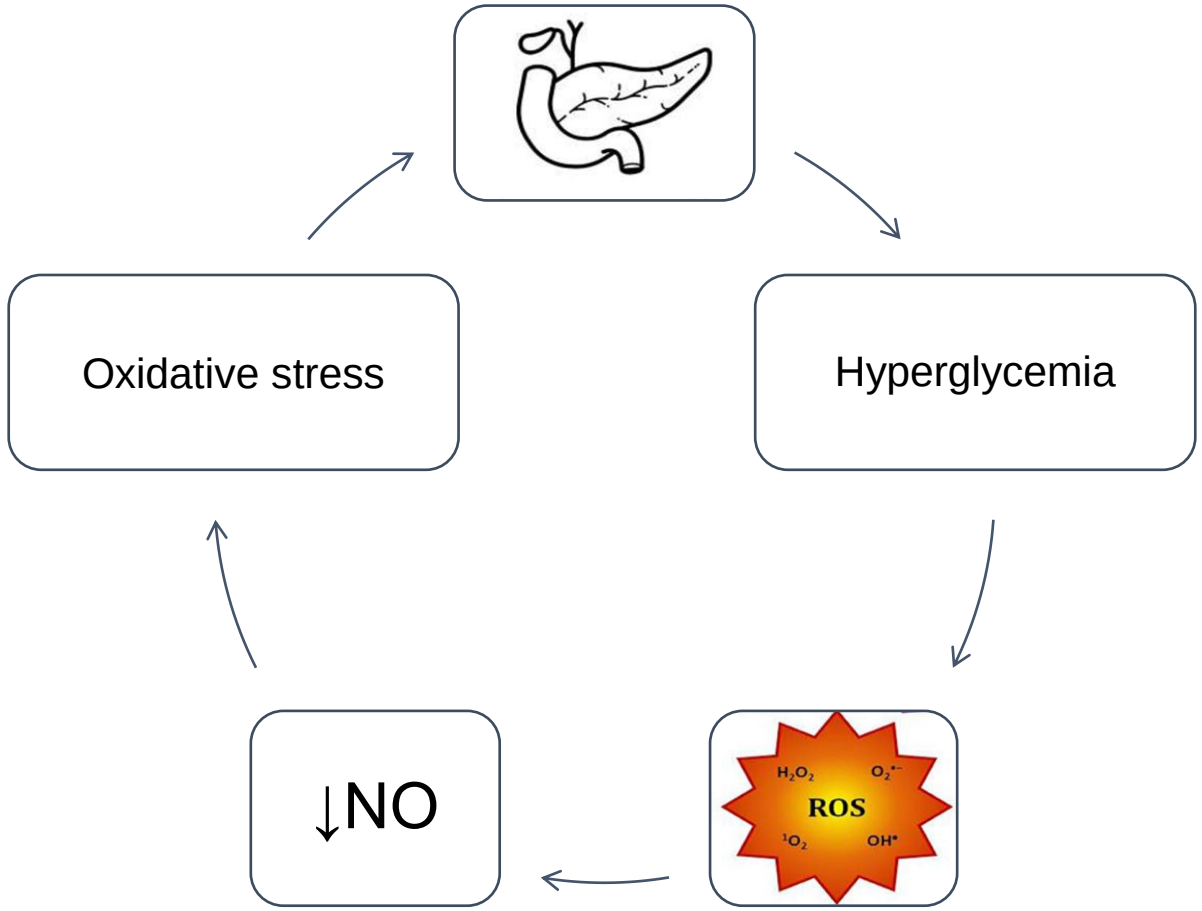
University of Kragujevac
Faculty of Medical Sciences

Effects of hyperbaric oxygen therapy on the cardiovascular system and oxidative stress in diabetic rats

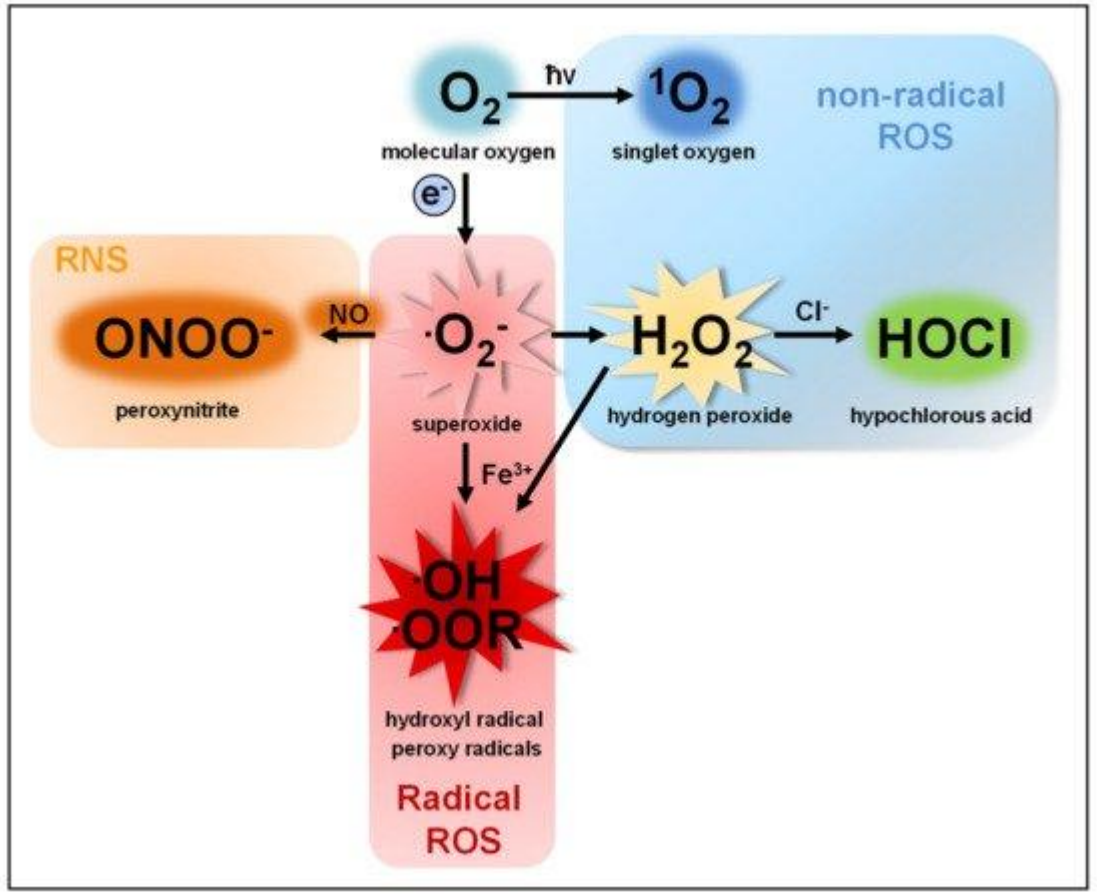
Prof. dr Vladimir Jakovljevic, MD, PhD

President of Serbian Physiological Society
President of IACS-European Section
President of International Society of Pathophysiology

Diabetes mellitus and Oxidative stress



↑ROS - HBOT???



Hyperbaric oxygenation – exposure to 100% oxygen at elevated pressure (2-3 ATA).

