Anticonvulsant like Activity of Silybin Correlates Blood Glucose Levels in PTZ induced Chemo-convulsive Rats

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ABSTRACT

The objective of this study is to observe the impact of Silybin (SIB) on the link between blood glucose and seizure prevention in chemo-convulsive rats. To produce seizures, adult male rats were administered with pentylenetetrazol (PTZ-105 mg/kg i.p.) at the CD97 dose. SIB (100 mg/kg.p.o.) was administered for two weeks. The animals were subjected to chemo convulsions on the 14th day, and the effectiveness of SIB to reduce clonus-type chemo convulsions (CC) was recorded, as well as blood glucose levels measured with a one-touch glucometer. Morphometric analysis and chimney tests were evaluated to know the neurotoxic profile of SIB, while actophotometer, rota rod, and hole board tests were used for behavioural analysis. Pre-treatment with SIB (100 mg/kg p.o.) resulted in a significant delay in the onset of CC (*p<0.05) and a significant reduction in the duration of CC (**p<0.01) as compared to controls. The acute dose of SIB significantly reduced blood glucose levels. In addition, acute administration of SIB considerably lowered blood glucose levels and promoted body weight of animals whereas chimney tests, indicating that, non-neurotoxic profile of silybin to animals. Additionally, behavioural tests revealed that no significant alterations in muscular coordination, locomotion, or anxious behaviour were noticed in animals. In summary; results reveal that the NDH + PTZ groups had considerably greater hyperglycemia levels, which is reflected in the early onset and longer duration of chemo convulsions in rat. SIB treatment, However, there was a significant reduction in elevated glucose levels, as well as a considerable delay in the onset and duration of seizures, demonstrating the relationship between blood glucose and Anticonvulsant like activity against chemo convulsive episodes.

Keywords: Silybin, Hyperglycaemia, Pentylenetetrazol, chemo convulsions, seizure protection
1. INTRODUCTION:

*In-vivo* and *in-vitro* studies have demonstrated the relevance of glucose homeostasis in the human body, demonstrating that synaptic transmission requires a certain glucose threshold. Hyperglycaemia, on the other hand, increases brain harm by generating ischemic conditions and hyper excitability in the neuronal environment, which can lead to provoking of epileptic seizures [1]. Moreover, Hyperglycaemia is the major symptom of Diabetes mellitus, which has a detrimental effect on the neuroendocrine axis, which may exacerbate the effects of diabetes on various systems of the human body and that rely on it. However, if left untreated, metabolic disorders such as hyperglycaemia and hypoglycaemia have been reported to cause seizures as a chronic neurological disorder due to neuronal firing.

According to existing research, lowering extracellular glucose levels can reduce seizure activity by lowering neuronal excitability [2]. In the present investigation, to evaluate the neuronal excitability, pentylenetetrazol [PTZ] has been used to induce chemo convulsions in rats. This model is considered as one of the gold standard and most popular approaches for evaluating brain excitability during provocation of convulsions in laboratory animals.

On the other hand, synthetic anti-diabetic medications such as sulphonylureas, biguanides, a-glycosidase inhibitors, and thiazolidines are associated with undesired side effects and may produce severe glycemic responses on chronic usage basis [3]. Alternatively, several bioflavonoid derived from plants have been reported to control blood glucose levels on a regular basis. Silybin (SIB) is one of the oldest traditional herbal medicine with diverse pharmacological effects and used to combat several human sufferings. SIB, is a blend of two stereoisomers (Silybin A and Silybin B) and the main active component (constitutes 34% ) of silymarin, which is derived from blessed milk thistle (*Silybum marianum*). Silybin inhibits many cytochrome P450 enzymes, alters P-glycoprotein-mediated cellular efflux, and exhibits free radical scavenging properties [4]. A number of studies have shown that SIB is beneficial in type 2 diabetes patients, including reductions in blood glucose, and various lipoproteins including triglycerides and total cholesterol levels etc [5,6]. As per the literature, Anti-diabetic effect of SIB is attributed to inhibition of gluconeogenesis in the liver and decrease of glucose-6 phosphatase activity [7, 8]. As a result, the goal of this study was to see how SIB affected the correlation between blood glucose and seizure prevention in chemo convulsive rats.

