenomenon. In the present study, scorpion envenomation increased the serum insulin levels by >4 times. At this concentration of insulin, a drastic decrease in plasma glucose is expected. On the contrary, there was an increase in plasma glucose (Fig. 5) indicating the development of insulin resistance. The ability of exogenous insulin to reverse the venom-induced toxicity either in this study or elsewhere suggests that the insulin receptors remain functional after envenomation. Thus, there is a possibility of abnormal synthesis of insulin. This is supported by the failure of protection by repaglinide.

Repaglinide, stimulates the release of insulin from the β-cells of the pancreas but has no role in the biosynthesis of peptides in isolated rat pancreatic islet cells. It is shown that repaglinide increases the insulin level hence, we expected a similar increase
of insulin and protection against toxicity induced by venom. On the contrary, repaglinide failed to protect the animals against venom-induced toxicity. The lack of protection by repaglinide indicates that insulin released after envenomation is non-functional. After envenomation increased level of free fatty acid, phospholipids and insulin are reported\textsuperscript{17,25}. Further, it is shown that exogenous insulin protects against scorpion toxicity either in this study or elsewhere\textsuperscript{3,11,12}. These facts along with our observation of hyperinsulinemia and hyperglycemia indicate that scorpion envenomation produces insulin resistance syndrome.

Protection by 2-DG against scorpion envenomation suggests that 2-DG counters the effects of envenomation syndrome probably by releasing functionally active insulin. In addition, 2-DG is shown to increase the insulin sensitivity in obese rats\textsuperscript{19}. In conclusion, present findings provide evidences for the protective effect of 2-DG against scorpion envenomation. Further, the altered insulin sensitivity induced by venom can be prevented by 2-DG, but not by insulinotropic agent (repaglinide). These findings open a new dimension in the treatment of scorpion envenomation and also in understanding the insulin resistance syndrome.

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**References**


![Fig. 5—Effect of venom on plasma glucose level and serum insulin concentration in presence and absence of 2-DG pretreatment. Note the significant (>4 times) increase in serum insulin level that was not associated with decrease in plasma glucose level. Instead, there was slight increase in plasma glucose level after MBT venom (5 mg/kg) in 2-DG (0.5 g/kg) pretreated group. The values are obtained from 4-6 different experiments. $P_{<0.05}$, * as compared to saline group; @ as compared to corresponding values in venom only group (Student’s $t$ test for unpaired observations).](image-url)

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