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EBSELEN, UM COMPOSTO COM PROPRIEDADE INSULINO-MIMÉTICA, REDUZ A HIPERGLICEMIA TEMPORÁRIA INDUZIDA PELO DIAZINON EM RATOS

DISSERTAÇÃO DE MESTRADO

Michael da Costa

Santa Maria, RS, Brasil
2012

EBSELEN, UM COMPOSTO COM PROPRIEDADE INSULINO-MIMÉTICA, REDUZ A HIPERGLICEMIA TEMPORÁRIA INDUZIDA PELO DIAZINON EM RATOS

Michael da Costa

Dissertação apresentada ao Programa de Pós-Graduação em Ciências Biológicas, Área de Concentração em Bioquímica Toxicológica, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do grau de **Mestre em Bioquímica Toxicológica**.

Orientadora: Profª. Drª. Cristina Wayne Nogueira
Co-orientador: Prof. Dr. Ricardo Brandão

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elaborada por
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como requisito parcial para obtenção do grau de
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Santa Maria, 01 de março de 2012

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Aqueles que sempre estiveram ali quando eu precisei de alguém pra conversar, ou aqueles, que lembraram-se de mim quando precisavam de alguém pra conversar...

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Muito obrigado a todos.

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica
Universidade Federal de Santa Maria

EBSELEN, UM COMPOSTO COM PROPRIEDADE INSULINO-MIMÉTICA, REDUZ A HIPERGLICEMIA TEMPORÁRIA INDUZIDA PELO DIAZINON EM RATOS

AUTOR: Michael da Costa
ORIENTADORA: Cristina Wayne Nogueira
CO-ORIENTADOR: Ricardo Brandão
LOCAL E DATA DA DEFESA: Santa Maria, março de 2012

O diazinon (DI) é um dos organofosforados (OP) mais utilizados no controle de pragas ao redor do mundo. Desta forma, são cotidianos os relatos de intoxicações acidentais por estes. Além disso, muitos casos de intoxicações devido à ingestão aguda por OP em tentativas de suicídio foram relatadas durante os últimos anos. Sabe-se que o principal efeito tóxico dos OP é através da inibição da acetilcolinesterase (AChE), levando a um maior estímulo das sinapses colinérgicas no sistema nervoso central (SNC) o que pode provocar à morte. A intoxicação por estes compostos também pode levar a um estado de hiperglicemia. Ainda desconhece-se o mecanismo pelo qual os OP levam a este efeito adverso, contudo, sugere-se que as vias da gliconeogênese e da glicogenólise estejam envolvidas. O ebselen (EB) apresentou propriedades benéficas em modelos experimentais de diabetes induzida com aloxano ou geneticamente, assim como contra complicações consequentes da diabetes. Sendo considerado assim, como um agente farmacológico em potencial para o tratamento da hiperglicemia provocada pela intoxicação por OP. Dessa forma, o presente trabalho avaliou os efeitos do EB *in vivo* no metabolismo da glicose e em outras alterações bioquímicas induzidas pelo DI em ratos, assim como, o efeito insulino-mimético do EB na captação de glicose em tecido muscular esquelético e na síntese e degradação do glicogênio hepático *in vitro*. Para os experimentos *ex vivo*, ratos *Wistar* machos adultos foram pré-tratados com uma única injeção de EB (50 mg/kg) pela via intraperitoneal (i.p.). Trinta minutos depois, os animais foram tratados com uma única dose de DI (200 mg/kg) pela via oral (v.o). Vinte e quatro horas após, parâmetros indicativos de dano pâncreatico e hepático, como as atividades da amilase, lipase, aspartato aminotransferase (AST), alanina aminotransferase (ALT), fosfatase alcalina (ALP) e lactato desidrogenase (LDH), bem como níveis séricos de glicose, conteúdo de glicogênio hepático e atividade da glicose-6-fosfatase (G6Pase) hepática foram determinados. O pré-tratamento com EB preveniu contra os danos pâncreaticos e hepáticos provocados pelo DI, como mostrado pela redução nas atividades da amilase, lipase, AST, ALT, ALP e LDH. Alem disso, o EB reduziu a hiperglicemia, a qual foi aumentada nos animais expostos ao DI e aumentou o conteúdo de glicogênio hepático, o qual tinha diminuido nestes. Em ensaios *in vitro*, o EB (150 mM) ou insulina (IN 10 mM, controle positivo) foram incubados com amostras de músculo esquelético ou tecido hepático, com o objetivo de medir a captação de glicose, síntese e quebra de glicogênio. Assim, o EB aumentou a captação de glicose pelo músculo esquelético, estimulou a síntese de glicogênio hepático e inibiu a quebra do glicogênio em uma maneira similar à IN. Neste contexto, o EB apresentou propriedades insulino-miméticas, diminuindo os níveis de glicose sérica *in vivo* e aumentando a captação de glicose pelo tecido esquelético, aumentando a síntese e diminuindo a quebra de glicogênio *in vitro*. Desta forma, o EB protegeu da hiperglicemia induzida por OP, agindo de uma forma mimética à insulina.

ABSTRACT

Dissertation of Master's Degree
Graduating Program in Toxicological Biochemistry
Federal University of Santa Maria, RS, Brazil

EBSELEN REDUCES HYPERGLYCEMIA TEMPORARILY-INDUCED BY DIAZINON: A COMPOUND WITH INSULIN-MIMETIC PROPERTIES

AUTHOR: Michael da Costa
ADVISOR: Cristina Wayne Nogueira
CO-ADVISOR: Ricardo Brandão
PLACE AND DATA OF THE DEFENSE: Santa Maria, March, 2012

The diazinon (DI) is a commonly organophosphorus (OP) used in pest control around the world. Severe acute poisoning due to ingestion of OP in suicide attempts were reported during the past years. The main toxic effect of OP is through acetylcholinesterase (AChE) inhibition, leading to a greater stimulation of cholinergic synapses in the central nervous system (CNS) which can lead to death. However, poisoning can lead to such state of hyperglycemia. However, the mechanism by which OP lead to this adverse effect is still unknown, even so, it is suggested that glycogenolysis and neoglycogenic pathways are involved. In recent studies, ebselen (EB) has shown pharmacological properties in experimental models of genetically or alloxan induced diabetes, as well as complications resulting from diabetes. Being considered a potential pharmacological agent for hyperglycemia treatment when caused by OP poisoning. Thus, this study evaluated the effects of EB on the glucose metabolism and other biochemical changes induced in rats by DI, as well as the insulin-mimetic effect of EB on glucose uptake in skeletal muscle tissue and the synthesis and breakdown of liver glycogen in tissues of rats. In *in vivo* experiments, rats were pretreated with a single injection of EB (50 mg/kg) intraperitoneally (i.p.). Thereafter, the animals were treated with a single oral (p.o.) dose of DI (200 mg/kg). Parameters indicative of liver and pancreas damage such as amylase, lipase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activities and serum glucose, hepatic glycogen content and hepatic glucose-6-phosphatase (G6Pase) activity were determined. Pretreatment with EB was effective in preventing against pancreatic and liver damage, as shown by the reduction in the activities of amylase, lipase, AST, ALT, ALP and LDH. EB was able to reduce blood glucose and hepatic glycogen content increased in the animals exposed to DI. In *in vitro* assays, EB (150 mM) or insulin (IN 10 mM as a positive control) were incubated with either skeletal muscle or liver tissue, in order to measure the absorption of glucose, glycogen synthesis and glycogen breakdown. Thus, EB increased glucose uptake in skeletal muscle, stimulated glycogen synthesis and inhibited hepatic glycogen breakdown in a manner similar to IN. In this context, EB demonstrated insulin-mimetic properties, lowering blood glucose levels *in vivo* and increasing glucose uptake by skeletal tissue, increasing glycogen synthesis and decreasing glycogen degradation *in vitro*. In conclusion, EB, possibly through its insulin-mimetic action, protected against pancreatic and hepatic damage caused by DI in rats.

LISTA DE TABELAS

Manuscrito - Ebselen reduces hyperglycemia temporarily-induced by diazinon: A compound with insulin-mimetic properties.

RESULTADOS

LISTA DE FIGURAS

Manuscrito - Ebselen reduces hyperglycemia temporarily-induced by diazinon: A compound with insulin-mimetic properties.