2. MATERIALS AND METHODS:

2.1 DRUGS AND CHEMICALS:

Silybin, diazepam, and pentylenetetrazol were procured from Yarrow chem. Pvt LTD, India, which were dissolved in compatible solvents. SIB and diazepam and dose of ETC is based on approved human therapy regimens have been used successfully in similar animal models [9]. The chemicals that were acquired were all of analytical purity.

2.2 ANIMALS AND LABORATORY CONDITIONS:

Albino male adult Wistar rats weighing 160-175 g were picked and housed in standard laboratory conditions, which includes 22°C temperature, 55.5 percent humidity, and light-dark cycles. All animals had unfettered access to food and water throughout the testing. The Institutional Animal Ethics Committee approved the entire study procedure with protocol permission No:SVCP/IAEC/I-01/MAR-2021, in line with the guidance of the Committee for
Control and Supervision on Animal Experiments [CPCSEA], New Delhi, India. To prevent bias, animals were randomly allocated to treatments as different treatment groups using randomised approaches. [10].

2.3 CHEMOCONVULSION BY PTZ:

In the present study, 30 mature albino males Wistar rats were separated into five groups of six animals each randomly. As a control, Group I was given normal saline [2ml/kg of NS 0.9%]. Non-Diabetic Hyperglycaemia [Group II] was given 5ml of 20% glucose intraperitoneally 30 minutes before PTZ induction of chemo convulsion. Groups III and IV mice were given SIB and conventional diazepam before receiving PTZ, whereas Group V animals were given SIB and glucose [20 % mg/kg] before receiving chemo convulsions. On the 14th day, PTZ was administered at a dose of 105 mg/kg i.p to induce chemo convulsions at its CD97 convulsive dose. Animals were kept separately in clear cages [25x15x10cm] for the next 30 minutes to observe the occurrence of clonus-like convulsions [11-12].

Group- I is regarded as Control animals and received NS-2ml/kg.p.o
Group-II is regarded as NDH animals and received Glucose-20% + PTZ [105 mg/kg i.p]
Group-III is regarded as NDH +DIAZ [2mg/kg.i.p] + PTZ [107 mg/kg i.p] group
Group-IV is regarded as NDH [Glucose-20%+ SIB [100 mg/kg.p.o] + PTZ [105 mg/kg i.p] group.

2.4 ANALYSIS OF MORPHOMETRIC PARAMETERS:

All experimental animals' body weights were meticulously assessed at periodic intervals to examine the effect of acute SIB treatment on them.

2.5 MEASUREMENT OF BLOOD GLUCOSE:

Glucose concentrations were assessed before and after the seizure tests in all of the experimental groups. A little drop of blood was obtained from the rat's tail tip to assess blood glucose content. and placed on a test stripe [glucose oxidase / peroxidase reactive strips] of a Dr. Morepen one touch glucometer. In 5 seconds, the glucose level [mg/100ml] was computed in millimolars and available. The glucose tolerance was measured every hour for three hours.

2.6 ASSESSMENT OF NEUROTOXICITY:

The neurotoxic profile of SIB was determined using a chimney test which is considered as. This is one of the gold standard tests for identifying a test chemical's neurotoxic profile. The animals were placed in a horizontal cylindrical tube measuring 25cm in length and 3cm in diameter. A criterion for neurotoxicity has been the capacity to exit the tube backwards in a specific amount of time [13].

2.7 MOBILITY, EXPLORATORY BEHAVIOUR AND MUSCLE COORDINATION:

Throughout the study, the animals were observed for any behavioural changes. The rats were placed individually in the activity cage before the challenge, and the overall activity count was monitored for 5 minutes. Reduced count was assumed to indicate depressive activity, and increased count was thought to be stimulant activity in the central nervous system. [14] The head dip test was used to examine the animals' exploratory behaviour in which the total numbers of head dipping were noted for three minutes. [15–17].
The rota rod is one of the most commonly utilised experimental tools for determining a test medication’s muscular grip strength and coordination [18-20]. The rota rod system produced by Inco Instruments, Ambala, India, was used to automatically record the latency to fall animals from the rotating bar, which revolves at a speed of 25 rpm. All of the animals were given enough trials to avoid experimental bias.

