Other mechanisms involved in the development of insulin resistance include the P110 heterodimer being responsible for the PI3-kinase activity [1]. Originally identified as a regulator of glycogen synthase (GS), a rate-limiting enzyme that promotes glycogen deposition. In basal state, glycogen synthase kinase-3 (GSK-3) activation results in the dephosphorylation of substrates including GS, resulting in their functional activation and consequent increased glycogen synthesis. In insulin-resistant state, in the absence of insulin and the insulin receptor may thus represent a novel therapeutic strategy for severe insulin-resistant patients, TIDM, and T2DM [6].

Vanadium compounds act in an insulin-mimetic manner both in vitro and in vivo have been well established. Vanadium is an ultra trace element, widely distributed in the nature and its compounds would closely mimic the physiological action of insulin by initial entry into the portal system [7].

In this regard and supporting a possible therapeutic use of vanadium, the present study aimed to investigate sodium orthovanadate (SOV), the inorganic salt compound of vanadium, influence in the reduction of P85 expression and the elevation of GSK-3 expression to the reduction of blood glucose levels of alloxan-induced diabetic mice.
MATERIALS AND METHODS

Animals and experimental groups
A group of 25-40 male mice of Balb/C strain, weighing between 20-30 g and 6-8 weeks of age, was obtained from Animal Laboratory at the Pharmacy Faculty, Airlangga University, Indonesia. Animals were maintained in the climatically controlled animal house facility of Animal Laboratory at the Pharmacy Faculty, for one week before the initiation of the experimentation.

The animals were fed ad libitum with mice feed and tap water. All of mice were divided into five groups. Group 1 was non-diabetic control mice (n=5). Group 2 was diabetic-un-treated control mice (n=5). Group 3 (n=5), Group 4 (n=5) and Group 5 (n=8) were diabetic-treated mice. The methodology of this experiment was performed after the approval by Airlangga University Animal Care and Use Committee (ACUC).

Animal model of diabetes mellitus
Diabetes was induced by a single intraperitoneal injection of alloxan monohydrate (Sigma Aldrich Inc.) at the dose of 200 mg/kgBW, was dissolved freshly in cold normal saline. Non-diabetic control mice received an injection of the vehicle. After 3 days, mice (n=23) with marked hyperglycemia (fasting blood glucose levels exceeding 140 mg/dL) were selected and used for the study [8].

Experimental protocol
Group 1 and Group 2 were administered orally with aquadest. Group 3 (n=5), Group 4 (n=5) and Group 5 (n=8) was forced-fed orally with sodium orthovanadate (Calbiochem Inc.) at the dose of 16, 32 and 64 mg/kg BW/day for 7 days respectively. Fasting blood glucose levels were determined on day 0 (before the induction of diabetes), day 3 (start of treatment), and day 10 (end of treatment). At the end of treatment (day 10), mice were anesthetised and skeletal muscle tissue were collected and fixed in 10% neutral buffered formalin for histochemistry and immunohistochemistry analysis.
nucleus (Fig. 4, sign  ) and inflammatory cells, the boundary of perimisium and endomisium seems clearly and closely so that the tissue looks more solid by muscle cells. The higher dose of sodium orthovanadate, the greater improvement in skeletal muscle cells.

Table 2: Fasting blood glucose levels on day 0 and 3

<table>
<thead>
<tr>
<th>Group</th>
<th>Total mice</th>
<th>Blood glucose levels on day ± SD (mg/dL)</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive</td>
<td>5</td>
<td>71.6 ± 10.2</td>
<td>78.4 ± 8.0</td>
</tr>
<tr>
<td>DM</td>
<td>23</td>
<td>59.1 ± 11.2</td>
<td>310.6 ± 107.2</td>
</tr>
</tbody>
</table>

Values are statistically significant at ’p < 0.05 vs day 0.

Table 3: Fasting blood glucose levels on day 10

<table>
<thead>
<tr>
<th>Group</th>
<th>Fasting blood glucose levels average on day 10 ± SD (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive</td>
<td>64.6 ± 18.3</td>
</tr>
<tr>
<td>DM</td>
<td>406.4 ± 64.1</td>
</tr>
<tr>
<td>DM+Sodium orthovanadate 16 mg/kgBW</td>
<td>30.3 ± 126.8</td>
</tr>
<tr>
<td>DM+Sodium orthovanadate 32 mg/kgBW</td>
<td>231.8 ± 57.1</td>
</tr>
<tr>
<td>DM+Sodium orthovanadate 64 mg/kgBW</td>
<td>75.6 ± 40.8</td>
</tr>
</tbody>
</table>

Values are statistically significant at ’p < 0.05 vs DM group.