A phenomenon known as hormesisit is a process that results

↓

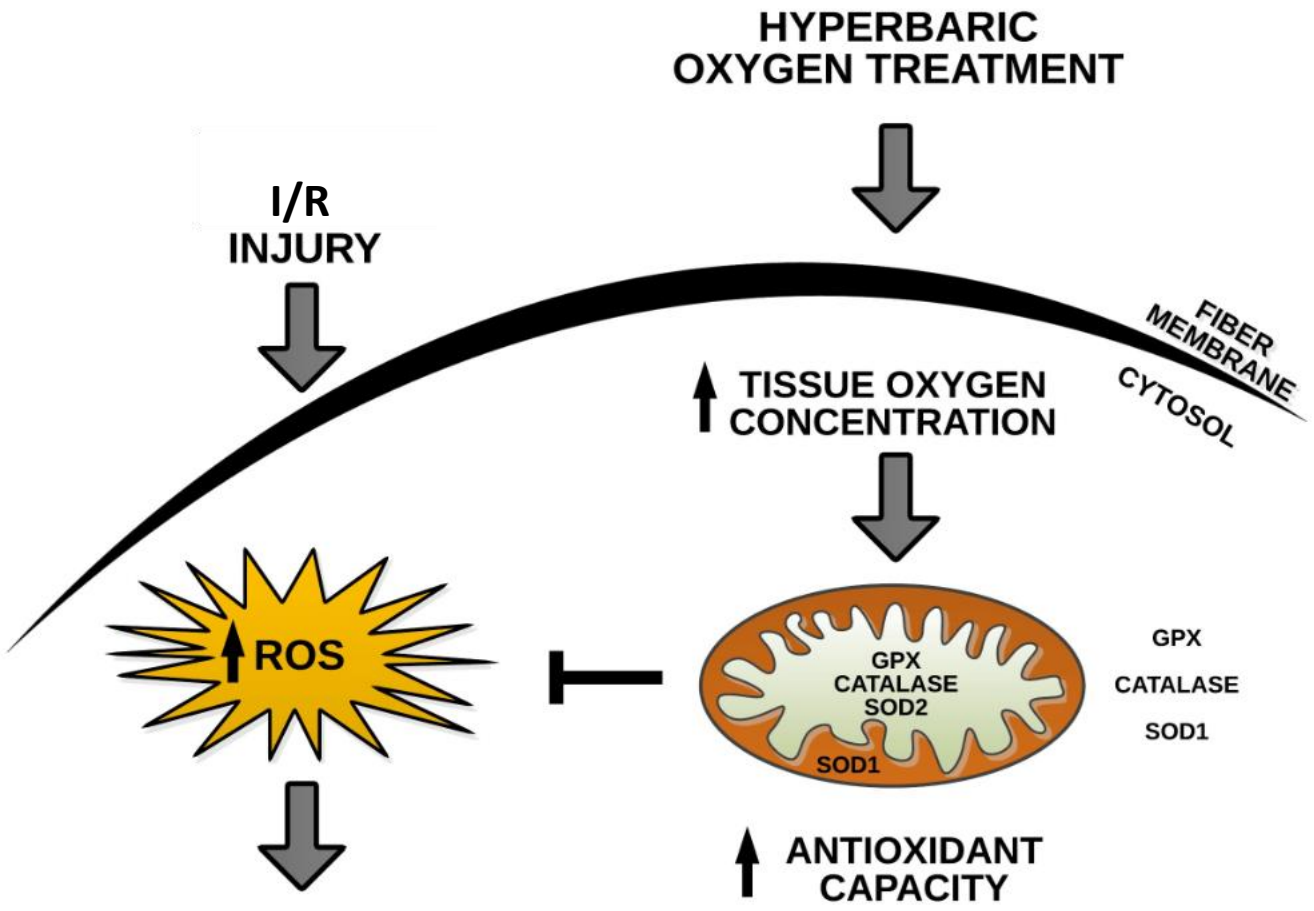
<u>functional improvement of cell resistance to stress</u>

↓

AS AN ANSWER

↓

to a sublethal level of (oxidative) stress.



depletion of ROS production capacity

↓

cytoprotective antioxidant effects

↓

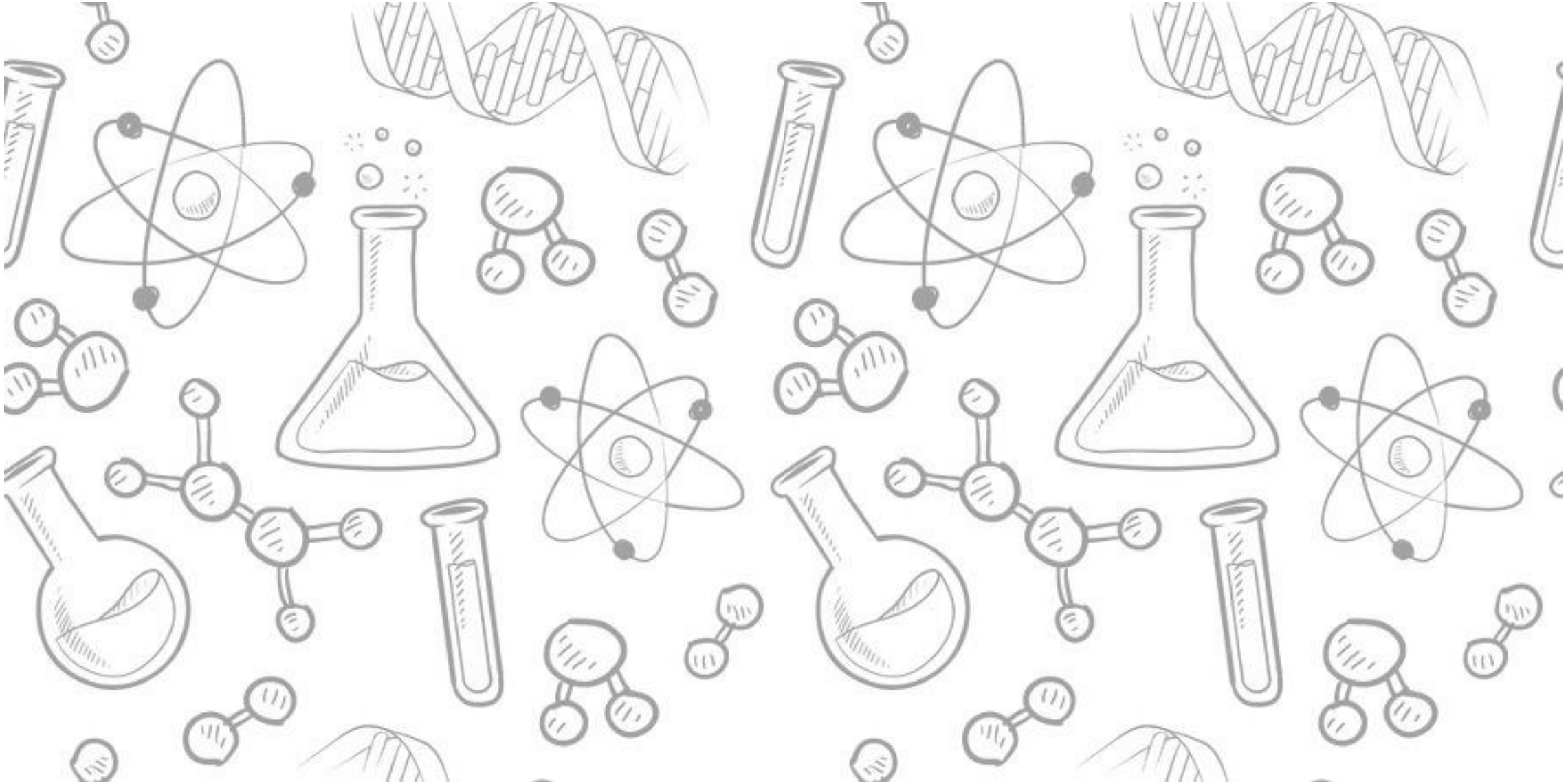
more pronounced after repeated exposure to HBOT

AIM

To determine the effects of HBO on:

- glucose homeostasis
- oxidative status and
- cardiac function in experimentally induced diabetes type 1 and insulin treated and non-treated rats