RESULTADOS

LISTA DE ABREVIAÇÕES

ACh – Acetilcolina

AChE – Acetilcolinesterase

ALP – Fosfatase alcalina

ALT – Alanina aminotransferase

AMPc – Adenosina monofosfato cíclico

AST – Aspartato aminotransferase

ATCh – Acetiltiocolina

BChE – Butirilcolinesterase

BTCh – Butiriltiocolina

ChE – Colinesterase

DI – Diazinon

DX – Diazoxon

EB – Ebselen

EROs – Espécies reativas de oxigênio

G6Pase – Glicose 6-fosfatase

GLUT – Transportador de glicose

GPx – Glutationa peroxidase

IN – Insulina

LDH – Desidrogenase láctica

OMS – Organização mundial da saúde

OP – Organofosforado

$(PhSe)_2$ – Disseleneto de difenila

SNC – Sistema Nervoso Central

SUMÁRIO

1. INTRODUÇÃO

Inseticidas organofosforados (OP) constituem uma das classes de pesticidas mais utilizadas no controle de pragas na agricultura e pecuária (AURBEK E COL., 2009). A exposição à OP causa um número significativo de envenenamento e mortes a cada ano (SHADNIA E COL., 2005). Apesar de intoxicações acidentais ocorrerem devido à exposição à pele ou inalação, também são descritos casos graves de intoxicações agudas devido à ingestão destes OP em tentativas de suicídio (KARKI E COL., 2004). De acordo com a Organização Mundial da Saúde (OMS), mais de um milhão de acidentes e dois milhões de tentativas de suicídio por envenenamento com OP são relatados ao redor do mundo anualmente e, destes, aproximadamente duzentos mil levam à morte em países desenvolvidos (SIVANGNANAM, 2002). De acordo com Gulr e col. (1996), os OP são responsáveis por mais intoxicações do que qualquer outra classe de pesticidas.

O diazinon (DI) (0,0-dietil-0-[2-isopropil-6-metilpirimidina-4-il] fosforotionato) é um exemplo clássico desta classe de pesticida. Este composto tem demonstrado efeito tóxico no Sistema Nervoso Central (SNC), Reprodutor, Respiratório e Cardiovascular em ratos (ALTUNAS E COL., 2004). O principal efeito tóxico do DI, assim como dos demais OP, é por meio da inibição da acetilcolinesterase (AChE), resultando no acúmulo de acetilcolina (ACh) e, como consequência, um maior estímulo das sinapses colinérgicas no SNC, nervos parassimpáticos e glândulas (AURBEK E COL., 2009). Porém, o efeito tóxico de alguns OP não se limita unicamente à inibição da AChE, seguida de crises colinérgicas (TONKOPII, 2003). Em estudos *in vitro* e *in vivo*, realizados por Gultekin e col. (2001), foi revelado que os OP induzem a produção de espécies reativas de oxigênio (EROs) e alteração no mecanismo de defesa antioxidante em humanos (RANJBAR E COL., 2002) e animais experimentais (KAMATH E COL., 2008).

A hiperglicemia também tem sido descrita como um dos efeitos adversos ocasionados pelo envenenamento por OP em humanos e animais experimentais (ABDOLLAHI E COL., 2004; HAGAR E COL., 2002; KALENDER E COL., 2005). Porém, o mecanismo preciso pelo qual os OP levam a um estado de hiperglicemia ainda não foi elucidado. Estudos realizados por Rahimi e Abdollahi (2007) sugerem o envolvimento da glicogenólise, gliconeogênese e um estado de resistência à insulina (IN) como possíveis mecanismos que podem explicar a indução da hiperglicemia pelos OP. De fato, foi demonstrado que a

exposição aguda ao DI causou a depleção do glicogênio hepático com um aumento na atividade da enzima glicogênio fosforilase, além de aumentar as atividades de enzimas da gliconeogênese e glicogenólise, como a glicose 6-fosfatase (G6Pase) hepática (KUZ´MINKAIA E COL., 1978). A pancreatite aguda também é uma complicação bem relatada em envenenamentos por OP em humanos e animais experimentais uma vez que o pâncreas é um dos tecidos alvo na intoxicação por OP (HSIAO E COL., 1996). Contudo, ainda desconhece-se o mecanismo molecular preciso pelo qual os OP induzem a pancreatite aguda. Acredita-se que envolva a obstrução dos ductos pancreáticos devido ao aumento na produção de EROs (SEVILLANO E COL., 2003).

O selênio (Se) é um elemento traço essencial, o qual possui funções cruciais como componente integral de enzimas antioxidantes como a glutationa peroxidase (GPx), selenoproteínas P e W e deiodinases (STAPLETON, 2000). As propriedades antioxidantes do Se contribuem para preservar uma condição saudável, já sua deficiência tem sido relacionada com um aumento no risco de doenças cardiovasculares, disfunções imunes, câncer, convulsões e diabetes (COMBS E GRAY, 1998; NAVARRO-ALARCON E LOPES-MARTINEZ, 2000). Assim, o ebselen (EB) (2-fenil-1,2-benzoisoselenazol-3(2H)-um), um composto orgânico sintético de Se, vem sendo considerado como um potencial agente farmacológico (NOGUEIRA E COL., 2004). Este composto apresenta propriedades antioxidantes, anti-nociceptiva, neuroprotetora e antiinflamatória em diversos modelos experimentais (MEOTTI E COL., 2004; NOGUEIRA E COL., 2003; PORCIUNCULA E COL., 2003). Ainda, o EB tem exibido propriedades protetoras em modelos experimentais de diabetes induzida geneticamente (CHANDER E COL., 2004) e diabetes induzida com aloxano (MIRANDA E COL., 2006), assim como contra complicações consequentes da diabetes como a retinopatia e a disfunção endotelial (CHANDER E COL., 2004; MIRANDA E COL., 2006).

Outro composto orgânico de Se, o disseleneto de difenila $(PhSe)_2$, foi eficaz em reduzir a hiperglicemia induzida por estreptozotocina em ratos (BARBOSA E COL., 2006). Além disso, alguns trabalhos têm demonstrado efeitos benéficos de compostos inorgânicos de Se em modelos de hiperglicemia. O mecanismo exato pelo qual estes compostos inorgânicos exibem ação anti-hiperglicêmica é ainda incerto, mas parece se tratar de uma propriedade mimética à IN (BERG E COL., 1995; EZAKI E COL., 1990; STAPLETON, 2000). De fato, a administração de compostos inorgânicos de Se a ratos diabéticos é capaz de reduzir a

concentração de glicose plasmática e aumentar a captação tecidual desta. Isto possivelmente, devido à translocação dos transportadores de glicose do citoplasma à membrana plasmática (STAPLETON E COL., 1997) e também através de um possível aumento na expressão de enzimas associadas ao metabolismo de carboidratos e ácidos graxos *in vitro* (BERG E COL., 1995). Entretanto, com relação ao EB, não existem relatos na literatura sobre uma possível ação insulino-mimética deste composto. Além disso, não existem estudos relacionando os efeitos do EB em alterações do metabolismo da glicose e ao dano tecidual provocado pelo DI. Sendo assim, torna-se interessante a procura para tratamentos alternativos para casos de intoxicação por DI, assim como estudos que visem elucidar os efeitos do EB e uma possível ação insulino-mimética deste composto de Se em casos de hiperglicemia induzida por OP.

2 OBJETIVOS

2.1 Objetivo geral

Avaliar os efeitos farmacológicos da pré-administração de EB em ratos com hiperglicemia induzida pelo DI e os efeitos insulino-miméticos deste composto orgânico de Se.

2.2 Objetivos específicos

✓ Avaliar os efeitos do EB no metabolismo da glicose e a outras alterações bioquímicas induzidas pelo DI em ratos *ex vivo*.

✓ Avaliar o efeito insulino-mimético do EB na captação de glicose em tecido muscular esquelético e na síntese e degradação de glicogênio hepático em tecidos de ratos *in vitro*.

3. DESENVOLVIMENTO

Os resultados que fazem parte desta dissertação estão apresentados sob a forma de manuscrito intitulado "**Ebselen reduces hyperglycemia temporarily-induced by diazinon: A compound with insulin-mimetic properties"**. Os itens Materiais e Métodos, Resultados, Discussão dos Resultados e Referências (do manuscrito), encontram-se no próprio manuscrito, estruturados de acordo com as normas da revista *Chemico-Biological Interactions* para a qual este foi submetido.

