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Research Article

Neuroprotective and antioxidant activities of saponins' mixture from *Astragalus glycyphylloides* in a model of 6-hydroxydopamine-induced oxidative stress on isolated rat brain synaptosomes

Magdalena Kondeva-Burdina[1], Ilina Krasteva[3], Georgi Popov[2], Vasil Manov[2]

1 *Laboratory of Drug Metabolism and Drug Toxicity, Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University of Sofia, 2 Dunav St., 1000, Sofia, Bulgaria*

2 *Department of Internal Non-communicable Diseases, Pathology and Pharmacology, Faculty of Veterinary Medicine, University of Forestry, 10 St. Kliment Ohridski Blvd., 1797, Sofia, Bulgaria*

3 *Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Sofia, 2 Dunav St., 1000, Sofia, Bulgaria*

Corresponding author: *Magdalena Kondeva-Burdina* (magdalenakondeva@gmail.com)

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Abstract

The aim of the study was to investigate the possible neuroprotective and antioxidant activity of purified saponins'mixture (PSM), isolated from *Astragalus glycyphylloides* (Fabaceae), in a model of 6-hydroxydipamine (6-OHDA)-induced oxidative stress on isolated rat brain synaptosomes. Synaptosomes were incubated with 3 different concentrations of PSM: 60 μg/mL; 6 μg/mL; 0.6 μg/mL. The effects of PSM were compared to those of silymarin (S), at the same concentrations. The main parameters, characterized functional and metabolic status of synaptosomes, were investigated: viability (MTT-test) and level of reduced glutathione (GSH). At isolated rat brain synaptosomes, in conditions of 6-OHDA-induced oxidative stress (150 μM), PSM revealed statistically significant, concentration-dependent, neuroprotective and antioxidant effects, compared to those of silymarin. Effects were most prominent at concentration 60 μg/mL. These neuroprotective effects of PSM might be due to the possible activity as scavenger of reactive oxygen species (ROS), produced by p-quinone (toxic metabolite of 6-OHDA).

Keywords

Astragalus glycyphylloides, saponins' mixture, synaptosomes, neuroprotection, antioxidant

Introduction

Oxidative stress is connected with the pathogenesis of many diseases like neurodegenerative disorders (Parkinson`s and Alzheimer`s disease), atherosclerosis, diabetes, cancer disease. Oxidative stress leads to disabilities, as free radicals damaging a number of structures such as lipids, proteins and DNA. Oxidative stress plays an important role in physiological adaptation and regulation of the signaling cellular transduction as well.

In our previous investigations was found antioxidant and neuroprotective activity of saponins and saponins' mixtures, obtained from different species of genus *Astragalus* – *A. corniculatus*, *A. hamosus*, *A. monspessulanus* (Mitcheva et al. 2008, 2009; Kondeva-Burdina et al. 2014). The effect of saponins from *A. glycyphylloides*, administered alone, on isolated rat brain synaptosomes and hepatocytes were also studied (Popov et al. 2018).

These data give us the ground to investigate the effects of purified saponins' mixture (PSM), isolated from *Astragalus glycyphylloides*, on isolated rat brain synaptosomes in conditions of 6-OHDA-induced oxidative stress.

Materials and methods

Plant material

The overground parts of *Astragalus glycyphylloides* DC. (Fabaceae) were collected in July 2016 in Rila Mountain, Bulgaria. The species was identified by Dr D. Pavlova from Faculty of Biology, Sofia University, Bulgaria, where voucher specimen was deposited (SO-093817).

Obtaining of PSM

The air-dried and powdered plant material was exhaustively extracted with 80% methanol via percolation. After evaporated of the solvent the dry residue were suspended in water and sequentially extracted with dichloromethane, ethyl acetate and n-butanol. The n-butanol extract was separated by column chromatography over Diaion HP-20, using H_2O-MeOH (100:0 → 0:100, v/v) as eluent to give ten main fractions (I-X).

Fraction IX was analysed by LC-HREISMS and a total saponin content of 60% w/w was detected. There was one main oleanan type triterpene saponin 3-*O*-β-D-glucopyranosyl-28-*O*-[β-D-xylopyranosyl-(1 → 2)-β-D-glucopyranosyl] oleanolic acid, with a molecular mass of 623 u (Shkondrov et al. 2018).

Chemicals

In our experiments: HEPES (Sigma Aldrich, Germany), NaCl (Merck, Germany), KCl (Merck, Germany), D-Glucose (Merck, Germany), $CaCl_2 \times 2H_2O$ (Merck, Germany), Trichloroacetic acid (TCA) (Sigma Aldrich, Germany), 2,2'-dinitro-5,5'-dithiodibenzoic acid (DTNB) (Merck, Germany), D(+)Sucrose (Fluka AG, Germany), $MgCl_2 \times 6H_2O$ (Merck, Germany), Percoll (Sigma Aldrich, Germany), NaH_2PO_4 (Merck, Germany), 6-hydroxydopamine (Sigma Aldrich, Germany), MTT (3-[4,5-dimethylthiazol-2-yl]-2,5diphenyl-tetrazolium bromide) (Sigma Aldrich, Germany), were used.