Material and methods



Induction of Diabetes Mellitus type 1

Male *Wistar albino* rats

↓

24 hour fasting

↓

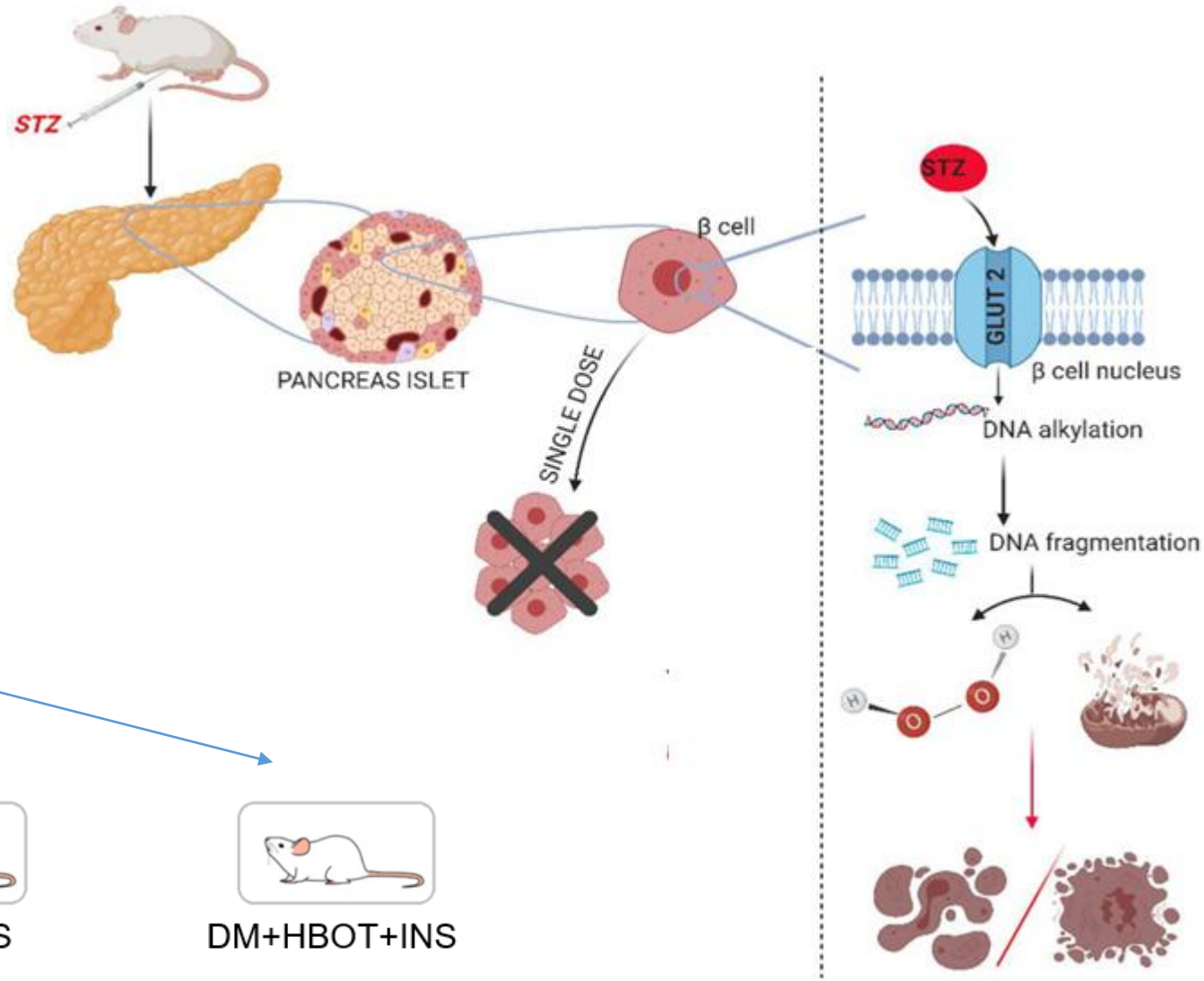
i.p. 60 mg/kg streptozotocin

↓

apoptosis of β-cells

↓

>11,1 mmol/l



Hyperbaric oxygen treatment

- 2 weeks treatment; 5 days per week, 1-hour session at 2,7 AT

HBO chamber for small animals (rodents)

Protocol of *human NPH insulin treatment*

- Glycemic level control of diabetic rats was made with subcutaneous injections of exogenous human NPH insulin*.
- The objective of the insulin therapy was to keep the glycemia of these animals as close as possible to the normoglycemia (from 60 to 150 mg/dL) throughout the 24h day.
- Initially, the administration chosen was 4 U/day of NPH insulin.
- Throughout the treatment, daily dose of insulin was adjusted on average every 3 days according to the glycemia of each animal (from 3 to 5 U/day)

**Pinheiro L. et al. Protocol of Insulin Therapy For Streptozotocin-Diabetic Rats Based on a Study of Food Ingestion and Glycemic Variation. Scand. J. Lab. Anim. Sci. 2011; 38:2.*

Estimating of cardiac function and oxidative status

- Retrograde perfusion according to the Langendorff
- Monitoring of dp/dt min and max, SLVP, DLVP,
- Heart rate and coronary flow

Global ischemia induced by occlusion of the retrograde flow of Krebs-Henseleit solution through the coronary vessels of the isolated rat heart for 20 minutes