3.1. Manuscrito 1

Ebselen, um composto com propriedade insulino-mimética, reduz a hiperglicemia temporária induzida pelo diazinon em ratos

Ebselen reduces hyperglycemia temporarily-induced by diazinon: A compound with insulin-mimetic properties

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Ebselen reduces hyperglycemia temporarily-induced by diazinon: A compound with insulin-mimetic properties

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Abstract

The present study investigated the effect of ebselen (EB) against hyperglycemia induced by the organophosphate (OPI) diazinon (DI) in rats. The insulin-mimetic properties of EB were investigated *in vitro* with the aim of better understanding the hypoglycemic effect of this compound. The protective effect of EB against pancreatic and hepatic damage caused by DI in rats was also appraised. In the *in vivo* experiments, rats were pre-treated with a single injection of EB (50 mg/kg, intraperitoneal, i.p.). Afterward, animals were treated with a single injection of DI (200 mg/kg, i.p.). The parameters indicative of pancreatic and hepatic damage such as, serum amylase, lipase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activities as well as serum glucose levels, hepatic glycogen content and glucose-6-phosphatase (G6Pase) activity were determined. EB pre-treatment was effective in reducing serum amylase, lipase, AST, ALT, ALP, and LDH activities, protecting against pancreatic and hepatic damage. EB reduced hyperglycemia and increased hepatic glycogen content in animals exposed to DI. In the *in vitro* assays, EB (150 μM) or insulin (IN 10 μM, positive control) was incubated with either skeletal muscle or hepatic tissue with the aim of measuring glucose uptake, glycogen synthesis and glycogen breakdown. EB increased the glucose uptake in skeletal muscle, stimulated hepatic glycogen synthesis and inhibited glycogen breakdown in a similar way to IN. In conclusion, EB, possibly through its insulin-mimetic action, protected against pancreatic and hepatic damage caused by DI in rats.

Key words: Insulin-mimetic; organophosphate; ebselen; pancreatic; hepatic, hyperglycemia.

1 Introduction

Diazinon (DI) (O,O-diethyl-O-[2-isopropyl-6-methylpyrimidin-4-yl] phosphorothionate) is one of the most common used organophosphate insecticides (OPI) in the control of a range of crop pests and as a veterinary ectoparasiticide around the world [1]. DI has some serious effects on the living organisms [2]. OPI compounds are primary recognized for their ability to induce toxicity in mammals through inhibition of acetylcholinesterase activity (AChE; EC 3.1.1.7) leading to accumulation of acetylcholine (ACh) and later activation of cholinergic muscarinic and nicotinic receptors [3,4], the central nervous system (CNS) and the respiratory system are also affected causing symptoms such as weakness, convulsion and respiratory failure [5]. DI itself is not a potent cholinesterase (ChE) inhibitor, however; it is converted to diazoxon (DX), a compound that is a strong enzyme inhibitor *in vivo* [6].

However, the toxic effects of some OPI are not limited to AChE inhibition leading to accumulation of acetylcholine (ACh). It is well known that hyperglycemia is one of the side-effects in sub-chronic and acute OPI exposure [7], whereas, studies have reported that this hyperglycemic state is only temporary [8,9]. Some of the proposed mechanisms for hyperglycemia following OPI poisoning are physiological stress, inhibition of paraoxonase, pancreatitis, oxidative stress and ChE inhibition [5].

It is well reported that pancreas is severely affected by some ChE inhibitors like OPI [10,11]. It is suggested that pancreatic tissue-fixed butyrylcholinesterase (BChE; EC 3.1.1.8) is the target enzyme of OPI toxicity. The inhibition of pancreatic BChE activity causes excess of cholinergic stimulation resulting in ductular hypertension [10,12], leading to acute pancreatitis [13]. Other mechanisms by which OPI induces pancreatitis may be due to

nitrosative stress generation, resulting in β-cells destruction during the development of type 1diabetes [14].

Studies conducted by Gopinath et al. [15] and Nishimura et al. [16] have reported that exposure to DI causes an increase in serum alanine aminotranferase (ALT), aspartate aminotranferase (AST) and alkaline phosphatase (ALP) activities in rats through the hepatic degeneration. Thus, DI also affects the liver tissue [17], resulting in disturbed metabolism of liver tryptophan, increasing xanthurenic acid formation which has been reported to form a complex with insulin and damage pancreatic β-cells. This phenomenon could also lead to the alteration of glucose metabolism [18].

Treatment of OPI poisoning includes administration of atropine as muscarinic antagonist and oximes, like pralidoxime and obidoxime as reactivators of OPI-inhibited AChE activity. This treatment is efficient in reactivating AChE activity and recovery the muscular tonus; however, it is insufficient in protecting the animal against OPI exposure side-effects, such as hyperglycemia. An additional approach for OPI poisoning is the development of bioscavengers [19] that sequester OPI before they are able to inhibit ChE.

Ebselen (EB) (2-phenyl-1,2-benzisoselenazol-3(2H)-one) is a synthetic organoselenium compound that has been considered a potential pharmacological agent [20] with low toxicity [21]. In fact, this compound exhibits antioxidant and anti-inflammatory properties in different experimental models [22,23]. Some studies have also reported that EB protects against experimental diabetes and diabetic complications such as nephropathy and endothelial dysfunction [23,24].

Based on the above considerations, the purpose of the present study was to investigate the effect of EB against hyperglycemia induced by DI in rats. The insulin-mimetic properties of EB were investigated *in vitro* with the aim of better understanding the hypoglycemic effect

of this compound. The protective effect of EB against pancreatic and hepatic damage caused by DI in rats was also appraised.

2 Materials and Methods

2.1 Animals

Adult male Wistar rats from our own breeding colony (280 - 320 g) were used. The animals were housed in cages and kept on a 12 h light/dark cycle, at a room temperature of 22 ± 2 °C, with free access to food (Guabi, RS, Brazil) and water. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, the Federal University of Santa Maria, Brazil. All efforts were made to minimize the number of animals used and their suffering.

2.2 Chemicals

Diazinon (DI) was purchased from Lusa S.A. (Montevideo, Uruguay); ebselen (EB) was synthesized according to the literature method [25]. Analysis of the ^{1}H NMR and ^{13}C NMR spectra showed that the compound obtained presented analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of EB (99.9%) was determined by GC/MS. Cyclic adenosine monophosphate (cAMP), 5,5´-dithiobis(2-nitrobenzoic acid) (DTNB), glucose-6-phosphate (G6P), glycogen, ethopropazine, acetylthiocholine (ATCh) and butyrylthiocholine (BTCh) were obtained from Sigma (St. Louis, MO, USA).

2.3 Experimental Design

2.3.1 Exposure protocol

Rats were divided into four groups: *I* control (C) n = 6; *II* ebselen (EB) n = 6; *III* diazinon (DI) n = 8; *IV* ebselen + diazinon (EB+DI) n = 8. EB and EB+DI groups were intraperitoneally (i.p.) injected with a single dose of 50 mg/kg body weight of EB [24] dissolved in dimethyl sulfoxide (DMSO).

DI and EB+DI groups were treated orally (p.o.), through oral gavage, with a single DI dose of 200 mg/kg body weight dissolved in canola oil. DI was administered 30 min after EB injection. Canola oil and DMSO in equal volume (1 ml/kg) were given to the control group. The dose and time of DI administration used in this experimental model were based on a previous study of Alpaslam et al. [17].

After 24 h, rats were killed by decapitation and hepatic, pancreatic and blood tissues were collected. All rats were fasted for 12 h.

2.3.1.1 Biochemical profile

Blood was collected through heart puncture and was centrifuged at 4,000 × g for 10 min, to obtain serum. The contents of glucose and hemoglobin were measured using commercial kits (LABTEST, Diagnostica S. A., Minas Gerais, Brazil) as well as the enzymatic activities of AST, ALT, lactate dehydrogenase (LDH) and alkaline phosphatase (ALP), which were used as markers of hepatic injury, and amylase and lipase as markers of pancreatic injury.

2.3.1.2 Erythrocyte AChE activity

AChE activity was determined according to Worek et al. [26] with some modifications. Heparinized total blood was incubated with Triton X-100 1:100 (v/v), after that, the hemolized product was added to 0.1 M phosphate buffer (pH 7.4), containing 3 mM

ethopropazine (to avoid plasma ChE interference). Then, 0.3 mM DTNB was added and the enzymatic reaction was initiated by addition of 0.45 mM ATCh. The absorbance was measured for one min at 436 nm (to avoid hemoglobin interference). The AChE activity of each sample was corrected based on the respective hemoglobin sample and was expressed in nmol/min/mg protein.

2.3.1.3 Plasma BChE activity

Following centrifugation at 4,000 × g for 10 min, plasma was added to 0.1 M phosphate buffer (pH 7.4). Then, 0.3 mM DTNB was added and the enzymatic reaction was initiated by addition of 0.45 mM BTCh. The absorbance was measured for one min at 412 nm. Enzyme activity was determined according to Worek et al. [26] with some modifications and expressed in nmol/min/mg protein.

