University of Kragujevac
Faculty of Medical Sciences

The effects of diferent plant extracts on cardiovascular function: focus on oxidative stress

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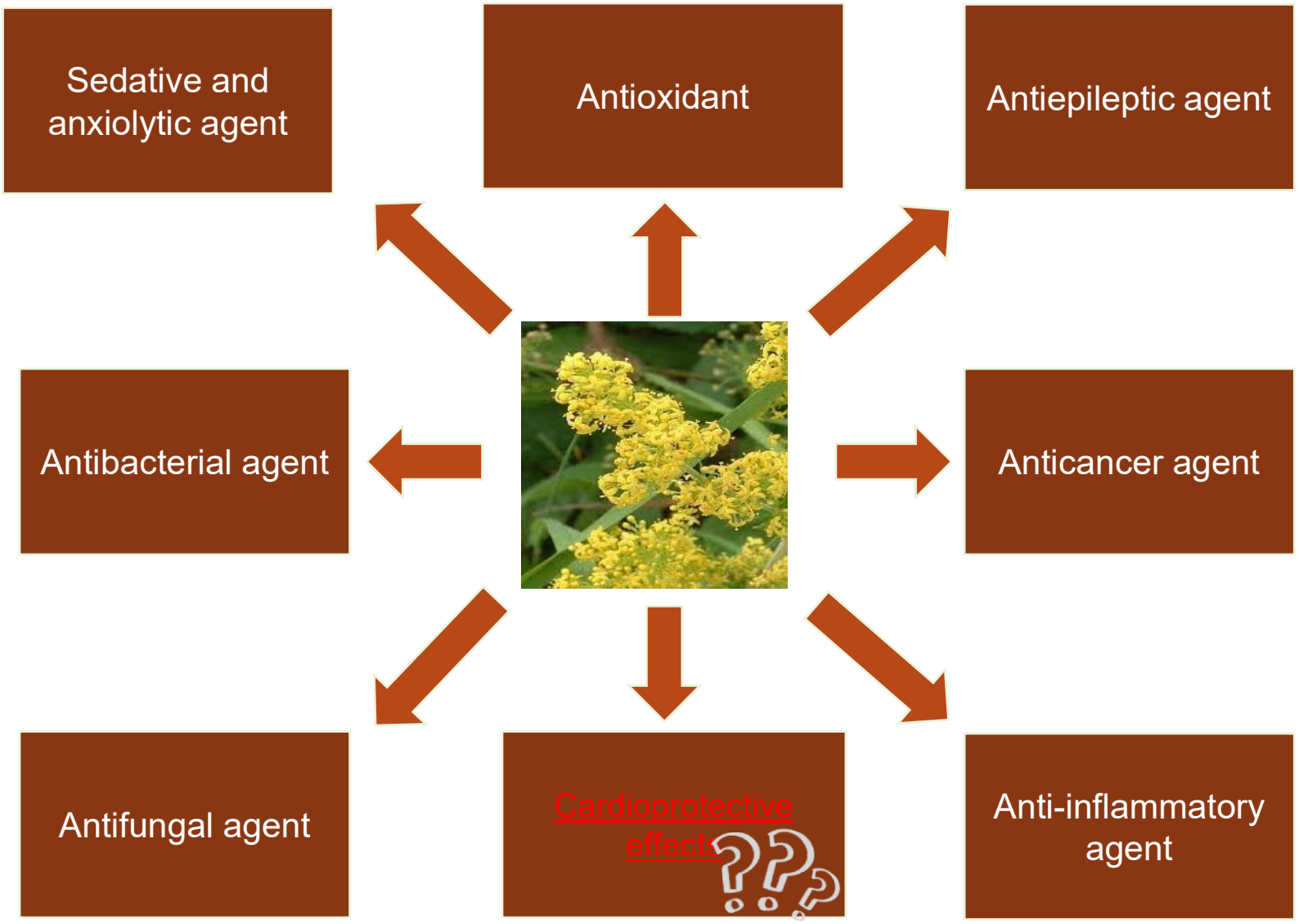
President of International Society of Pathophysiology

Galium verum

- Galium verum (*G verum*, synonyms Lady's bedstraw the Rubiaceae family).

- It has been used for centuries in traditional medicine both as prophylactic and therapeutic agent.