Immunohistochemistry of P85 and GSK-3 expressions in skeletal muscle cells

The results of IRS scoring of the muscle cells that expressed the P85 and GSK-3 were summarized in Table 4. Administration of sodium orthovanadate for 7 days, reduced the excessive P85 expressions and increased GSK-3 expressions in skeletal muscle tissue. The higher dose of sodium orthovanadate, the greater reduction in P85 expressions, was characterized by fewer brown color produced (Fig. 5). The higher dose of sodium orthovanadate, the greater enhancement of GSK-3 expressions, was characterized by the growing strength of brown color produced (Fig. 6).
Table 4: P85 and GSK-3 expressions

<table>
<thead>
<tr>
<th>Group</th>
<th>Protein expressions average per field of-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P85</td>
</tr>
<tr>
<td>Naïve</td>
<td>2.7 ± 1.4*</td>
</tr>
<tr>
<td>DM</td>
<td>7.6 ± 1.6</td>
</tr>
<tr>
<td>DM+Sodium orthovanadate 16 mg/kgBW</td>
<td>6.4 ± 2.1*</td>
</tr>
<tr>
<td>DM+Sodium orthovanadate 32 mg/kgBW</td>
<td>4.5 ± 2.1*</td>
</tr>
<tr>
<td>DM+Sodium orthovanadate 64 mg/kgBW</td>
<td>3.9 ± 2.0*</td>
</tr>
</tbody>
</table>

Values are statistically significant at \( p < 0.05 \) vs DM group.

**DISCUSSION**

The main finding of our study was that sodium orthovanadate reduced the excess of P85α expressions (Table 4; Fig. 5) and increased phosphorilation at serin-9 of GSK-3β expressions (Table 4; Fig. 6), would lead to the reduction of blood glucose levels (Table 3) and improved a necrosis in skeletal muscle cells alloxan-induced diabetic mice that given sodium orthovanadate treatment for 7 days (Fig. 4).

Sodium orthovanadate adopts a trigonal bipyramidal structure that mimics the transition state of the phosphoryl transfer reaction, thereby acting as a competitive inhibitor of PTP-1B [11], allowing the phosphoester bond (which also forms an autophosphorylation) and thus the insulin signal transduction remain intact [12]. Inhibition of PTP-1B activity effectively raises the concentration of phosphorylated insulin receptor and IRS-1 [13], thereby allowing the tyrosine-phosphorylated IRS-1 to dock with the p85 regulatory subunit of phosphatidylinositol-3-kinase (PI3-kinase). This interaction unregulated the p110 catalytic subunit of PI3-kinase, which catalyzes the production of phosphoinositide moieties that subsequently activate 3-phosphoinositide-dependent kinases (PDK), including PDK1. One downstream target of PDK1 is the serine/threonine kinase Akt. Interestingly, one substrate for phosphorylation by Akt action is GSK-3. Akt phosphorylates specific serine residues on GSK-3 [14], inhibit GSK-3 via Ser 9/21 phosphorylation, results in the dephosphorylation and activation of GS, leading to increased rates of glycogen synthesis [5], thereby improved a necrosis in skeletal muscle cells in diabetic state. The activation of these steps up to and including PI3-kinase and Akt ultimately results in the translocation of a specific glucose transporter protein isoform (GLUT-4) to the membranes of the sarclemma and the t-tubules, where glucose transport takes place via a facilitative diffusion process. The amount of GLUT-4 protein incorporated into the sarcolemmal membrane correlates closely with the degree of insulin-stimulated glucose transport [14], thereby reduced the elevation of blood glucose levels in diabetic state.

Based on that finding, the reduction of P85 expressions and the elevation of GSK-3 expressions can be used for new therapeutic strategy to reduce the elevating blood glucose levels and to improve a necrosis that may happened in patients with T2DM.

**CONCLUSION**

Vanadium compounds improved metabolic disorders in models of type 2 diabetes mellitus. In type 2 diabetes mellitus, sodium orthovanadate treatment normalized hyperglycemia by reduced P85 expressions and increased GSK-3 expressions in insulin signaling pathway. Hence, this compound is a potential candidate for oral therapy in diabetes as substitutes for insulin.

**CONFLICT OF INTERESTS**

Declared None

**ACKNOWLEDGEMENT**

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**REFERENCES**