2.3.1.4 Pancreas AChE and BChE activities

Pancreatic tissue samples were homogenized 1:20 (w/v) in Medium I (0.32 M sucrose, 5.0 mM Tris-HCl, 0.1 mM EDTA) buffer. The homogenate was centrifuged at 4,000 × g for 10 min to yield the low-speed supernatant (S1) fraction that was used in the assay. Enzyme activities were carried out according to the method of Worek et al. [23] using ATCh and BTCh as substrate for AChE and BChE, respectively. Enzyme activities were spectrophotometrically measured at 412 nm and expressed in nmol/min/mg protein.

2.3.1.5 Hepatic glucose-6-phosphatase (G6Pase) activity

G6Pase activity was determined based on a previous method [27]. Briefly, hepatic tissue samples were homogenized 1:100 (w/v), in 250 mM sucrose containing 1 mM EDTA

(pH 7.0), after that the homogenate was centrifuged at 4,000 × g for 10 minutes. The supernatant was incubated with 50 mM G6P for 30 min at 37 ° C. Following incubation, the reaction was stopped by addition of 1.0 ml of 10% trichloroacetic acid (TCA), and tubes were centrifuged at 4,000 × g to remove precipitated protein and the inorganic phosphate (iP) content of supernatant was measured. Enzyme activity was determined by subtracting the Pi content of null-time blanks from respective assay samples, and the results were expressed as µmol Pi released min/mg protein.

2.3.1.6 Hepatic glycogen content

The hepatic glycogen content was determined by the method described by Krisman [28]. Briefly, a known amount of liver tissue was digested in 2 ml of 30% KOH solution. Followed by 10 min on a boiling water bath, 2 ml of ethanol was added to the tubes to precipitate the glycogen. After precipitation, glycogen was re-suspended in 1 ml of 5N HCl 20%. The glycogen content was measured with iodine reagent at 460 nm and expressed as µg of glycogen/mg of tissue.

2.3.1.7 Protein quantification

Protein concentration was measured by the method of Bradford [29], using bovine serum albumin as the standard.

2.3.2 In Vitro Assays

For the following procedures (sections 2.3.2.1 to 2.3.2.3), a subset of three animals with 24 h of fasting was used. Insulin was used as positive control. The concentrations of insulin (10 µM) and ebselen (150 µM), the Krebs-Henseleit bicarbonate (KHB) buffer (118

mM NaCl, 25 mM $NaHCO_3$, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgCl_2$, 2 mM pyruvate, 2% albumin and 11 mM glucose, pH 7.4) as well as the time of the assay (30 min) were the same for all *in vitro* procedures and were based on our previous studies.

2.3.2.1 Glucose Uptake

Glucose uptake was determined based on a previous study [30] with some modifications. Briefly, slices of striated muscle tissue (soleus muscle) weighing approximately 500 mg were incubated for 30 min at 37° C with 1 ml of KHB buffer. The assay contained three samples; I control (C), II insulin (IN) and III ebselen (EB) containing the following:

C: KHB + tissue + DMSO (ebselen vehicle) + saline (insulin vehicle);

IN: KHB + tissue + DMSO + insulin;

EB: KHB + tissue + ebselen + saline.

The glucose concentration of each sample was spectrophotometrically determined at initial time (time zero). After 30 min of incubation, the glucose concentration of each sample was determined (final time).

The amount of the muscle glucose uptake was calculated as the difference of the glucose concentration in each sample (between the initial (0 min) and final (30 min) times). Glucose concentration was expressed as μM of glucose uptake/mg of tissue.

2.3.2.2 Glycogen Synthesis

Glycogen synthesis was determined based on a previous study of Tolman et al. [31] with some modifications. In this test, slices of hepatic tissue weighing approximately 200 mg were incubated for 30 min at 37° C with 1 ml of KBH. The assay contained three samples as

described in the above section (2.3.2.1). The hepatic glycogen content was spectrophotometrically measured according to Krisman [28] at the time 0 and 30 min after incubation.

The amount of hepatic glycogen for each sample was calculated as the glycogen content at the final time minus the concentration at the initial time. Glycogen content was expressed as µg of glycogen/mg of tissue.

2.3.2.3 Glycogen Breakdown Inhibition

For this test, samples of liver were homogenized 1:5 (w/v) in 10 mM Tris, pH 7.5 and centrifuged at 4,000 × g for 5 min to obtain the low-speed supernatant (S1). After that, a sample of S1 was incubated with 1 ml of KHB containing 1 mM cAMP (a glycogen phosphorylase stimulator) and 3 mg glycogen. Insulin, EB or DMSO was added to the samples, resulting in three samples (as described in the above section 2.3.2.1).

The amount of glycogen was spectrophotometrically measured according to Krisman [28] at time 0 and 30 min and expressed as µg of hydrolyzed glycogen/mg of tissue. The amount of hydrolyzed glycogen for each sample was calculated as the glycogen concentration at the final time minus the concentration at the initial time.

2.4 Statistical Analysis

All experimental results are given as the mean (s) ± S.E.M. The statistical analysis was performed using One-way (ANOVA) followed by the Newman-Keuls or Two-way (ANOVA) followed by the Duncan's test when appropriate. p values < 0.05 were considered to be significant.

3 Results

3.1 In Vivo

3.1.1 Biochemical Profile

Two-way ANOVA of AST activity showed a significant EB × DI interaction [$F_{(1,24)}$ = 21.09; p = 0.000117]. Post-hoc comparison revealed that DI increased AST activity (p = 0.000065) and pre-treatment of rats with EB protected against this increase (p = 0.000057) (Table 1).

Statistical analysis of ALT activity revealed a significant main effect of DI [$F_{(1,24)}$ = 5.31; p = 0.030053] and EB [$F_{(1,24)}$ = 7.11; p = 0.013488] showed by two-way ANOVA. Post-hoc comparison showed that ALT activity was significantly increased in DI group (p = 0.025685) and that EB pre-treatment was effective against this alteration (p = 0.018811) (Table 1).

Two-way ANOVA of ALP activity demonstrated a significant DI [$F_{(1,24)}$ = 6.63; p = 0.016594] and EB [$F_{(1,24)}$ = 8.48; p = 0.007613] main effects. Post-hoc comparison revealed that ALP activity was significantly increased (p = 0.015984) in DI group and that EB pre-treatment was effective against this increase (p = 0.012105) (Table 1).

Two-way ANOVA of LDH activity showed a significant EB × DI interaction [$F_{(1,24)}$ = 10.36; p = 0.003667]. Post-hoc comparison indicated that DI increased LDH activity (p = 0.000874) and that EB pre-treatment (p = 0.000094) was effective against the increase (Table 1).

Two-way ANOVA of amylase activity showed a significant EB × DI interaction [$F_{(1,24)}$ = 7.42; p = 0.011794]. Post-hoc comparison revealed that DI increased amylase activity (p = 0.001607) and that pre-treatment with EB was effective against the enzyme alteration (p = 0.006695) (Table 1).

Two-way ANOVA of serum lipase activity demonstrated a significant EB × DI interaction [$F_{(1,24)} = 10.36$; $p = 0.003667$]. Post-hoc comparison revealed that lipase activity increased in DI group ($p = 0.000874$) and this increase was partially prevented by EB pre-administration ($p = 0.000094$) (Table 1).

Two-way ANOVA of serum glucose levels showed a significant EB × DI interaction [$F_{(1,24)} = 14.79$; $p = 0.000777$]. Post-hoc comparison indicated that DI increased glucose levels ($p = 0.000057$) and pre-treatment with EB protected against this increase ($p = 0.000203$) (Fig. 1A).

3.1.1.2 Erythrocyte AChE activity

Two-way ANOVA of AChE activity showed a significant EB × DI interaction [$F_{(1,24)} = 20.48$; $p = 0.0106$]. Post-hoc comparison revealed that DI significantly inhibited AChE activity ($p = 0.0169$). EB at a dose of 50 mg/kg did not restore AChE activity but inhibited *per se* AChE activity (Table 2).

3.1.1.3 Plasma BChE activity

Two-way ANOVA of BChE activity indicated a significant EB × DI interaction [$F_{(1,24)} = 0.1406$; $p = 0.7119$]. Post-hoc comparison showed that BChE activity was significantly inhibited by DI ($p = 0.000001$) and that EB did not restore the activity of this enzyme (Table 2).