Therapeutic use of *G. verum*



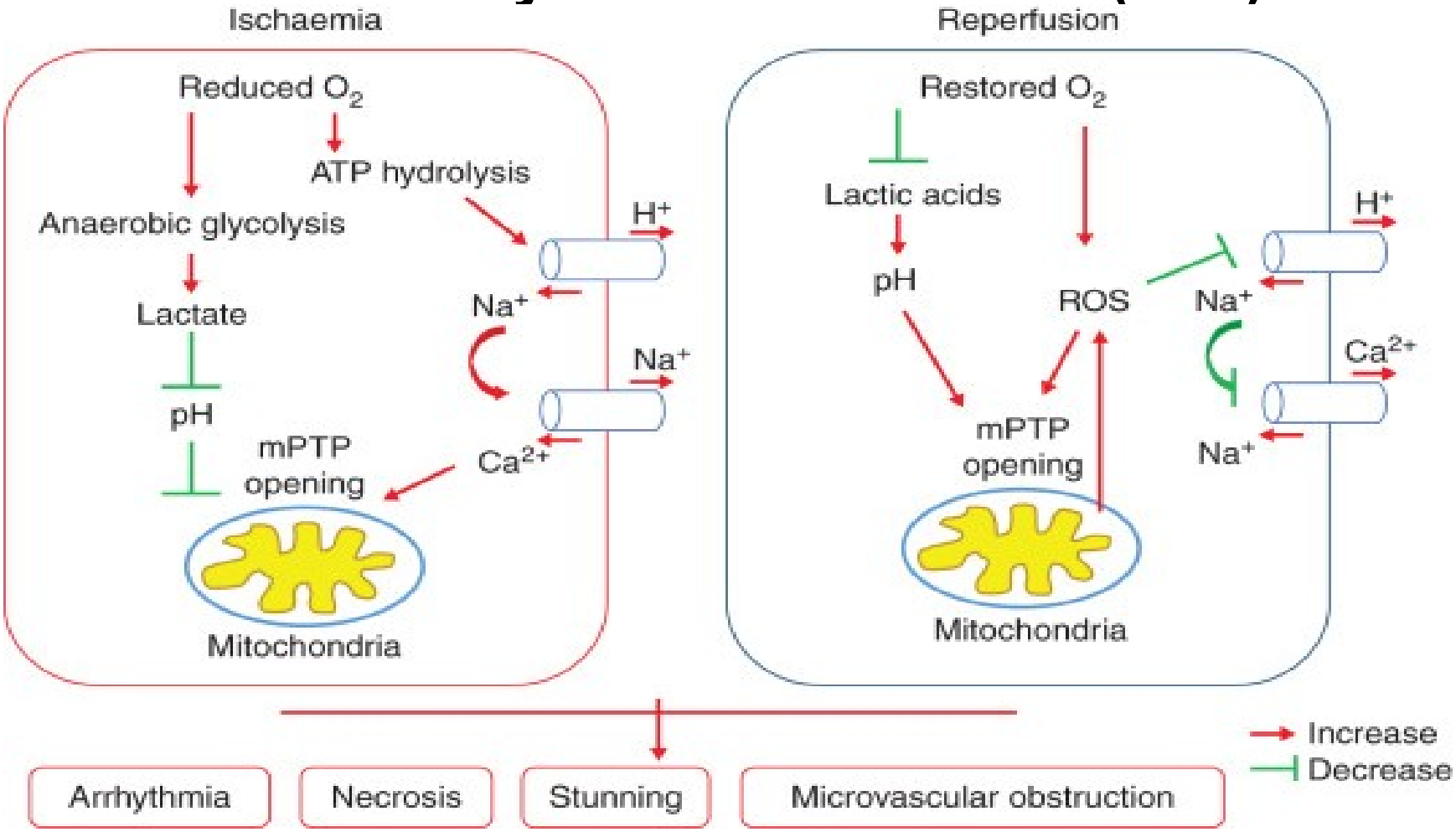
Chemical composition *of G. verum*

G. verum extract contain:

- iridoid glycosides
- phenolic compounds
- anthraquinones
- triterpenes etc

❑ **Phenolic compounds -** the most significant constituents efficient in achieving cardioprotection.

Acute myocardial infarction (AMI)



Paradies G, Paradies V, Ruggiero FM, Petrosillo G. Mitochondrial bioenergetics and cardiolipin alterations in myocardial ischemia-reperfusion injury: implications for pharmacological cardioprotection. Am J Physiol Heart Circ Physiol. 2018 Nov 1;315(5):H1341-H1352.

How can we prevent heart damage induced by I/R?

➨ **Myocardial preconditioning**- exposing heart to different agents prior ischemia reperfusion in order to reduce harmful effects of I/R injury.

Pharmacological agents

- The serious side effects of the available drugs limit their usage and still stayed as an important problem for the health system.

Plants and plant-derived products

- Intake of polyphenols from plants is associated with reduced cardiovascular risk.

Benefits of polyphenols in attenuation myocardial of I-R injury

❑ A class of naturally occurring compounds present in fruits, plants etc

❑ Numerous researches have confirmed the protective effects of plant extracts against reperfusion-induced heart damage, mainly attributed to:

✓ antioxidant
✓ anti-inflammatory activity
✓ anti-apoptotic activity of polyphenols
✓ ability to preserve nitrogen monoxide

First aim of the study

was to assess the effects of methanol extract of *G. verum* on myocardial ischemia/reperfusion injury.

Materials and methods

- The study was performed in the cardiovascular laboratory of the Faculty of Medical Sciences, University of Kragujevac, Serbia.

- Methanol extract was prepared extracting 100g of aerial part of plant with 500 ml of methanol by heat reflux extraction