2.8 STATISTICAL ANALYSIS:

The implication of the difference between the treatment and control groups was determined using a one-way analysis of variance [ANOVA] followed by the Dunnett test. p<0.05 was used to determine statistical significance.

3. RESULTS:

3.1 BLOOD GLUCOSE LEVEL:

Blood glucose values were observed hourly for three hours before and after PTZ-induced seizures in all experimental groups. The glucose levels were found to be normal prior to seizure induction, with no hyperglycaemia [Fig 1]. Except in the control groups, glucose levels increased after inducing chemo convulsions. Blood levels were found to be considerably lower in SIB-treated rats when compared to NDH+ PTZ groups (at 1hr *p<0.05, at 2h **p<0.01).

Fig 1: Effect of SIB on blood glucose level after seizure induction on rats
3.2 PTZ ELICITED CHEMO CONVULSIONS:

The commencement of clonus convulsion [Fig.1a] and its duration [Fig.1b] were observed and evaluated in all experimental animals in the PTZ model. Pre-treatment with SIB [100 mg/kg p.o] resulted in a significant delay in the onset of CC (*p<0.05) followed by a significant reduction in the duration of CC (**p<0.01) when compared to control mice. According to the findings, pre-treatment with SIB had a protective impact (**p<0.05) on the duration of the clonic seizure form in rats, as well as a delay in the onset of clonic seizures. [Fig.2a & 2b].
The effect of SIB on PTZ-induced chemo convulsions [b] is shown in Figures 1[a] and 1[b]. Pretreatment with SIB [100 mg/kg p.o.] provided considerable protection against PTZ-induced chemo convulsions, as demonstrated by a delayed start and a significant reduction in CC duration, according to the findings. Values are Mean ± SEM; *p<0.05, ** p<0.01 & *** p<0.001 compared to control groups; ns: not significant. Non-significant. [n=5] is the number of animals.

### 3.3 MORPHOMETRIC EVALUATION:

To demonstrate the effect of SIB treatment, all experimental animals were morphometrically assessed, and the findings are displayed in Table 1. From the results, acute administration of SIB showed significant increases in body weight, with a particularly apparent increase in NDH groups treated with SIB [164.30±4.07, p<0.05] on day 14 compared to control [145.70± 5.01] and NDH + PTZ [155.20± 5.47] treated groups, according to the findings. As a result, morphometric studies demonstrated that SIB has a positive influence on body weight gain, demonstrating that it has no negative effects.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Changes in Body weight [g]</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 7</td>
<td>Day 14</td>
</tr>
<tr>
<td>CONTROL</td>
<td>135.90 ± 3.16</td>
<td>142.40 ± 3.59</td>
<td>144.40 ± 3.19</td>
</tr>
<tr>
<td>NDH + PTZ</td>
<td>139.10 ± 3.47 ns</td>
<td>142.30 ± 2.73 ns</td>
<td>148.30 ± 2.20 ns</td>
</tr>
<tr>
<td>DIAZ + PTZ</td>
<td>136.70 ± 3.13 ns</td>
<td>141.50 ± 2.45 ns</td>
<td>141.50 ± 2.45 ns</td>
</tr>
<tr>
<td>NDH + SIB + PTZ</td>
<td>138.57 ± 4.72 ns</td>
<td>140.6 ± 5.39 ns</td>
<td>154.42 ± 2.35 ns</td>
</tr>
</tbody>
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**Statistical comparison:** Values are mean ± SEM; *p<0.05, ** p<0.01, and *** p<0.001 compared to the control group, NS: Non-significant. The number of animals [n=5].