3.1.1.4 Pancreas AChE and BChE activities

Post-hoc comparison revealed that AChE activity was significantly inhibited in EB (p = 0.000000) and DI (p = 0.000000] groups. The activity of BChE was not altered in all experimental groups (Table 2).

3.1.1.5 Hepatic glucose-6-phosphatase activity

The activity of G6Pase was not altered in all groups (Fig. 1B).

3.1.1.6 Hepatic glycogen content

Two-way ANOVA of glycogen content revealed a significant EB × DI interaction [$F_{(1,24)}$ = 203.22; p = 0.000000]. Post-hoc comparison revealed that glycogen levels were decreased in DI group (p = 0.000054) and that pre-treatment with EB was partially effective against this decrease (p = 0.000152) (Fig. 1C).

3.2 In Vitro Assays

3.2.1 Glucose Uptake

One-way ANOVA demonstrated that EB and IN increased the glucose uptake in skeletal muscle tissue [$F_{(2,20)}$ = 7.12; p = 0.0037] (Fig. 2A).

3.2.2 Glycogen Synthesis

One-way ANOVA revealed that EB and IN increased the synthesis of glycogen [$F_{(2,23)}$ = 4.13; p = 0.0201] in hepatic tissue (Fig. 2B).

3.2.3 Glycogen Breakdown Inhibition

Statistical analysis revealed that both IN and EB decreased glycogen breakdown [$F_{(2,23)} = 4.39$; $p = 0.0266$] in hepatic tissue (Fig. 2C).

4 Discussion

The present study revealed that pre-administration of EB protected against hepatic and pancreatic damage, which was demonstrated by the alteration in AST, ALT, ALP, LDH, amylase and lipase activities in rats acutely exposed to DI. EB also protected against the increase in serum glucose and was partly effective against the decrease in the hepatic glycogen content caused by this OPI exposure. By contrast, EB pre-administration did not protect against AChE and BChE activities inhibition in animals that received DI. Indeed, EB *per se* inhibited AChE activity in blood and pancreas. Additionally, the *in vitro* assays demonstrated the EB insulin-mimetic property, increasing the glucose uptake by the skeletal muscle, stimulating the synthesis of glycogen and decreasing the hydrolysis of glycogen by the hepatic tissue.

It is well documented that pancreas is the target organ of some OPI like diphonate, dimethoate, mevinphos and DI [32]. The current study showed that acute administration of DI caused pancreatic damage in rats, demonstrated by the increase in serum amylase and lipase activities. A previous study reported that OPI caused also liver damage [15]. In this way, the hepatic damage caused by DI in rats was also demonstrated and characterized by the increase in serum AST, ALT, ALP and LDH activities, results that are in better agreement with a previous study of Alpaslam et al. [17]. Nevertheless, animals that received pre-treatment with EB did not show any alteration in pancreatic and hepatic enzyme activities, indicating that EB protected against pancreatic and hepatic damage in rats. The hepatoprotective effect of EB

could be resulted of the antioxidant properties of this compound [20]. These findings are consistent with the hepatoprotective action of other organoselenium compounds [33,34].

On the one hand, the mechanisms accounting for OPI toxicity are related to AChE inhibition activity leading to ACh accumulation. On the other hand, the pancreatic BChE has been reported as the target enzyme of OPI toxicity [10,12]. With the aim of better explaining the mechanisms involved in DI toxicity, ChE activities were measured in blood and pancreas of rats exposed to DI. As expected, acute administration of DI inhibited AChE activity in blood and pancreas, and BChE activity in blood. Previous studies demonstrated that pancreatic BChE is target of OPI intoxication [35,36]. By contrast, DI did not alter BChE activity in pancreas of rats. The lack of BChE inhibition by DI could be tentatively explained by anatomic, physiologic and molecular cell biology of the rat pancreas which differs substantially from those of human and other experimental animals [37].

Moreover, the attempt to prevent ChE inhibition with pre-administration of EB was not successful. EB inhibited *per se* AChE activity in blood and pancreas, suggesting that EB could be selective for AChE. The selectivity for AChE enzyme is a behavior similar to that of galantamine, a reversible AChE inhibitor [38] and thus preserving plasma BChE to scavenge nerve agent. It is possible that AChE prevention has not been achieved in this protocol most likely due to the high DI dose acutely administrated to rats. Even so, we do not rule out the possibility that EB might have a beneficial effect against AChE inhibition. However, a broader range of doses and routes of administration should be further tested. Mazzanti et al. [39] showed that ebselen *per se* inhibited AChE activity in cerebral structures rich in cholinergic pathways. It has been demonstrated by Nizri et al. [40] that AChE inhibitors possess anti-inflammatory properties promoting a cholinergic upregulation by reducing lymphocyte proliferation and the secretion of pro-inflammatory cytokines. Since the anti-

inflammatory property of EB is well established in the literature [41], we suggest that ebselen could inhibit AChE activity through its anti-inflammatory effect.

In recent years studies have been performed in animals trying to explain the mechanisms by which OPI affect glucose homeostasis [5]. In the present study, acute administration of DI to rats led to a hyperglycemic state, even 24 h after DI administration. A possible mechanism to explain hyperglycemia found in DI-exposed rats is pancreatitis, clearly demonstrated in this study by an increase in serum amylase and lipase activities. It is known that ChE inhibition leads to muscular tremors and convulsions which increase release of glucose via stimulation of glycogenolysis to meet the energy requirement for these activities [42]. In fact, stimulation of the adrenal gland leads to adrenaline hypersecretion which promotes insulin resistance and glycogenolysis in hepatocytes and skeletal muscle [43]. Accordingly, adrenaline hypersecretion has been reported as a mechanism for hyperglycemia caused by OPI poisoning [5]. In the present study, a decrease in hepatic glycogen levels, which could indicate activation of glycogenolysis, was found in animals exposed to DI. Regarding gluconeogenesis pathway interference in hyperglycemia, we investigated G6Pase activity and this enzyme activity remained at normal in DI-exposed rats. This result leads us to suppose that gluconeogenesis is not involved in hyperglycemia induced by DI, however; we cannot completely rule out gluconeogenesis interference in this effect because other gluconeogenic enzymes might be altered and were not determined in this study.

Take into account metabolic pathways, another explanation for the hyperglycemia found in DI-exposed rats could be the stimulation of glycogenolysis. Although we did not measure glycogen phosphorylase activity, the enzyme that catalyzes the rate-limiting step in glycogen degradation, the hepatic levels of glycogen were determined in an attempt to

indirectly predict glycogenolysis activity. As a result, hyperglycemia was accompanied by a decrease in hepatic glycogen levels.

A single administration of EB before DI administration was effective against hyperglycemia and the decrease in hepatic glycogen content, suggesting an insulin-mimetic effect of EB in rats. Accordingly, inorganic selenium stimulates glucose uptake in insulin-responsive tissues; translocates glucose transporter to the plasma membrane and stimulates the tyrosyl phosphorylation of proteins phosphorylated by insulin [44,45]. Although data on antidiabetic effect of organoselenium compounds in animal models are scarce in the literature, Chander et al. [19] and Gealekman et al. [20] have reported that EB was effective against experimental diabetes development and diabetic complications through its antioxidant property. By contrast, EB was not effective in reducing hyperglycemia induced by streptozotocin in rats [46]. The explanation for discrepancies in EB efficiency against hyperglycemia found in the present study and the other [46] would be the differences between the experimental protocols such as via, dose and time of EB administration. It is also important to consider that diazinon and streptozotocin induce hyperglycemia by different mechanisms, thus this certainly is another reason for discrepancies in EB efficiency against hyperglycemia.

The insulin-mimetic properties of EB were investigated *in vitro* with the aim of better understanding EB antihyperglycemic effect. The *in vitro* data revealed that EB increased glucose uptake by the skeletal muscle, showing an insulin-mimetic effect presumably due to its capacity of enhancing glucose transporter translocation from the intracellular site to the plasma membrane. Most of the glycogen synthesis occurs in liver and is insulin-mediated. In this *in vitro* study, EB increased glycogen synthesis in hepatic tissue, presumably through glycogen synthase (UDP-glucose-glycogen glucosyltransferase) stimulation, an enzyme

involved in converting glucose in glycogen. EB also decreased glycogen breakdown, probably inhibiting glycogen phosphorylase activity. Taken together, these *in vitro* results correlate with effects of EB *in vivo*, i.e., the reduction in serum glucose levels and the increase in hepatic glycogen content in animals exposed to DI.