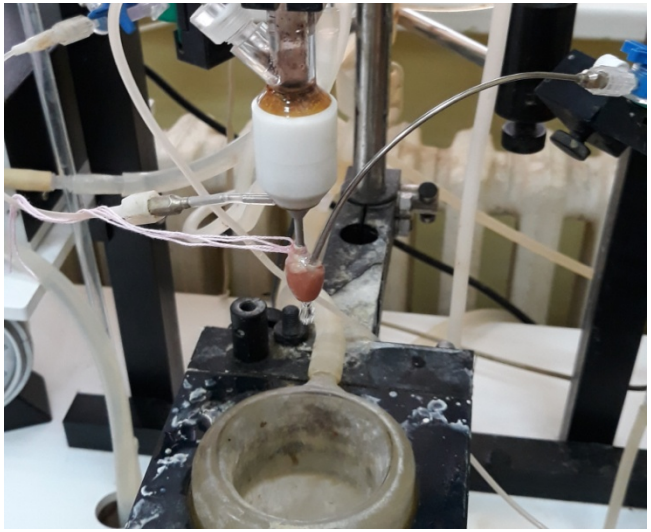
Treatment with *G. verum* extract

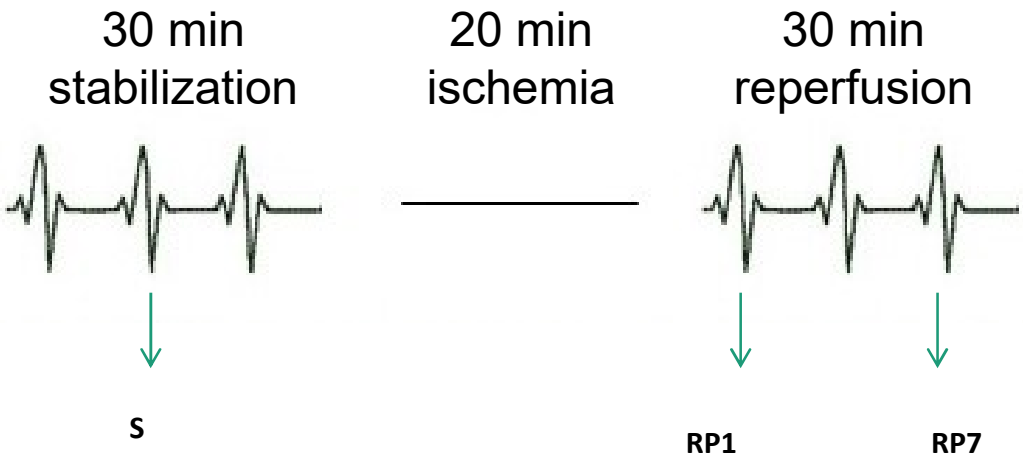
Male, healthy (n=20)

1. Control — *Untreated rats subjected to I/R injury*

2. GVE — *Methanol extract of G. verum* 250 mg/kg every day, 4 weeks, *per os before I/R injury*

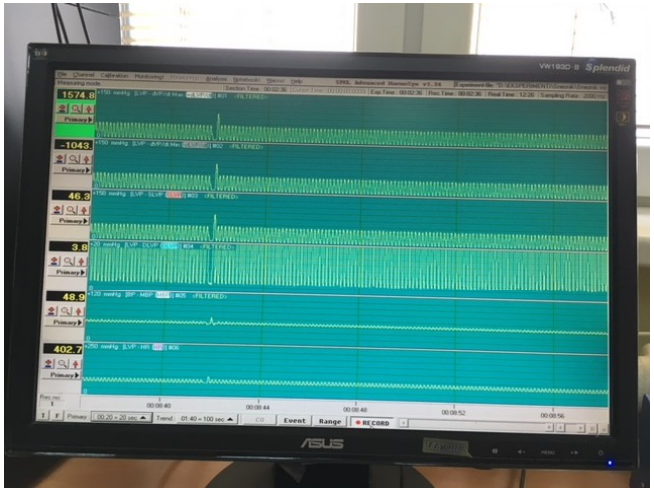
- After a short-term narcosis rats were sacrificed by cervical dislocation.
- After urgent thoracotomy, hearts were rapidly excised, the aortas were cannulated and retrogradely perfused according to Langendorff technique, under a constant perfusion pressure of 70 cmH_2O.





Cardiodynamic parameters

After placing the sensor in the left ventricle, the following parameters of myocardial function have been continuously registered:



- maximum rate of pressure development in the left ventricle (dp/dt max)
- minimum rate of pressure development in the left ventricle (dp/dt min)
- systolic left ventricular pressure (SLVP),
- diastolic left ventricular pressure (DLVP)
- heart rate (HR)

Coronary flow (CF) was measured flowmetrically.

Oxidative stress markers

After sacrificing animals blood samples were taken for determination of systemic redox state

In plasma samples:

- The index of lipid peroxidation, measured as thiobarbituric acid-reactive substances (TBARS)
- Nitrite (NO_2^-)
- Superoxide anion radical (O_2^-) and
- Hydrogen peroxide levels (H_2O_2)

In erythrocyte samples:

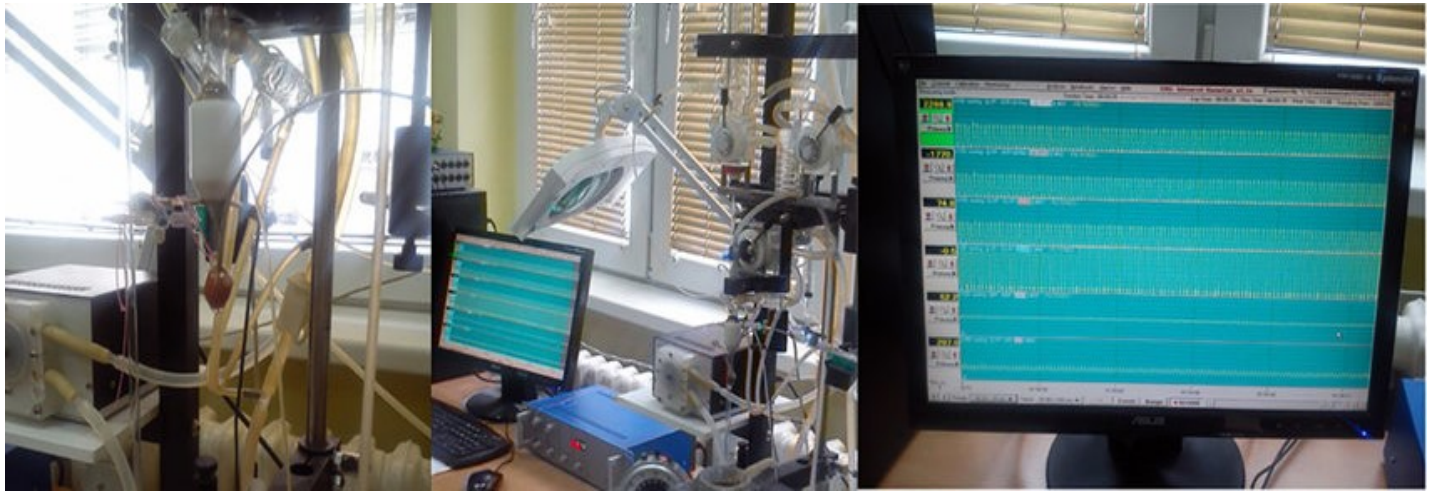
- Superoxide dismutase (SOD)
- Catalase (CAT)
- Reduced gluthatione (GSH)