↓

Reperfusion 30 minutes

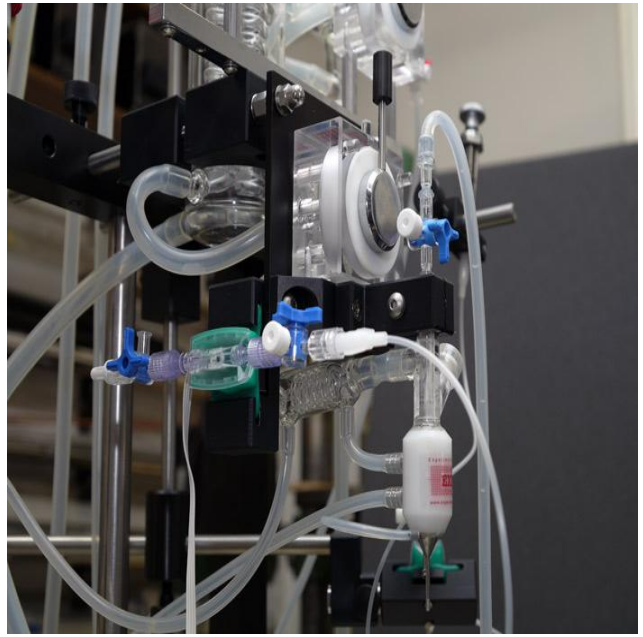
↓

Collection of coronary venous effluent every 5 minutes

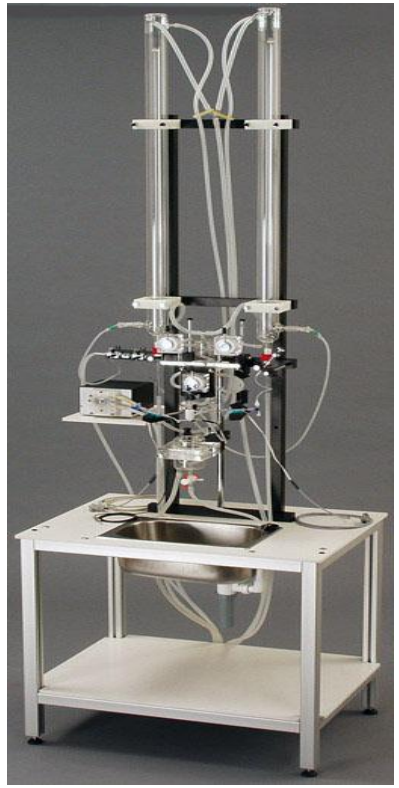
↓

Spectrophotometrically

$O_2^{-\cdot}$ H_2O_2 TBARS NO_2^-



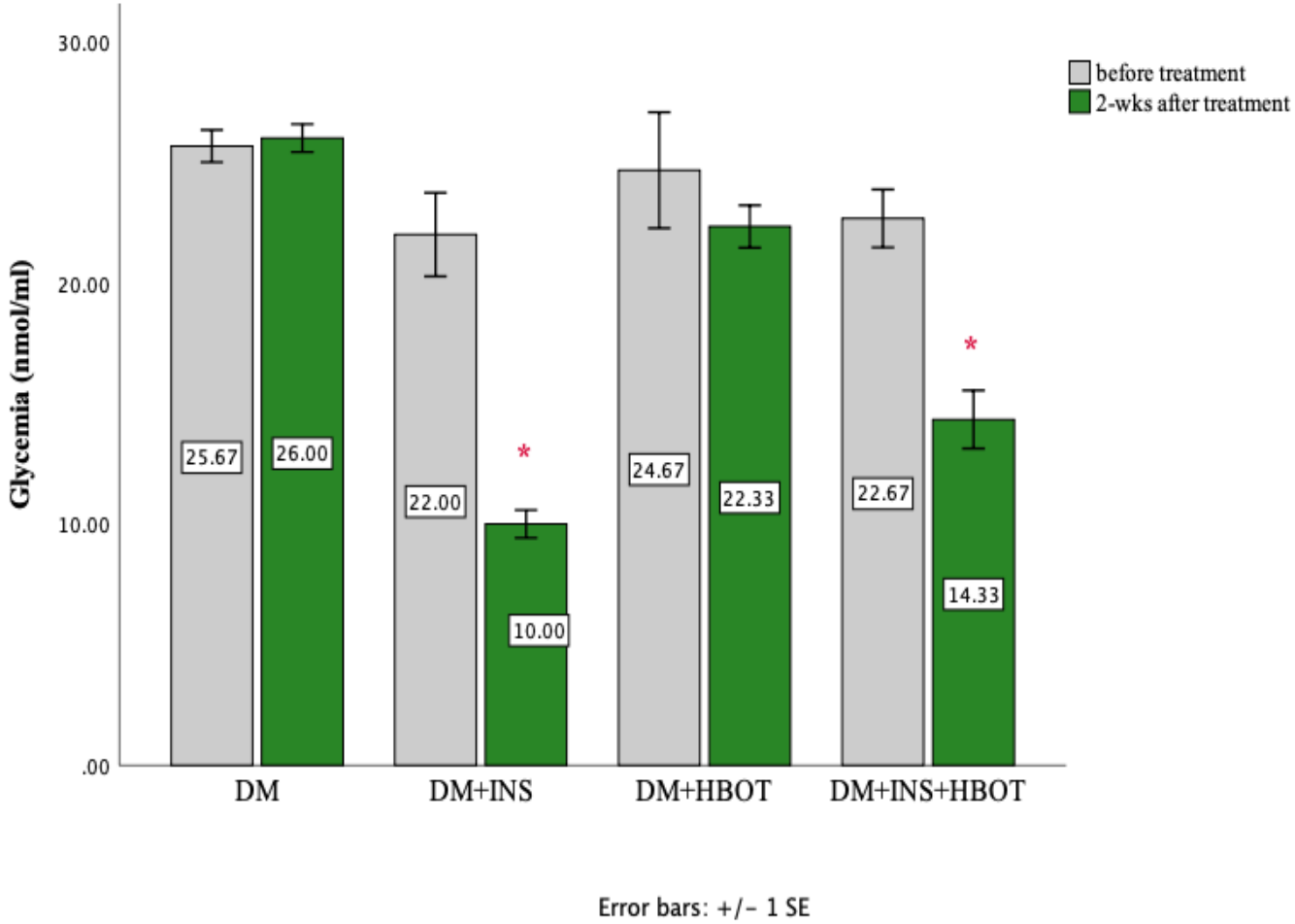
Canila for rat aorta



Langendorff apparatus

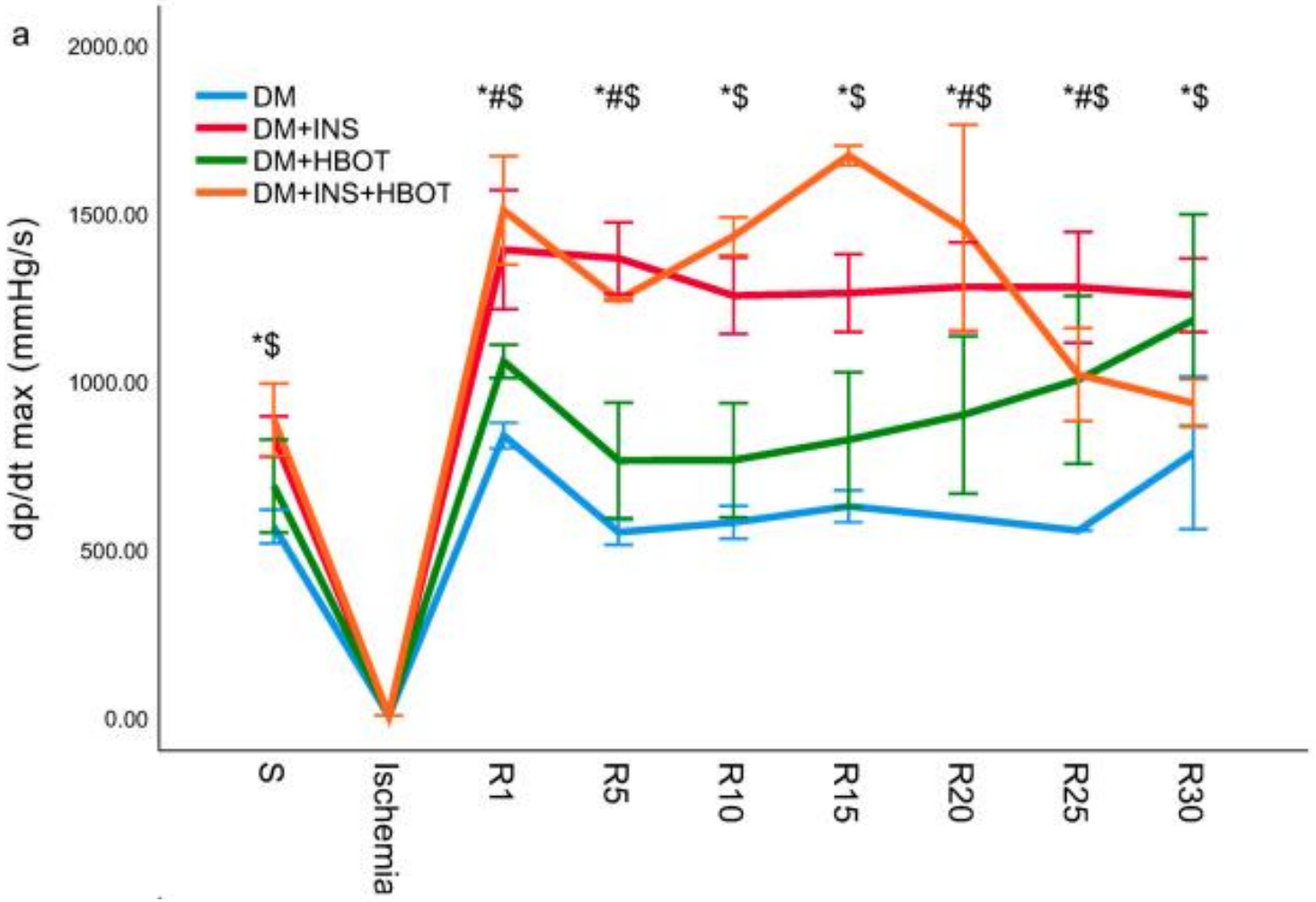
Results

Glycemia

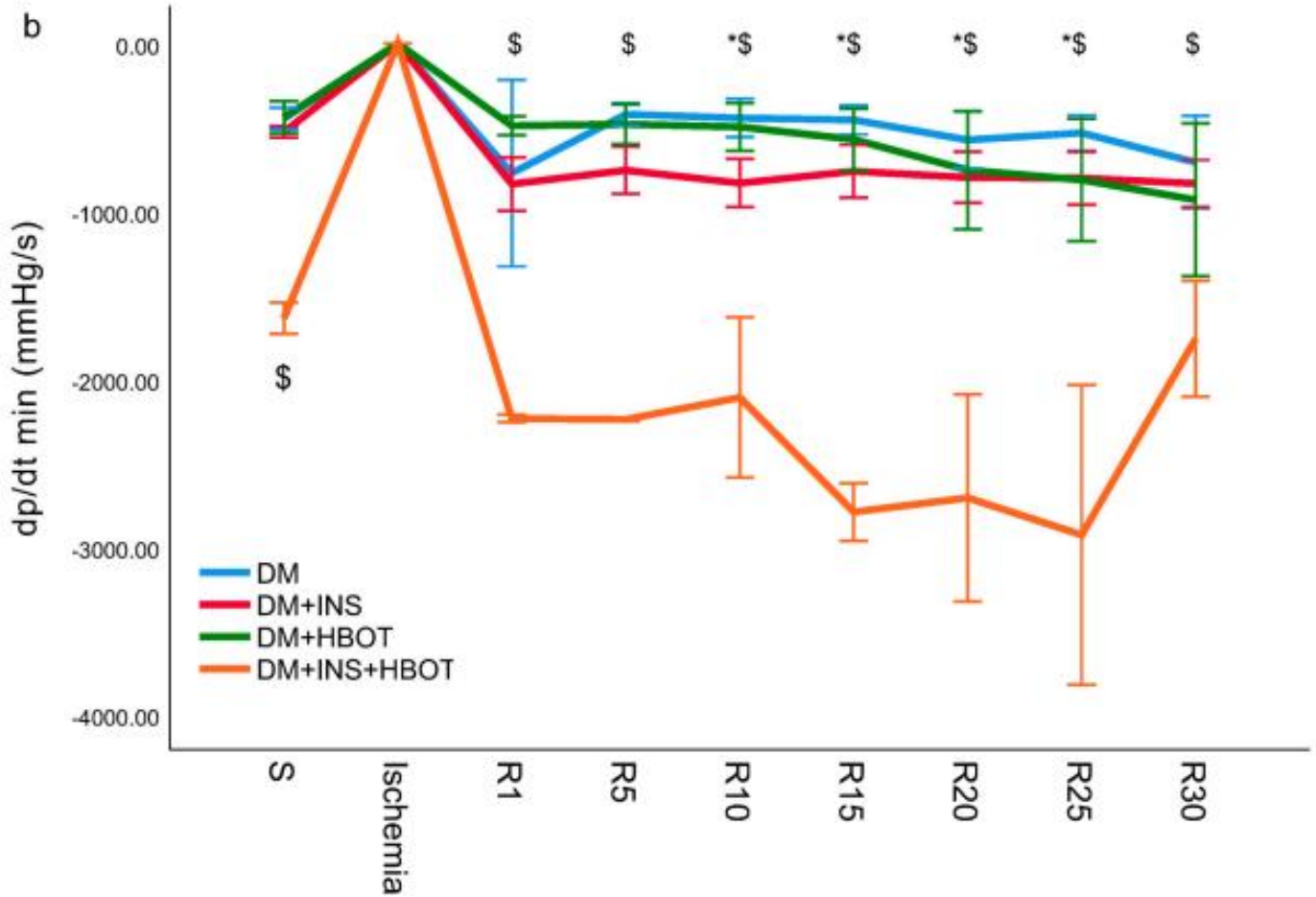


- NPH insulin treatment significantly reduced blood glucose levels
- NPH does not induce normoglicemia but significantly reduced hyperglicemia in treated groups