In addition to the above mentioned mechanisms of action, one can speculate that EB antioxidant property could be, at least in part, related to its antihyperglycemic action. Rahimi and Abdollahi [5] reported that the increase of oxidative stress increases glucose production via stimulation of glucose-6-phosphate dehydrogenase (G6PDH) in OPI intoxication.

In conclusion, EB, possibly through its insulin-mimetic action, protected against hyperglycemia induced by DI in rats.

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Figure legends

Fig. 1. Effect of EB on serum glucose levels (A), G6Pase activity (B) and hepatic glycogen content (C) of rats exposed to DI *in vivo*. Data are reported as mean ± S.E.M. of 6 to 8 animals per group. (a) $p < 0.05$ as compared to the control group (Two-way ANOVA/Duncan) and (b) $p < 0.05$ as compared to the DI group (Two-way ANOVA/Duncan).

Fig. 2. Effect of EB and IN (positive control) on glucose uptake in skeletal muscle (A), glycogen synthesis (B) and glycogen breakdown (C) in the hepatic tissue *in vitro*. Data are reported as mean ± S.E.M., for 3-4 independent experiments. (a) $p < 0.05$ as compared to the control (One-way ANOVA/Newman-Keuls).

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Table 1. Effect of EB on hepatic (*H*) and pancreatic (*P*) enzyme activities in rats exposed to DI.

	C	EB	DI	EB+DI
AST (U/L) ^{H}	163.6±10.1	158.3±4.4	233.8±8.5[a]	157.1±6.3[b]
ALT (U/L) ^{H}	69.1±4.5	61.3±4.5	85.5±4.5[a]	67.4±5.2[b]
ALP (U/L) ^{H}	183.1±20.4	149.9±19.7	250.3±13.5[a]	176.9±19.6[b]
LDH (U/L) ^{H}	374.8±25.2	348.7±14.8	527.8±40.4[a]	321.6±14.5[b]
Amylase (U/L) ^{P}	1259±46.7	1417±34.7	2048±99.0[a]	1403±51.3[b]
Lipase (U/L) ^{P}	8.4±1.0	5.9±1.7	26.9±6.3[a]	14.4±1.8[ab]

Data are expressed as mean ± S.E.M. (Two-way ANOVA/Duncan).
(a) $p < 0.05$, compared with the C group.
(b) $p < 0.05$, compared with the DI group.

Table 2. Effect of EB on pancreatic (*P*) and blood (*B*) AChE and BChE enzyme activities in rats exposed to DI.

	C	EB	DI	EB+DI
AChE (nmol/min/mg prot) ^{P}	3.03±0.16	0.00[a]	0.00[a]	0.00[a]
BChE (nmol/min/mg prot) ^{P}	2.21±0.03	2.24±0.00	2.22±0.05	2.21±0.01
AChE (nmol/min/mg prot) ^{B}	3.85±1.45	0.14±0.05[a]	0. 36±0.18[a]	0. 35±0.16[a]
BChE (nmol/min/mg prot) ^{B}	8.47±0.68	8.25±0.85	4.16±0.16[a]	4.10±0.42[a]

Data are expressed as mean ± S.E.M. (Two-way ANOVA/Duncan).
(a) $p < 0.05$, compared with the C group.
(b) $p < 0.05$, compared with the DI group.