Structural alterations of the heart tissue

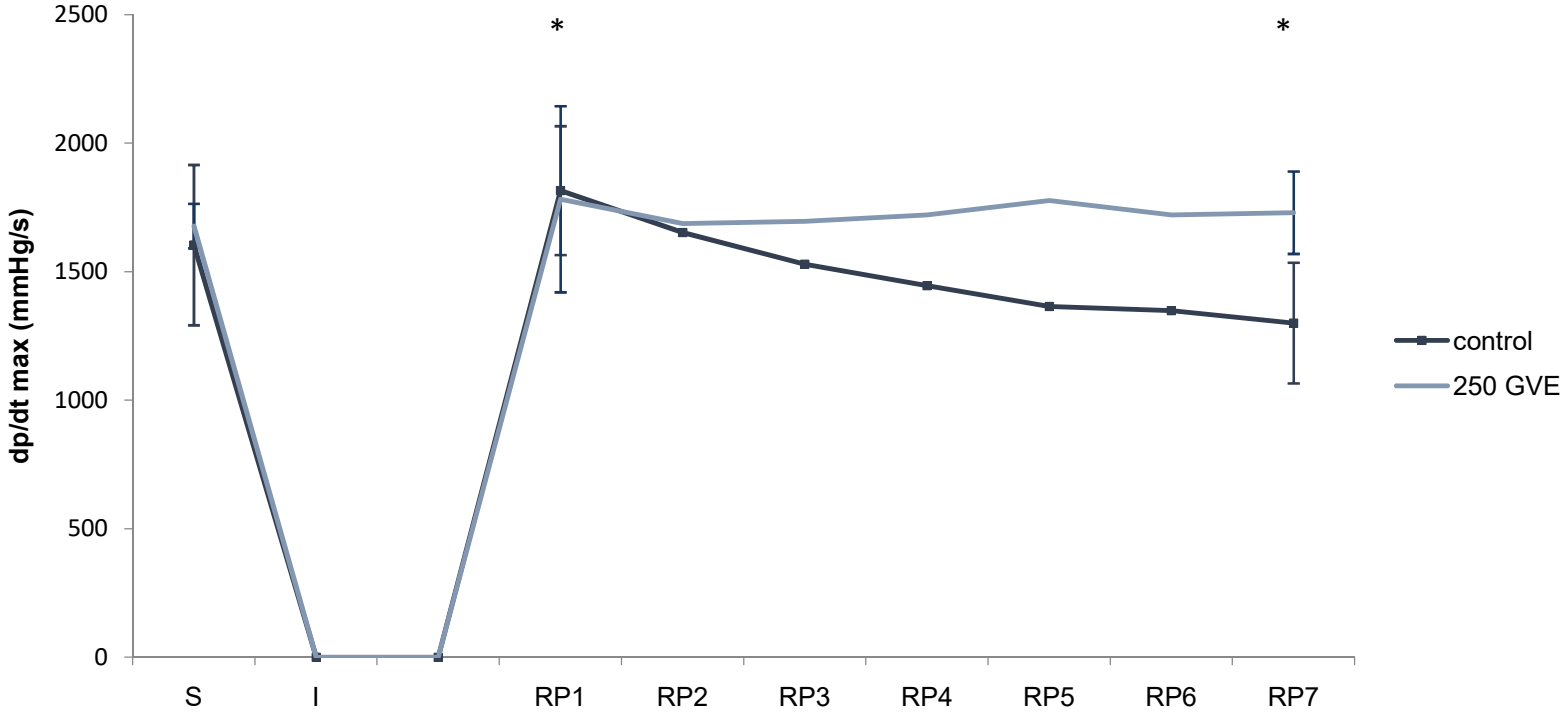
- Hematoxylin and eosin (HE) staining

Results

Cardiodynamic parameters



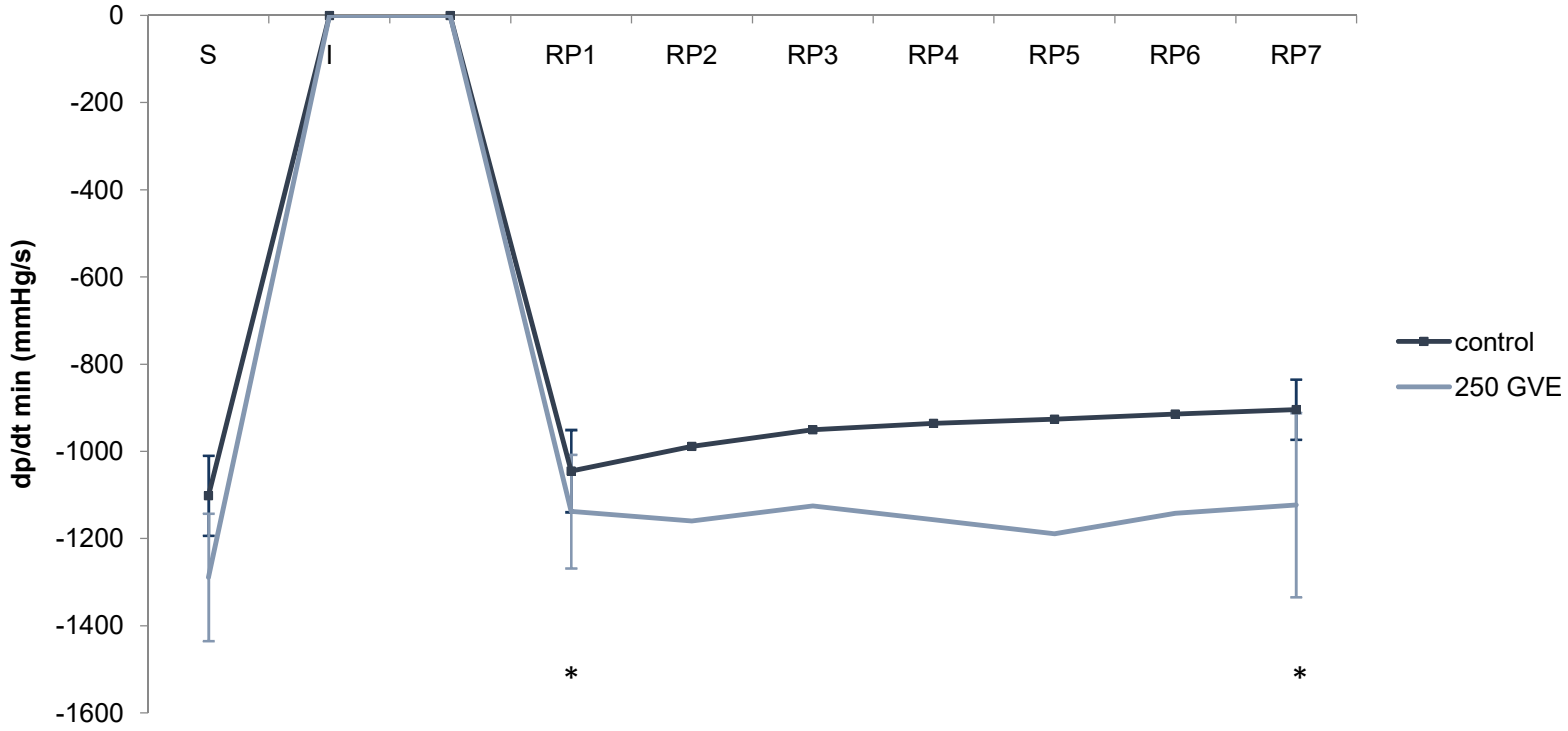