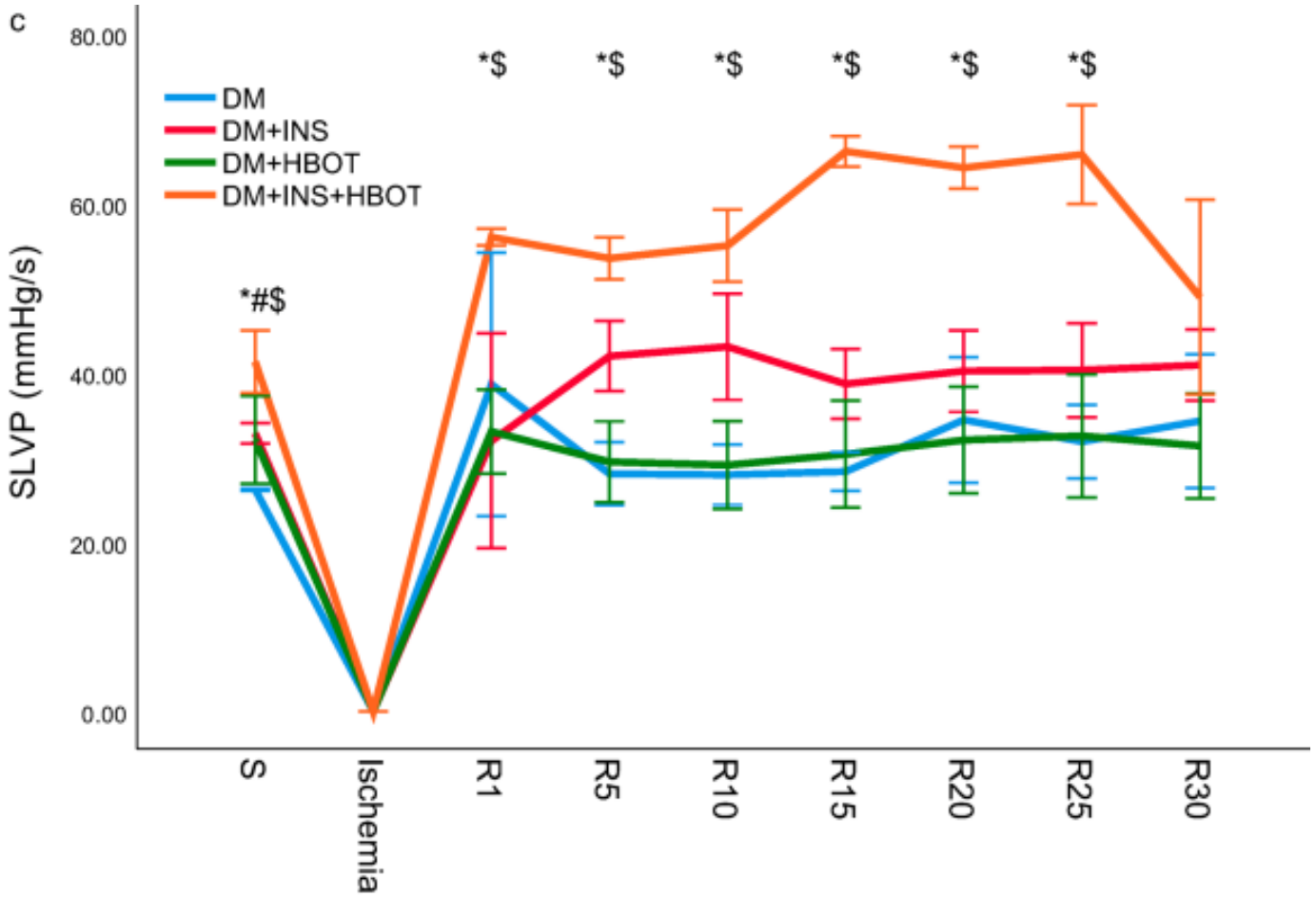
Cardiodynamic parameters



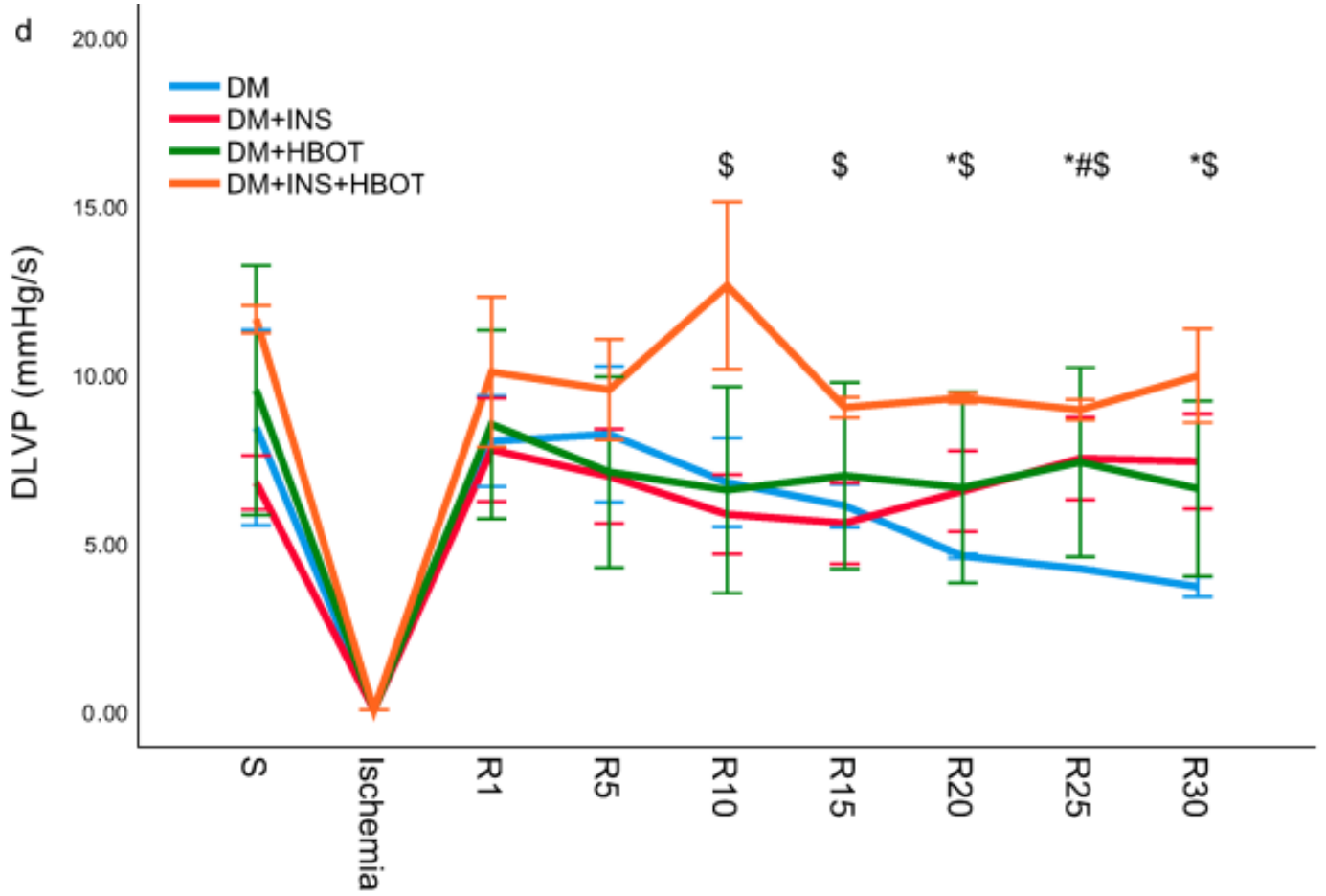
Cardiodynamic parameters



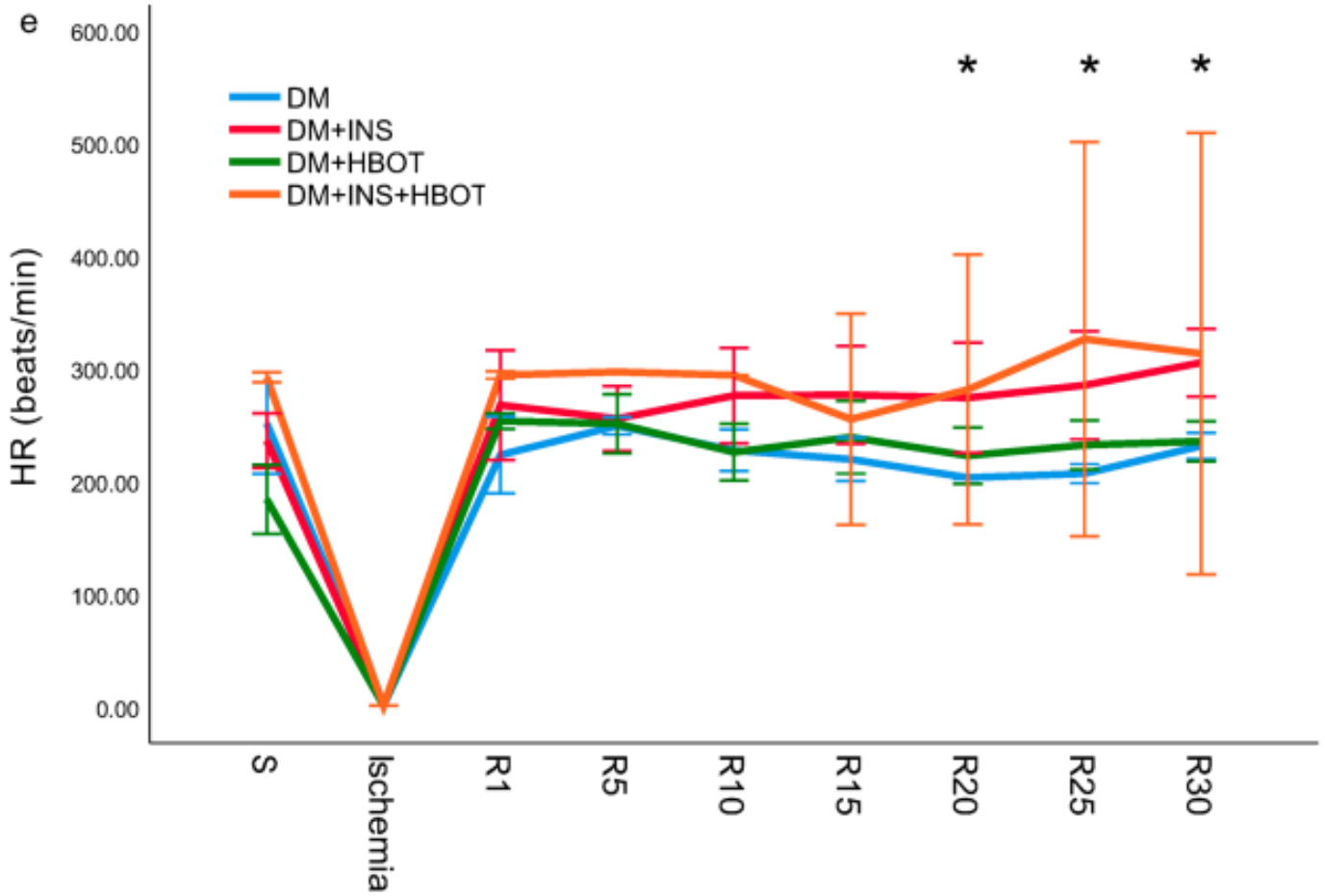
Cardiodynamic parameters



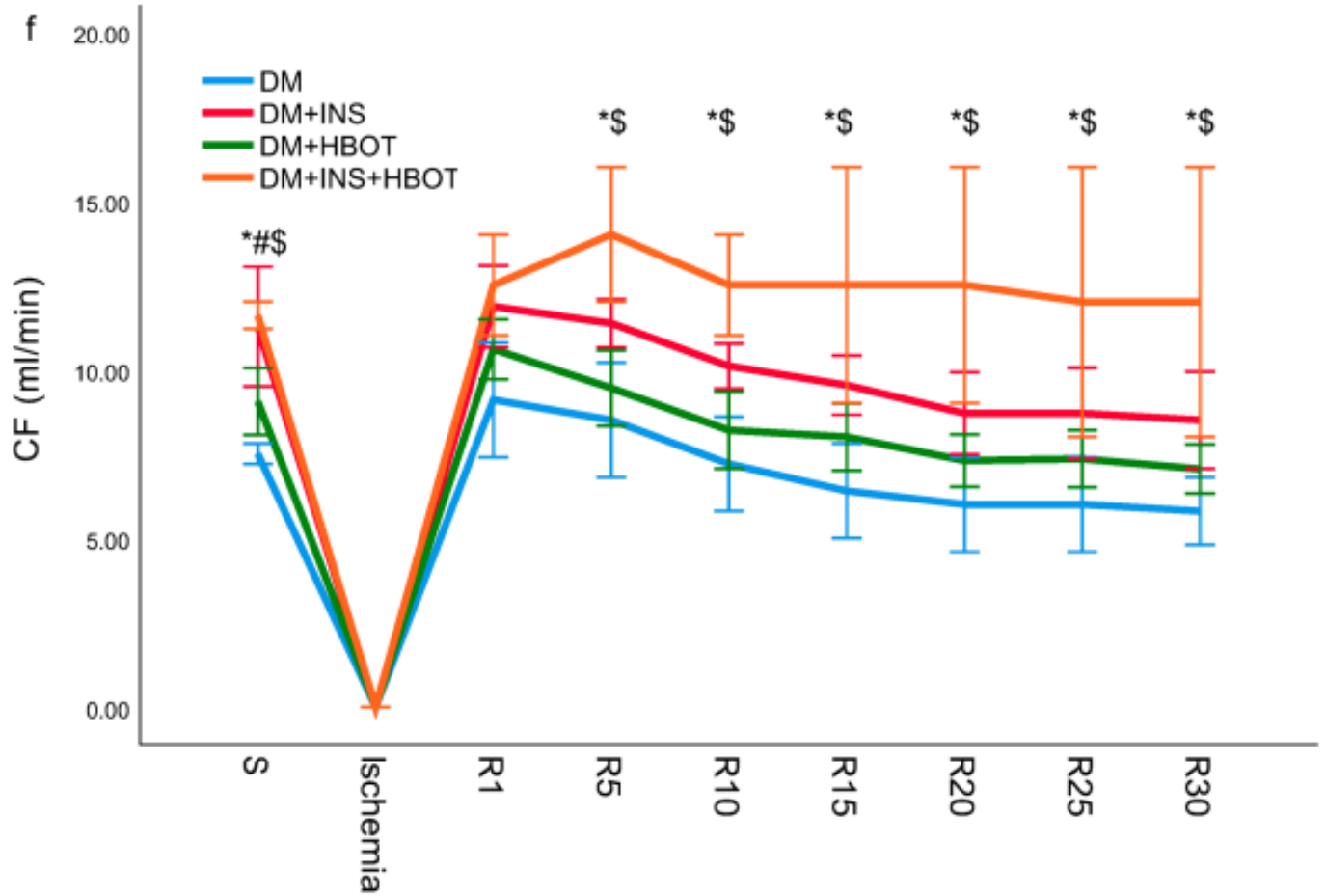
Cardiodynamic parameters



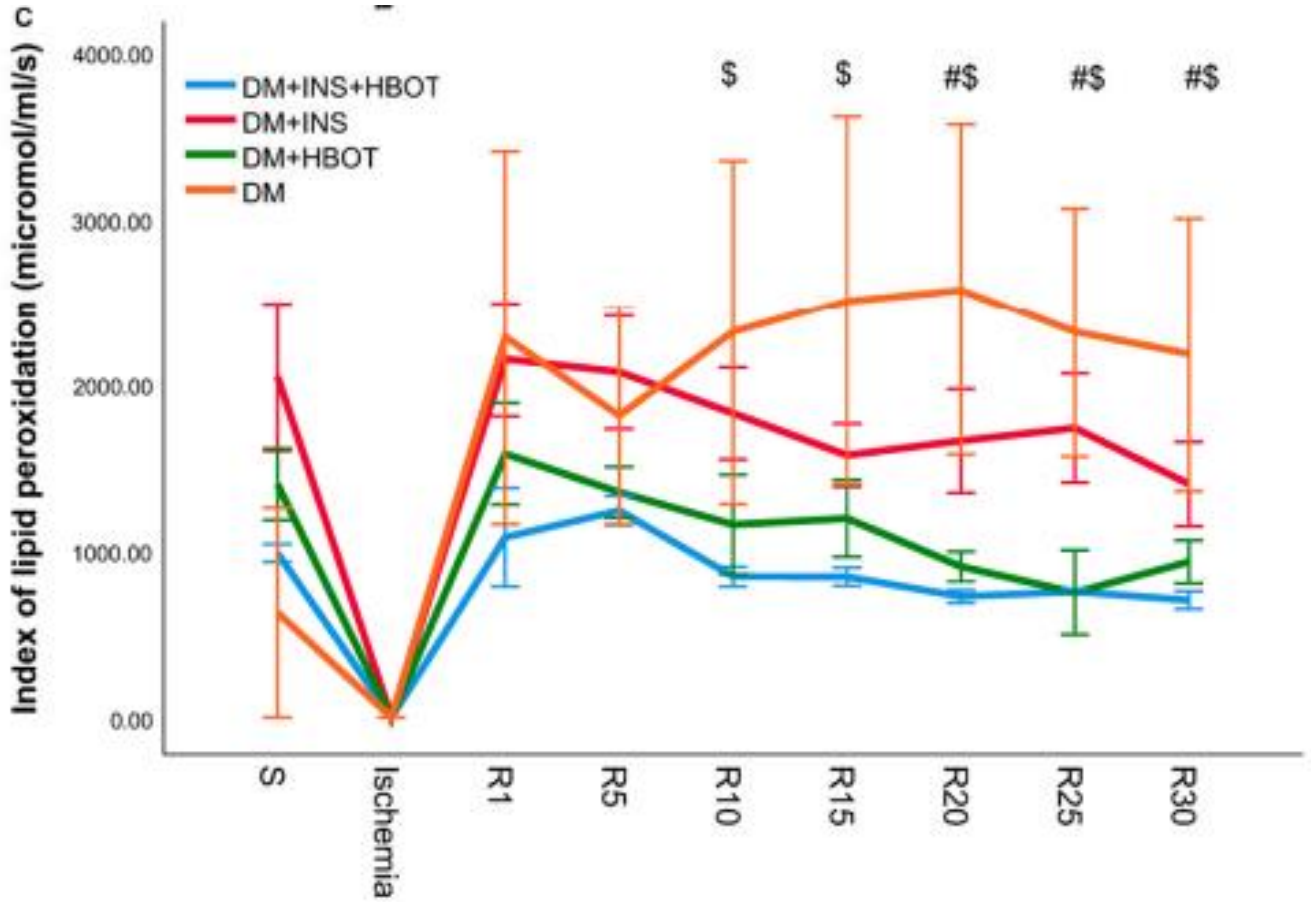
Cardiodynamic parameters



Cardiodynamic parameters

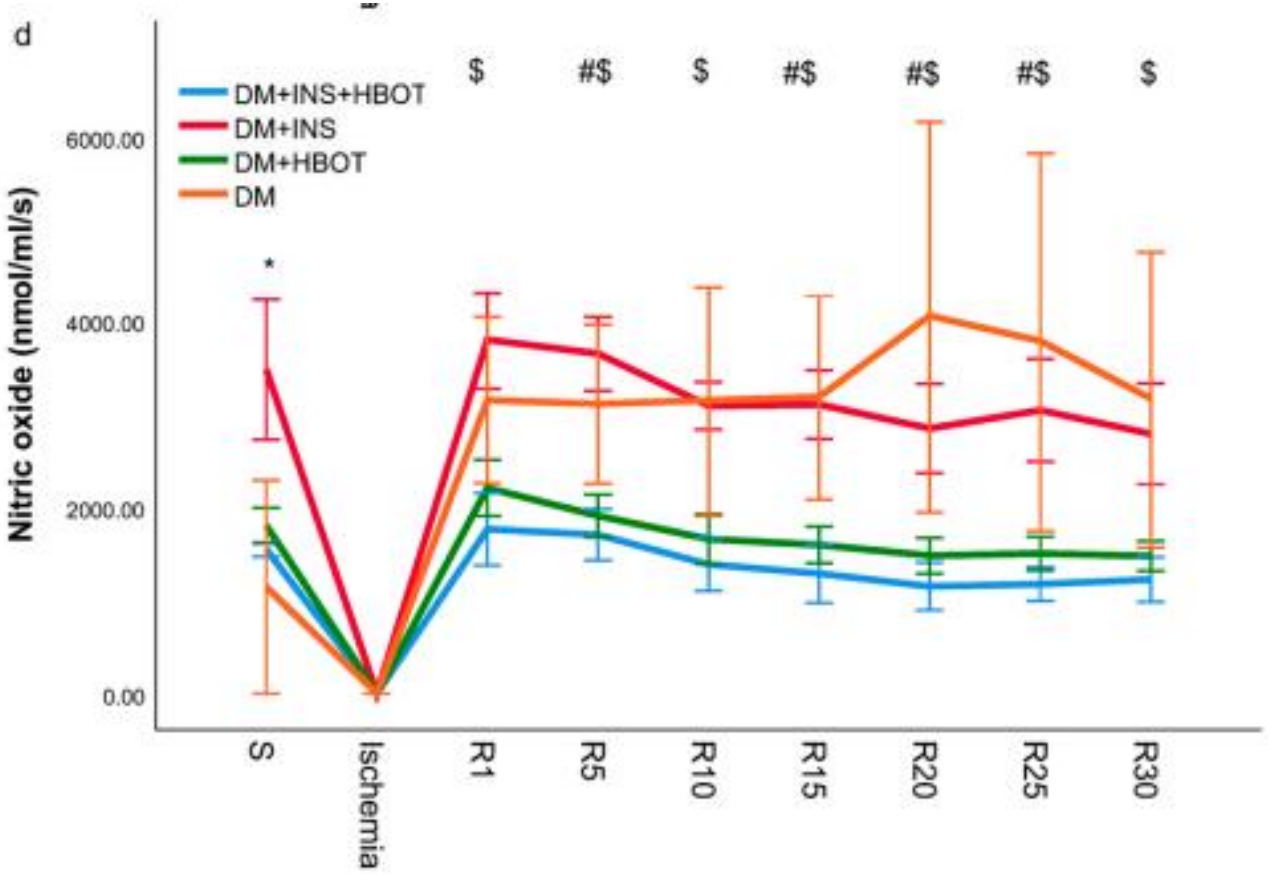


Biomarkers of oxidative stress



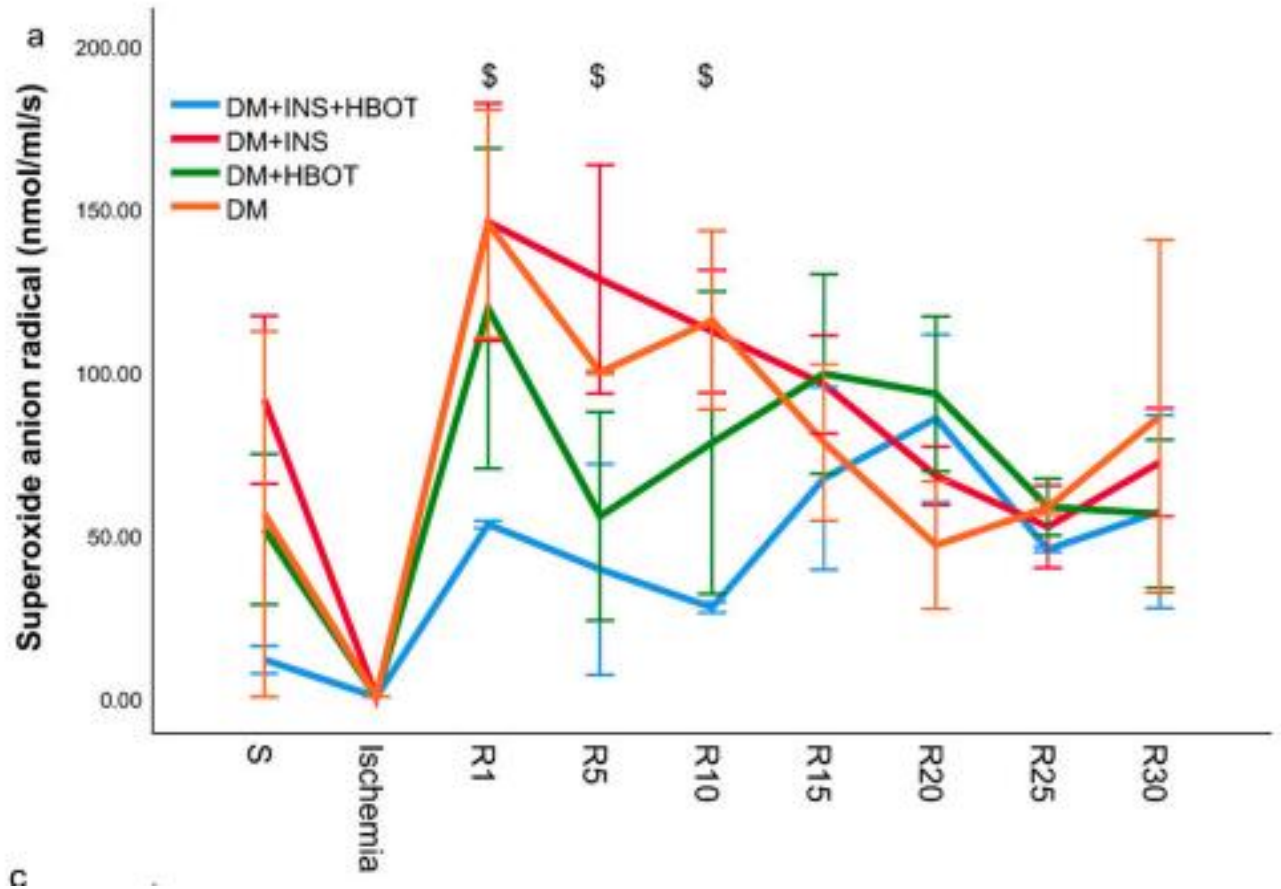
* DM vs. DM+INS
\# DM vs. DM+HBOT
$ DM vs. DM+INS+HBOT

Biomarkers of oxidative stress