Animals

Old male Wistar rats (body weight 300–400 g) were used. The rats were housed in Plexiglas cages (3 per cage) in a 12/12 light/dark cycle, under standard laboratory conditions (ambient temperature 20 °C ± 2 °C and humidity 72% ± 4%) with free access to water and standard pelleted rat food 53-3, produced according ISO 9001:2008.

Animals were purchased from the National Breeding Center, Sofia, Bulgaria. At least 7 days of acclimatization was allowed before the commencement of the study. The health was monitored regularly by a veterinary physician. The vivarium (certificate of registration of farm № 0072/01.08.2007) was inspected by the Bulgarian Drug Agency in order to check the husbandry conditions (№ A-11-1081/03.11.2011). All performed procedures were approved by the Institutional Animal Care Committee and the principles stated in the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes were strictly followed throughout the experiment.

Isolation and incubation of synaptosomes

Synaptosomes were prepared by brains from old male Wistar rats, as previously described by Taupin et al. (1994) with small modifications (Kondeva-Burdina et al. 2014). The brains were homogenized in 10 vol. of cold Buffer 1, containing: 5 mM HEPES and 0.32 M sucrose. The synaptosomes were isolated by using Percoll reagent to prepare the gradient. They were re-suspended and incubated in Buffer 2, containing: 290 mM NaCl, 0.95 mM $MgCl_2.6H_2O$, 10 mM KCl, 2.4 mM $CaCl_2.H_2O$, 2.1 mM NaH_2PO_4, 44 mM HEPES, 13 mM D-glucosa. Incubations were performed in a 5 % CO_2 + 95 % O_2 atmosphere. The content of synaptosomal protein was determined using serum albumin as a standard (Lowry et al. 1951).

Synaptosomes vibility' measured by MTT-test, described by Mungarro-Menchaca et al. (2002)

After incubation with PSM and silymarin, synaptosomes were treated with MTT solution (0.5 mg/mL) for 1 h in 37 °C and then were centrifuged at 15 000 × g for 1 min. The formed formasan crystals were dissolved in DMSO. The extinction was measured spectrophotometrically at λ = 580 nm.

GSH level in synaptosomes

GSH was determined with the Ellman reagent (DTNB), which forms color complexes with –SH group at pH = 8 with maximum absorbance at 412 nm (Robyt et al. 1971).

Statistical analysis

Statistical analysis was performed using statistical program 'MEDCALC'. Results are expressed as mean ± SEM for 6 experiments. The significance of the data was assessed using the nonparametric Mann-Whitney test. Values of $P \leq 0.05$; $P \leq 0.01$ and $P \leq 0.001$ were considered statistically significant. Three parallel samples were used.

Results and discussion

Administered alone, 6-OHDA revealed statistically significant neurotoxic effects: decreased synaptosomal viability and GSH level with 49% and with 58%, respectively, compared to the control (non-treated synaptosomes) (Figs 1, 2).

In conditions of 6-OHDA-induced oxidative stress, PSM and silymarin (S) revealed statistically significant concentration-dependent, neuroprotective effects, most prominent at concentration 60 µg/mL (Figs 1, 2).

PSM preserved statistically significant synaptosomal viability at concentration 60 µg/ml – with 36 %, at concentration 6 µg/mL – with 20%, and at concentration 0.6 µg/mL – with 16%, compared to 6-OHDA.

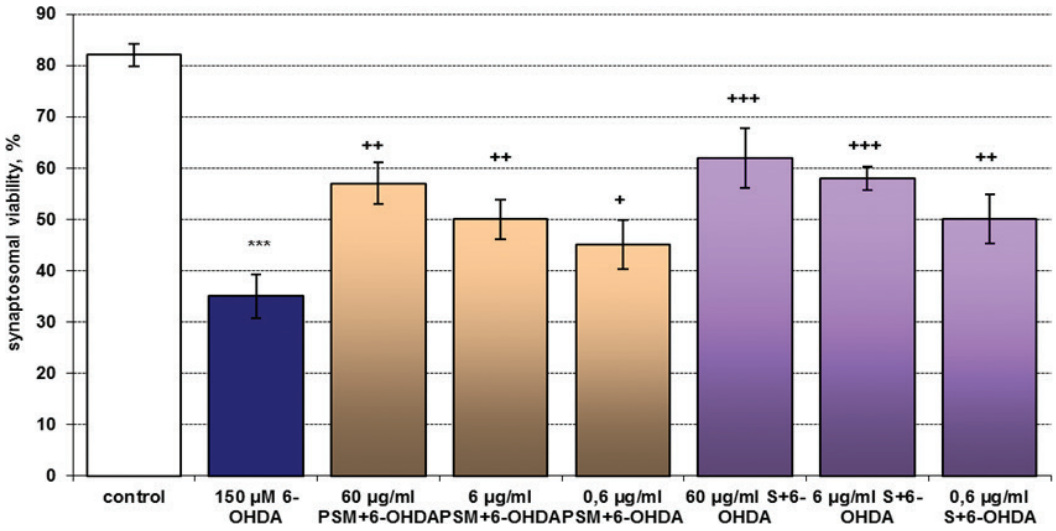

Figure 1. Effects of PSM and S on synaptosomal viability in conditions of 6-OHDA-induced oxidative stress; $^{***}P \leq 0.001$ *vs* control (non-treated synaptosomes); $^{+}P \leq 0.05$, $^{++}P \leq 0.01$, $^{+++}P \leq 0.001$ *vs* 6-OHDA.