dp/dt max (mmHg/s)



	CONTROL	250 GVE
S vs RP1	**p < 0.05**	**p < 0.05**
S vs RP7	**p < 0.05**	p > 0.05
RP1 vs RP7	**p < 0.05**	p > 0.05

* control vs GVE

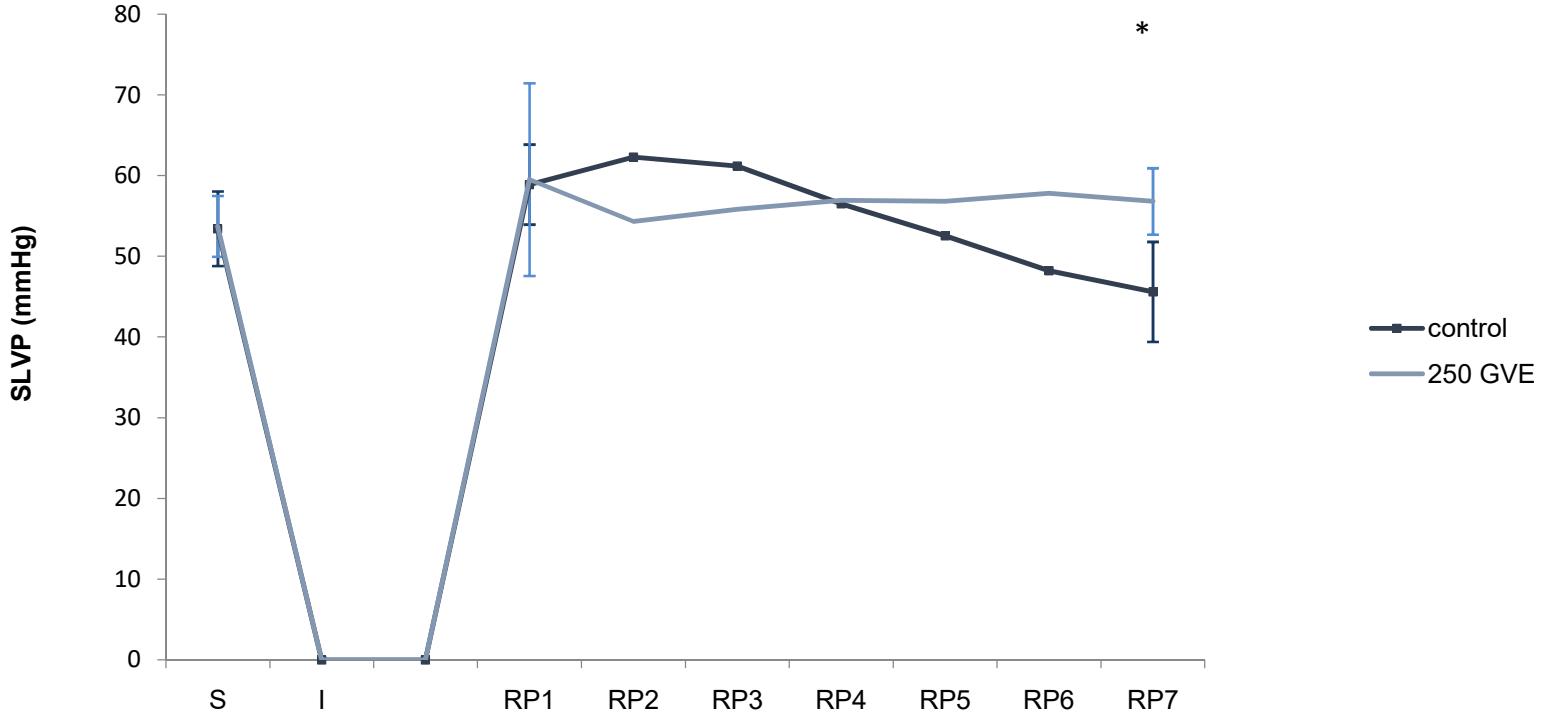
dp/dt min (mmHg/s)