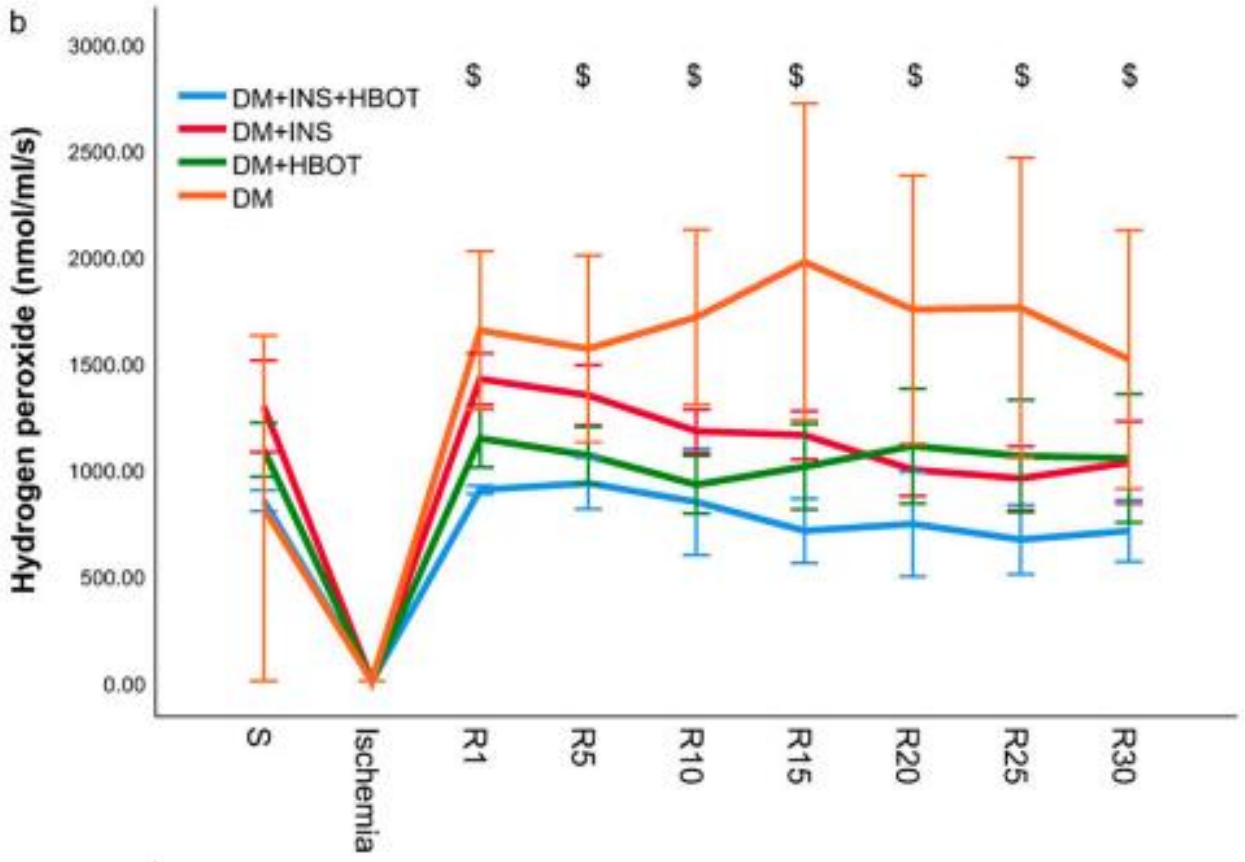
* DM vs. DM+INS
\# DM vs. DM+HBOT
$ DM vs. DM+INS+HBOT

Biomarkers of oxidative stress



* DM vs. DM+INS
\# DM vs. DM+HBOT
$ DM vs. DM+INS+HBOT

Biomarkers of oxidative stress



* DM vs. DM+INS
\# DM vs. DM+HBOT
$ DM vs. DM+INS+HBOT

Conclusions

- Cardiac function was significantly improved by NPH insulin, and combination of insulin and HBO treatment seems to be effective in restoring the cardiac function in diabetic animals.

- NPH insulin could not induce normoglicemia after 2 weeks treatment but can reduced extremme hyperglicemia.