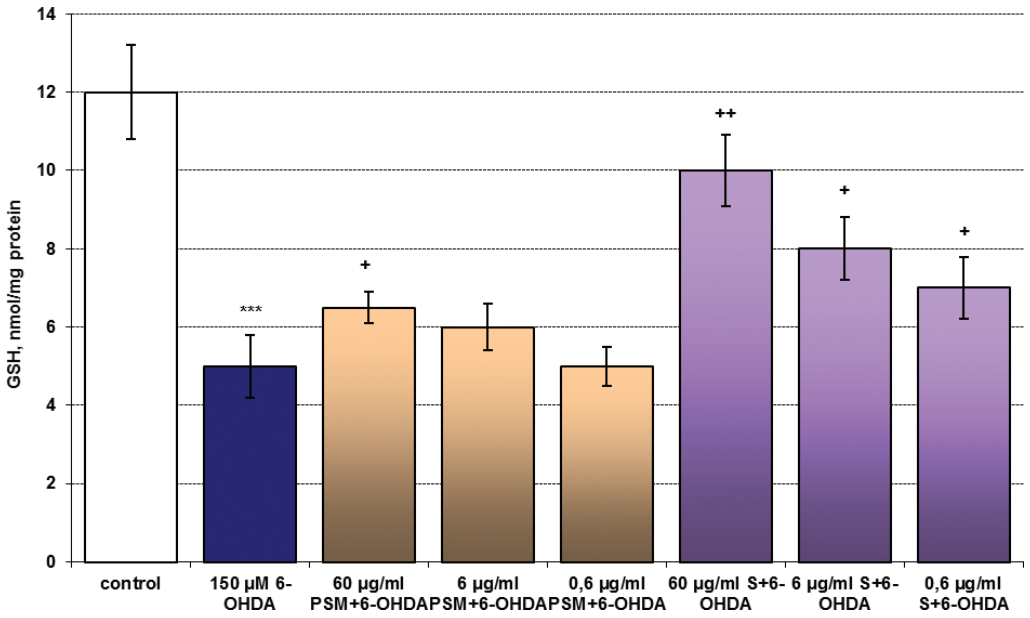

Figure 2. Effects of PSM and S on GSH level in conditions of 6-OHDA-induced oxidative stress $^{***}P \leq 0.001$ *vs* control (non-treated synaptosomes); $^{+}P \leq 0.05$, $^{++}P \leq 0.01$ *vs* 6-OHDA.

Silymarin preserved statistically significant synaptosomal viability at concentration 60 μg/mL – with 63%, at concentration 6 μg/ml – with 43%, and at concentration 0.6 μg/mL – with 31%, compared to 6-OHDA.

PSM preserved statistically significant GSH level only at concentration 60 μg/mL – with 30%, compared to 6-OHDA. Concentrations 6 μg/mL and 0.6 μg/mL didn't show statistically significant protective effects on this parameter.

Silymarin preserved statistically significant GSH level at concentration 60 μg/mL – with 100%, at concentration 6 μg/mL – with 60%, and at concentration 0.6 μg/mL – with 40%, compared to 6-OHDA.

Our study proved that at isolated rat brain synaptosomes, in conditions of oxidative stress, induced by 6-hydroxydopamine (6-OHDA) (150 μM), PSM revealed statistically significant, concentration-dependent, neuroprotective and antioxidant effects, similar to those of silymarin. Effects were most prominent at concentration 60 μg/mL. These neuroprotective effects of PSM might be due to the possible activity as scavenger of reactive oxygen species (ROS), produced by p-quinone (toxic metabolite of 6-OHDA). Our data correlate with other literature data about neuroprotective effects of other species from genus *Astragalus*. Some researchers report about neuroprotective effects of purified extract from *A. membranaceus*, which content saponin Astragaloside IV, at *in vivo* model of ischemic brain trauma. This saponin (40 mg/kg^{-1}) statistically significantly ($P < 0.03$) decreased the production of malondialdehyde and increased the levels of the antioxidant enzyme: glutathione peroxidase and superoxide dismutase at the ischemic brain tissue (Luo et al. 2004; Yang et al. 2012).

Conclusion

On isolated rat brain synaptosomes, in conditions of 6-OHDA-induced oxidative stress, PSM showed good statistically significant, concentration-dependent, neuroprotective and antioxidant effects, compared to those of silymarin and most prominent at concentration 60 μg/mL. These neuroprotective effects of PSM might be due to the possible activity as scavenger of ROS, produced by p-quinone (toxic metabolite of 6-OHDA).

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