	CONTROL	250 GVE
S vs RP1	p > 0.05	**p < 0.05**
S vs RP7	**p < 0.05**	p > 0.05
RP1 vs RP7	p > 0.05	p > 0.05

* control vs GVE

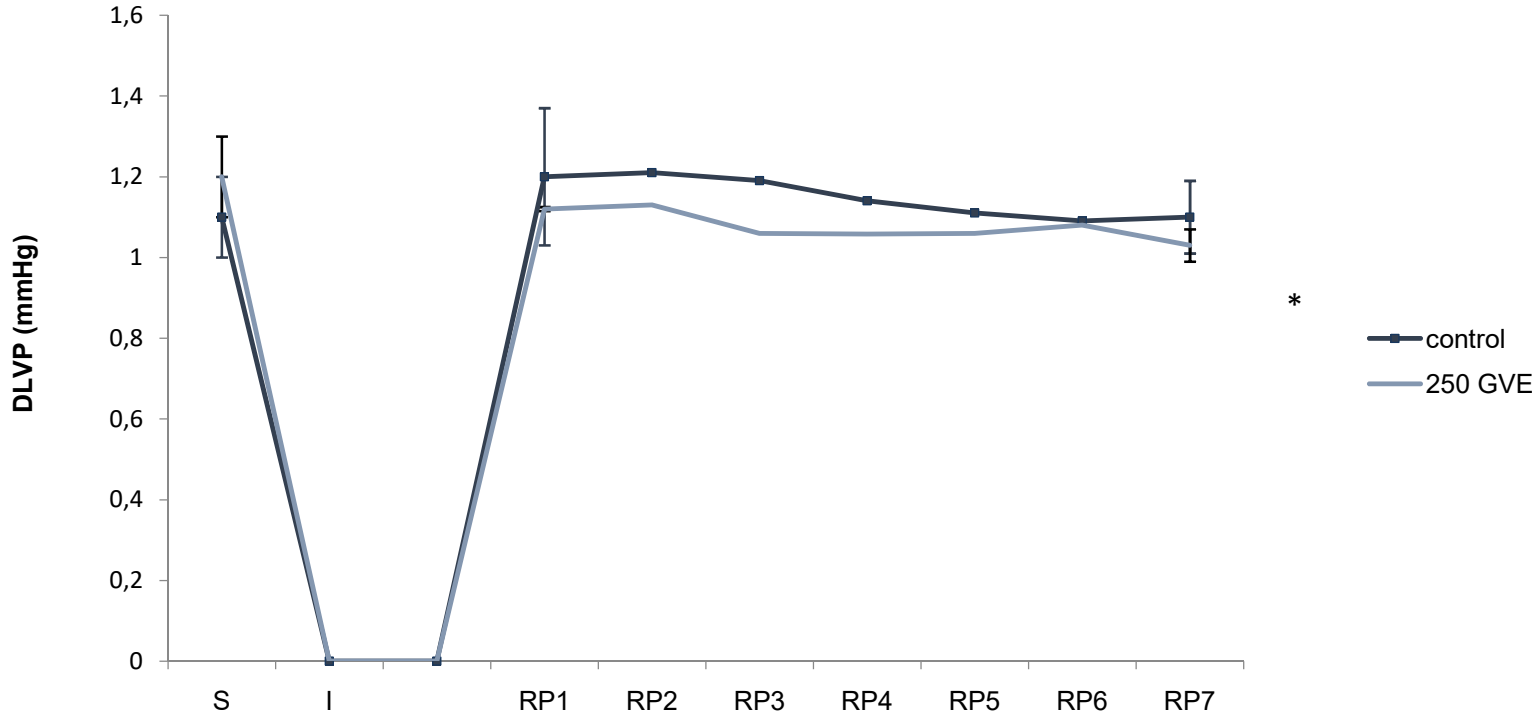
SLVP (mmHg)



	CONTROL	250 GVE
S vs RP1	p > 0.05	**p < 0.05**
S vs RP7	**p < 0.05**	p > 0.05
RP1 vs RP7	p > 0.05	p > 0.05