- HBOT+INS → synergistic antioxidant effect → redox homeostasis

- Our results provide, what is to our knowledge, the most detailed schedule of insulin therapy for treating STZ-diabetic rats and HBO treatment.

TEAM

Prof. Vladimir Jakovljevic, MD, PhD

Prof. Vladimir Zivkovic, MD, PhD

Prof. Ivan Srejovic, MD, PhD

Prof. Nevena Jeremic, MrPharm, PhD

Prof. Tamara Nikolic Turnic, MD, PhD

Ass. Prof. Isidora Milosavljevic, MrPharm, PhD

Ass. Prof. Jovana Joksimovic Jovic, MD, PhD

Ass. Prof. Jovana Jeremic, MrPharm, PhD

Ass. Prof. Jovana Bradic, MrPharm, PhD

Ass. Prof. Aleksandra Stojanovic, MrPharm, PhD

Ass. Prof. Jasmina Sretenovic, MD, PhD

Ass. Anica Petrovic, MrPharm, PhD

Ass. Andjela Milojevic Samanovic,DMD, PhD

Ass. Marijana Andjic, MrPharm, PhD student

Ass. Nevena Draginic, MrPharm, PhD student

Ass. Maja Nikolic, MD, PhD student

Ass. Marina Nikolic, MrPharm, PhD student

Ass. Aleksandar Kocovic, MrPharm, PhD student

Ass. Maja Savic, MrPharm, PhD

Ass. Katarina Radonjic, MrPharm, PhD

Milica Milinkovic, MrPharm, PhD student

Ass. Marko Ravic, MD, PhD student

dr Bozidar Pindovic, MD, PhD student

The team