Figure 1

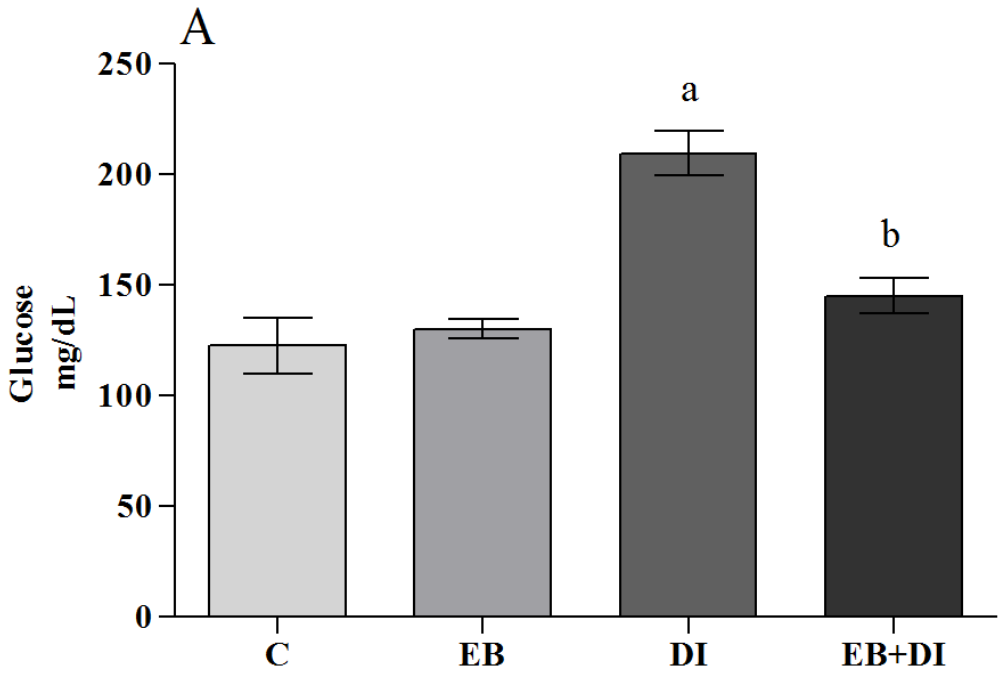


Figure 1

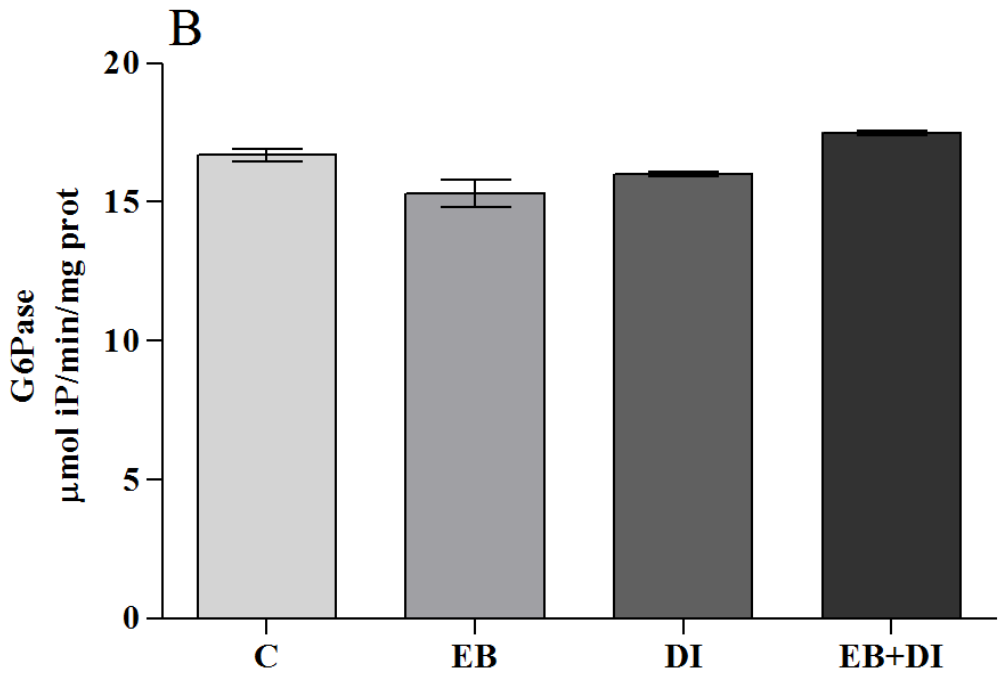


Figure 1

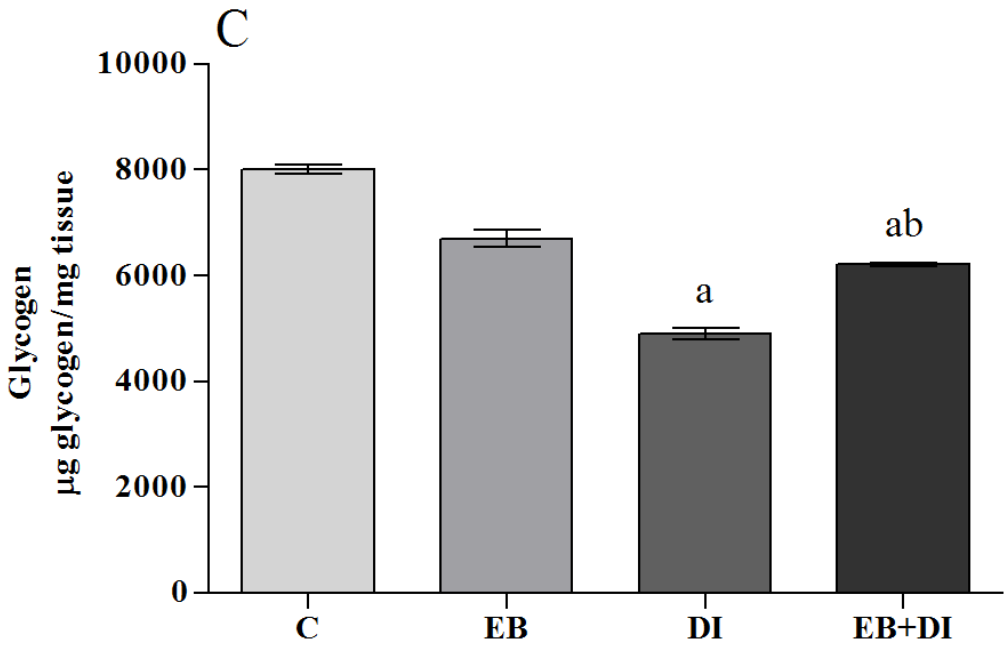

C
10000
8000
6000
4000
2000
0
Glycogen
µg glycogen/mg tissue
a
ab
C
EB
DI
EB+DI

Figure 2

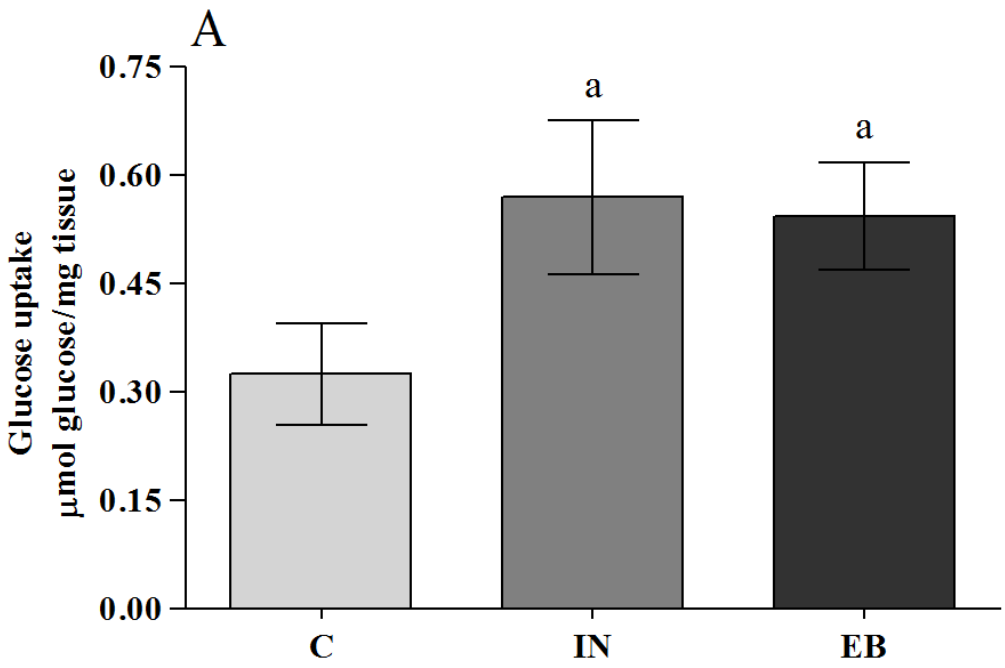

A
0.75
0.60
0.45
0.30
0.15
0.00
Glucose uptake
µmol glucose/mg tissue
a
a
C
IN
EB

Figure 2

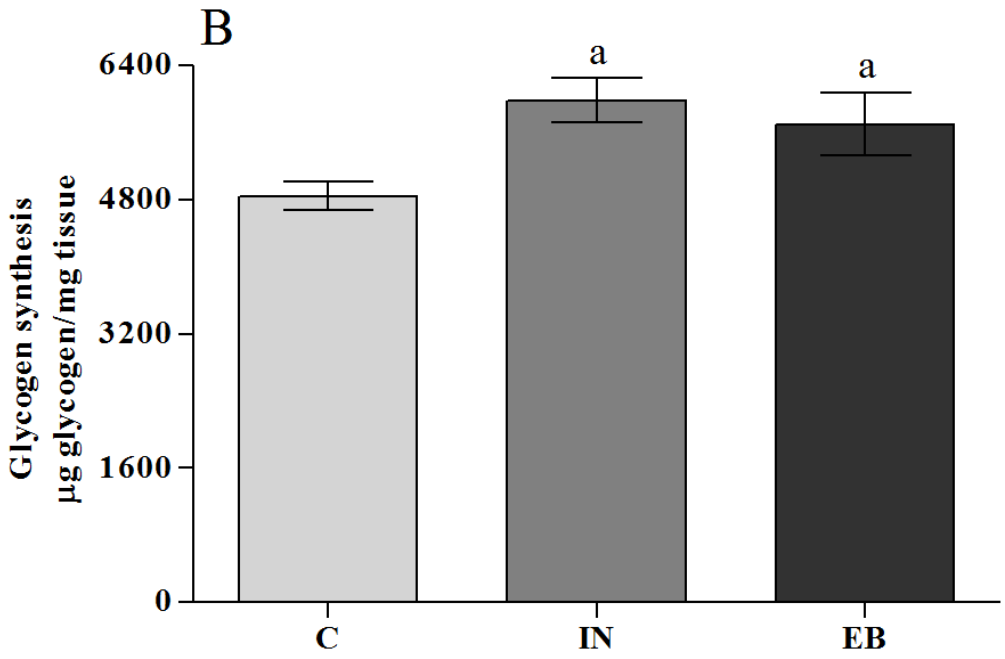
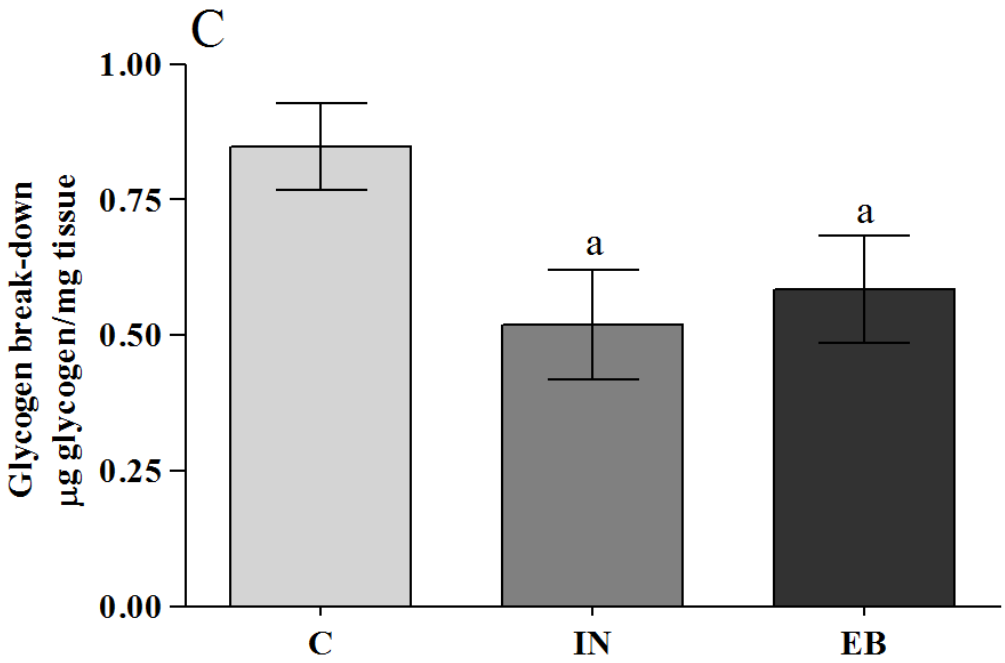

B
a
a
6400
4800
3200
1600
0
Glycogen synthesis
µg glycogen/mg tissue
C
IN
EB

Figure 2


C
1.00
0.75
0.50
0.25
0.00
Glycogen break-down
µg glycogen/mg tissue
a
a
C
IN
EB

4. DISCUSSÃO

No Brasil a exposição a pesticidas OP, representa um grande problema no que diz respeito à saúde da população (BROCARDO E COL., 2007). Sabe-se que estes compostos são potentes inibidores das colinesterases (ChE) e a intoxicação por estes pode levar à morte. Hoje em dia o tratamento imediato para envenenamento por OP consiste na administração de sulfato de atropina seguida da infusão de oximas e anticonvulsivantes (JOOSEN E COL., 2011). Este tratamento atual é muito eficaz na reativação enzimática e recuperação do tônus muscular.