* control vs GVE

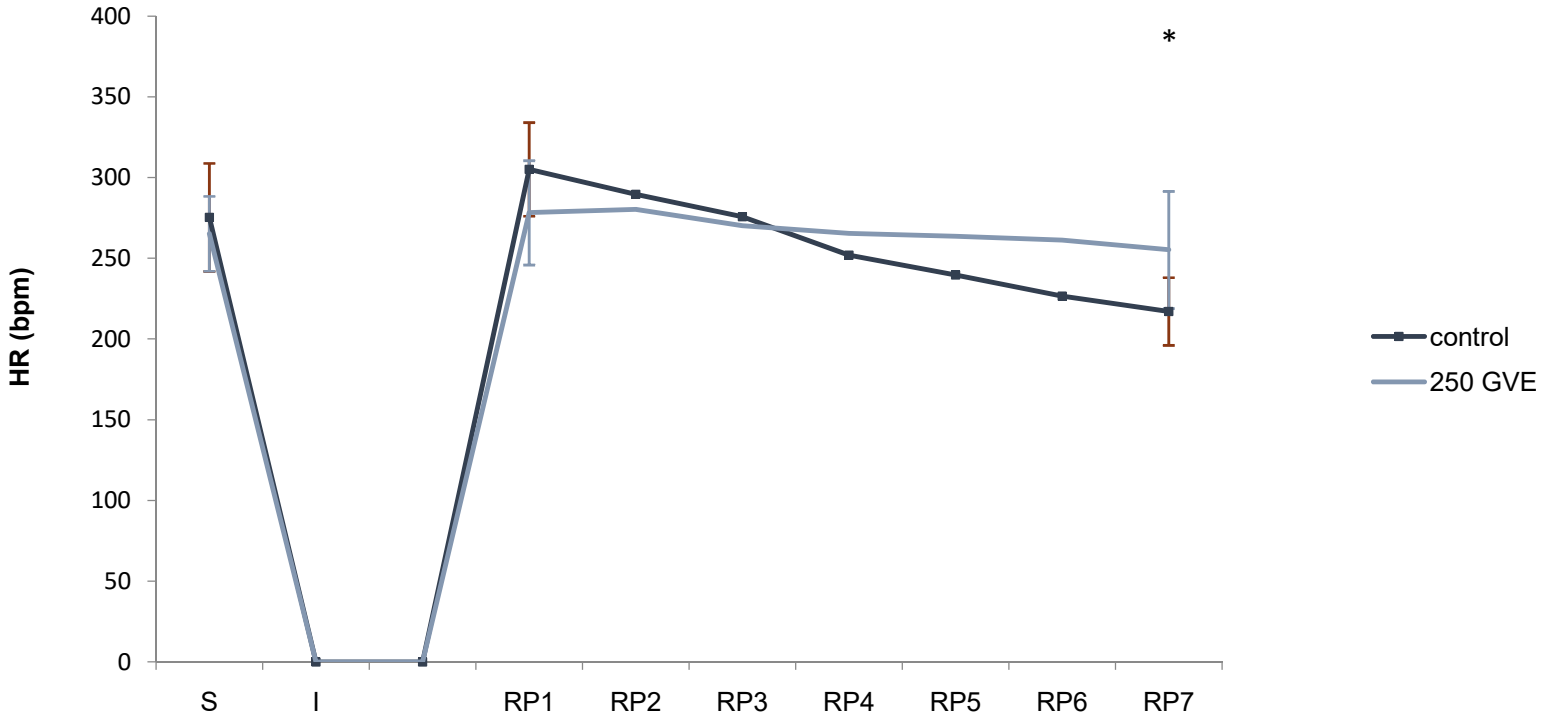
DLVP (mmHg)



	CONTROL	250 GVE
S vs RP1	p > 0.05	p > 0.05
S vs RP7	p > 0.05	p > 0.05
RP1 vs RP7	p > 0.05	p > 0.05

* control vs GVE

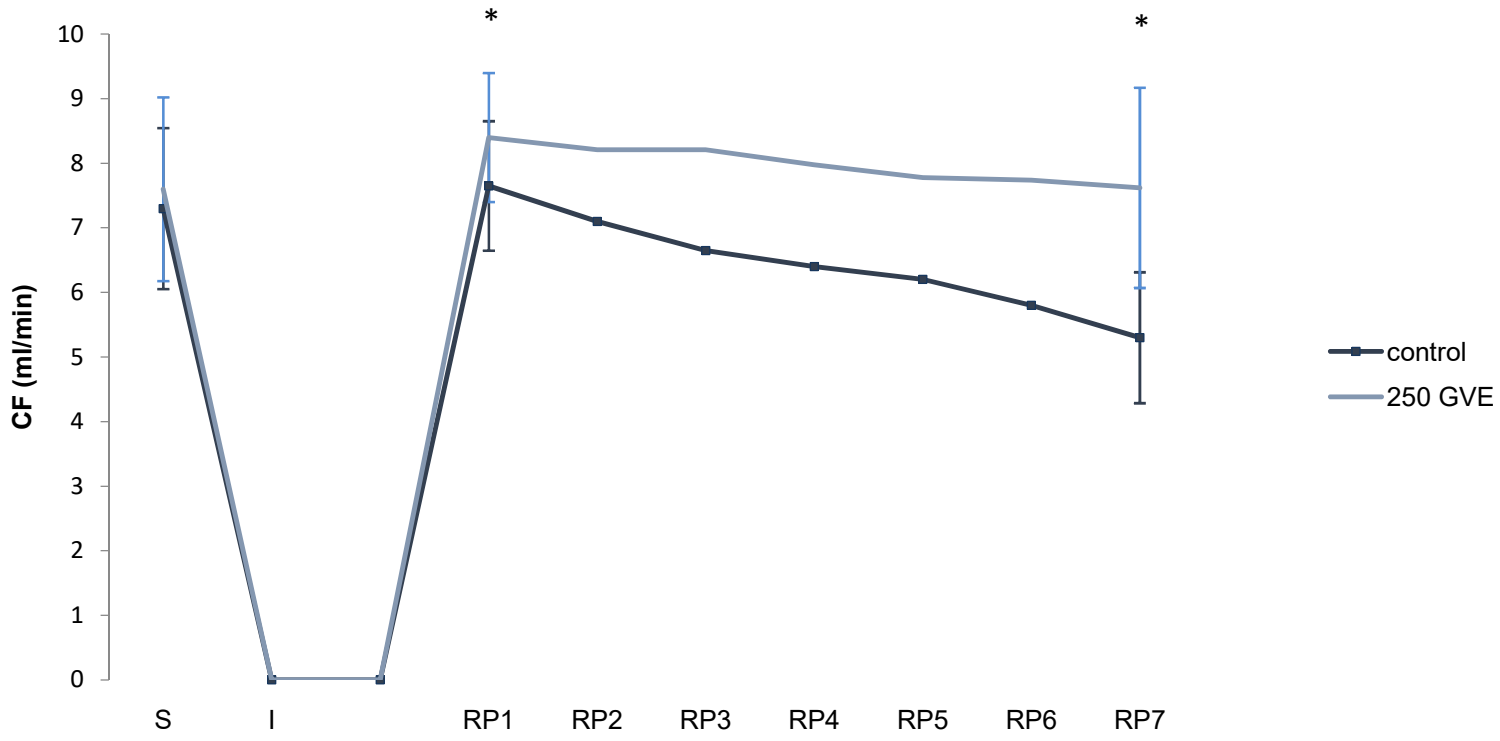
HR (bpm)