Figure 3 shows the effect of SIB on experimental animals' body weight on day 14. Values are mean ± SEM; *p<0.05, ** p<0.01, and *** p<0.001 compared to the control group, NS: Non-significant. The number of animals [n=5].
3.4 EVALUATING ACUTE NEUROTOXICITY:

A typical chimney test with rats was used to explore SIB's neurotoxic effects. According to the findings, all experimental rats given SIB at 100 mg/kg i.p. were able to evacuate the tube within one minute by climbing back up as soon as they reached the other end, demonstrating that SIB has no effect on motor control and is not neurotoxic [Fig 4].

![Image of chimney test results](image)

**Figure 4: Acute neurotoxicity by chimney test**

The results of the chimney test, shown in Figure 4, demonstrated that all of the experimental animals were able to climb backwards out of the tube within one minute, indicating that SIB has no effect on motor control and is not neurotoxic to the animals.

3.5 MOBILITY, EXPLORATORY BEHAVIOUR AND MUSCLE COORDINATION:

Fig. 5 depicts the effect of SIB on the rota rod test muscle grip strength of chemo convulsive rats. The effects of SIB on locomotion and the head tip test are seen in Fig. 6. In the rota rod test, animals given NDH+ PTZ or NDH + SIB + PTZ showed no significant differences in muscle grip strength when compared to control groups. The diazepam-treated groups, on the other hand, showed a continuous decrease in muscle grip strength, confirming the traditional medication’s calming characteristics. The locomotion results showed that the remaining experimental groups, such as NDH+ PTZ and NDH + SIB + PTZ, exhibited no significant differences in locomotion compared to control groups, with the exception of the normal diazepam receiving [*p<0.05] group.
4. DISCUSSION:

Seizures are expected to be among the neurological disorders caused by metabolic diseases. Synaptic transmission requires a specific level of glucose concentration, according to in-vivo and in-vitro research. As per the reports high glucose produces convulsions and has been associated to neuronal hyper excitability and ischemia-induced brain damage in rat models, whereas fasting-induced hypoglycaemia protects against this neurotoxicity. [21,22].

As a result, the intention of this research is to understand further about the connection between blood glucose and seizure-inducing responses in experimental groups. The results of this study back up prior research that demonstrated a significant increase in blood glucose in NDH groups when compared to control groups. Acute SIB administration, however, resulted in significant blood level reductions at 1 hour [*p<0.05] and 2 hours [**p<0.01] compared to the NDH + PTZ groups.

On the other hand, morphometric analysis revealed that SIB administration showed significant rise in body weight, with a particularly noticeable increase in NDH groups treated with SIB (127.8 ±2.557, p<0.05) on day 14 compared to control (148.2 ±3.693) and NDH + PTZ NDH + PTZ (181.6 ±2.657) treated groups. In addition, the results of a classic chimney test reveal that SIB has no effect on motor control because all animals were able to evacuate the tube backwards within a predetermined time limit, demonstrating that SIB is not neurotoxic. Behavioral testing revealed no significant changes in muscle coordination, locomotion, or nervous behavior in all experimental animals, with the exception of diazepam-treated groups. The diazepam-treated groups, on the other hand, demonstrated a consistent drop in muscular grip strength, indicating the relaxing properties of the traditional medication. The outcomes were comparable to those of the control animals.
5. CONCLUSION:

According to the findings of this study, the NDH + PTZ groups had significantly higher glycemic levels, as evidenced by the rat's early onset and longer duration of chemo convulsions. However, when compared to the control groups, acute administration of SIB significantly improved glycemic control, as well as the delayed onset and duration of seizures. As a result of these findings, seizure length is linked to blood glucose levels, signifying that seizures are longer in NDH + PTZ rats. As a result, our data corroborate and validate the link between blood glucose and seizure responses, and demonstrate a beneficial impact on blood glucose-level reduction and seizure protection in chemo-convulsive rats.

DECLARATION OF INTEREST

There are no conflicts of interest declared by the authors.

FUNDING

The submitted research work was funded and supported by Internal Research Grant from Sree Vidyanikethan Educational Trust (SVET, Tirupati, AP, India) with Approval No: SVCP-11 dated 29th June 2019.

ACKNOWLEDGMENTS

The authors express their gratitude to the management of Sree Vidyanikethan Educational Trust-Mohan Babu University for providing financial support as well as the necessary facilities and support.

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