Também são muitos os relatos dos efeitos adversos ocasionados pela intoxicação por OP, como é o caso da hiperglicemia (KALENDER E COL., 2005), pancreatite (HSIAO E COL., 1996), neuropatologias e convulsões (SHIH E McDONOUGH, 1997) entre outros. Porém, para estes efeitos adversos o tratamento atual não exerce nenhuma ação profícua. Nesse contexto, torna-se importante a procura por tratamentos alternativos os quais sejam eficazes contra estes. É assim, que o EB "um composto orgânico de selênio" surge como um tratamento potencial contra a hiperglicemia provocada pela intoxicação por OP, já que este composto tem demonstrado eficácia em diversos modelos de diabetes e contra complicações consequentes desta em camundongos (MIRANDA E COL., 2006). Contudo, hoje em dia não há relatos sobre o efeito do EB sobre a hiperglicemia induzida por OP.

Nesse contexto, o objetivo do presente trabalho consistiu em avaliar o efeito insulino-mimético do EB em um modelo de hiperglicemia aguda induzida por DI em ratos. Assim, os resultados *ex vivo* mostraram que a administração de EB preveniu a hiperglicemia induzida pelo DI, diminuindo os níveis de glicose plasmática e aumentando os níveis de glicogênio hepático. A administração de EB também foi eficaz em proteger os animais do dano hepático e pancreático provocados pelo DI, porém, não foi de suficiente eficácia em prevenir a inibição das ChE. Já *in vitro*, o EB possuiu propriedades miméticas à IN, aumentando a captação de glicose do meio através de amostras de tecido muscular esquelético (em fatias), aumentando a síntese e diminuindo a quebra do glicogênio hepático.

Neste trabalho foi claramente demonstrado que uma administração oral de DI na dose de 200 mg/kg levou a um aumento nos níveis de glicose plasmática. Além do mais, o DI também provocou uma diminuição na concentração de glicogênio hepático, o que pode ter contribuído para esse estado de hiperglicemia nos animais. Diferentemente do que ocorreu

com a concentração de glicogênio hepático a atividade da G6Pase não foi alterada nos animais tratados com DI. Porém, não é descartada em totalidade a interferência da via gliconeogênica na hiperglicemia. O EB, no protocolo de exposição, preveniu este aumento nos níveis de glicose plasmática e a diminuição na concentração de glicogênio hepático, demonstrando assim possuir um efeito insulino-mimético.

O aumento provocado pelo DI nas atividades das enzimas hepáticas AST, ALT, ALP e LDH, assim como, das enzimas pancreáticas amilase e lípase, prova aqui que este OP induz a um dano tecidual nestes órgãos. Já que, estas são enzimas intracelulares e a sua atividade plasmática encontra-se aumentada em casos de lise celular. Apesar de serem muitos os relatos na literatura o mecanismo preciso pelo qual o DI provoca este dano ainda é desconhecido (SEVILLANO E COL., 2003). Contudo, a pré-administração de EB foi capaz em proteger os órgãos deste dano provocado pelo DI. Assim, a dose de EB (50 mg/kg) utilizada neste estudo demonstrou ser muito eficaz, alem disso, não apresentou toxicidade hepática e pancreática aos animais, uma vez que não causou alteração *per se* na atividade destas enzimas no plasma.

Estudos prévios como os de Sahin e col. (2002) e de Harputluoglu e col. (2003) reportam que a BChE pancreática é a enzima alvo da toxicidade dos OP em humanos e animais experimentais, tornando o pâncreas um dos principais órgãos afetados pela intoxicação por OP, como o DI (HSIAO E COL., 1996). Porém, neste estudo a administração de DI levou a uma inibição da atividade da BChE plasmática, mantendo intacta a atividade desta enzima no pâncreas, contrariando assim, estes dados já reportados na literatura. Contudo, este resultado pode ser devido a que o pâncreas de roedores (ratos e camundongos) difere substancialmente do pâncreas humano e de outros animais experimentais, principalmente no que diz respeito a aspectos fisiológicos (CASE, 2006). Entretanto, ambas as atividades, pancreática e eritrocitária da AChE foram inibidas pelo DI. Tendo em vista que o EB possui uma afinidade maior pela AChE em ralação à BChE, como pode ser visto neste estudo, acredita-se que o EB possa vir a proteger da inibição da atividade da AChE por OP, assim também, preservando atividade da BChE a qual ira servir como uma proteção adicional contra esta inibição (HILMAS E COL., 2009). A dose de EB utilizada neste estudo não foi efetiva em proteger a inibição na atividade da AChE provocada pelo DI. Porém, não é descartado, que o EB possa vir a proteger da inibição da atividade da AChE provocada por OP. Contudo, a realização de uma curva de dose e de tempo seria necessária para uma melhor elucidação desta hipótese.

Sabe-se que o tecido muscular esquelético faz uso de glicose como fonte de energia, além do mais, no músculo esquelético ocorre à síntese de glicogênio, esta síntese ocorre geralmente para a reposição dos depósitos de glicogênio do músculo após terem sidos exauridos, por exemplo, pelo exercício físico (CHAMPE E HARVEY, 2000). Porém, este se trata de um tecido insulinodependente, o qual necessita da IN para estimular a captação da glicose do meio através dos transportadores de glicose (GLUT). Desta forma, foi demonstrado aqui que quando adicionado EB (150 μM) a um meio contendo amostras de músculo esquelético (em fatias) e uma solução tampão a qual contem glicose (EVANS E COL., 1997), este composto orgânico de selênio foi capaz de aumentar a captação da glicose do meio por parte do músculo esquelético. Assim, agindo de uma forma mimética à IN, já que este resultado também foi comparável com a adição de IN (10 μM) ao meio. Ainda é incerta a forma pela qual o EB age na captação de glicose. Contudo, sabe-se que o selenato, um composto inorgânico de selênio, afeta o transporte de glicose, assim como a translocação dos transportadores desta em tecidos insulinodependentes (EZAKI, 1990). Desta forma, não se exclui a hipótese de que o EB possa vir a agir de uma forma semelhante.

Já o tecido hepático não é um tecido insulinodependente no que diz respeito à captação de glicose do meio, uma vez que os GLUT encontrados nos hepatócitos não necessitam de IN para estimular o transporte de glicose do meio externo para o interior da célula. Porém, é no tecido hepático que ocorre a maior parte da síntese de glicogênio e este sim é um processo estimulado pela IN. Também é no fígado que ocorre a fosforilação do glicogênio, processo o qual é inibido pela IN (CHAMPE E HARVEY, 2000). Neste trabalho conseguiu-se estimular a síntese de glicogênio pelo tecido hepático ao adicionar EB a um meio contendo o tecido hepático e uma solução tampão a qual continha glicose. Da mesma forma provamos que a adição do EB a um meio contendo tecido hepático e uma solução tampão contendo AMPc diminuiu a quebra do glicogênio. Estes testes com o EB foram realizados em paralelo com a IN como controle positivo, e assim foi visto que este composto orgânico de selênio “em uma concentração quinze vezes (15x) maior que a da IN” obteve resultados semelhantes aos desta. Assim, este é o primeiro relato sobre o efeito insulino-mimético de um composto orgânico de selênio sobre a síntese e a quebra do glicogênio.

5. CONCLUSÕES

De acordo com os resultados apresentados nessa dissertação, é possível concluir que:

- ✓ A administração de EB pela via intraperitoneal foi eficaz na proteção contra a hiperglicemia induzida por DI em ratos.

- ✓ O EB possui propriedades insulino-miméticas, aumentando a captação de glicose em amostras de tecido esquelético, assim como, aumentando a síntese e diminuindo a quebra do glicogênio hepático.

6. PERPESCTIVAS

Considerando os resultados obtidos nesta dissertação, as perspectivas para trabalhos posteriores são:

- ✓ Investigar por quais outras vias o DI induz à hiperglicemia.
- ✓ Investigar por quais outras vias o EB exerce efeito insulino-mimético.
- ✓ Investigar a dose e o tempo para a administração do EB, os quais possam vir a exercer efeitos protetores sobre inibição da AChE pelo DI.

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