	CONTROL	250 GVE
S vs RP1	p > 0.05	p > 0.05
S vs RP7	**p < 0.05**	p > 0.05
RP1 vs RP7	p > 0.05	p > 0.05

* control vs GVE

CF (ml/min)

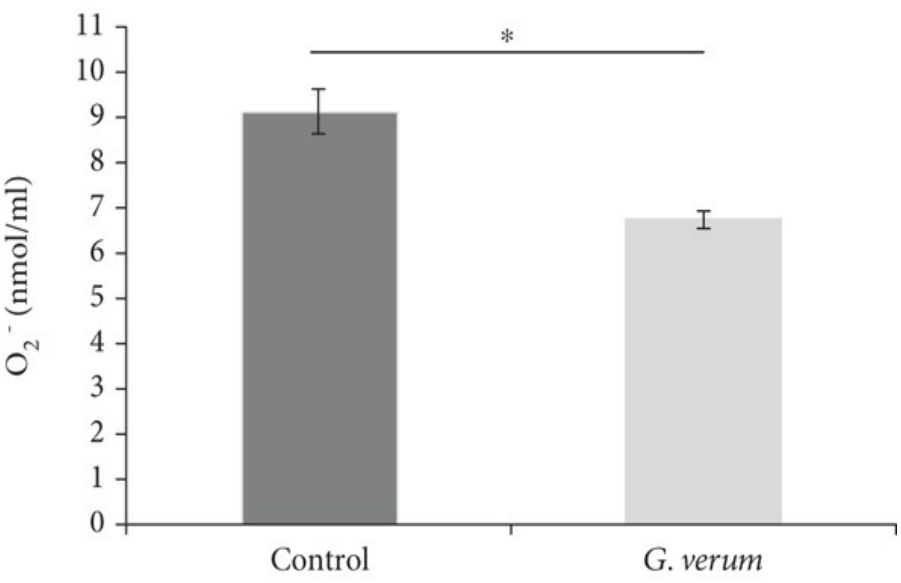


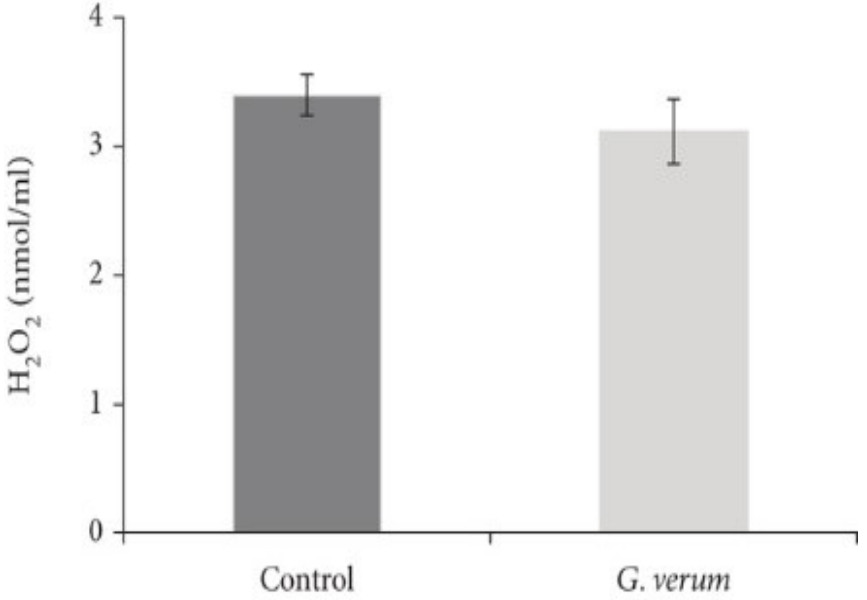
	CONTROL	250 GVE
S vs RP1	p > 0.05	p > 0.05
S vs RP7	**p < 0.05**	p > 0.05
RP1 vs RP7	p > 0.05	p > 0.05

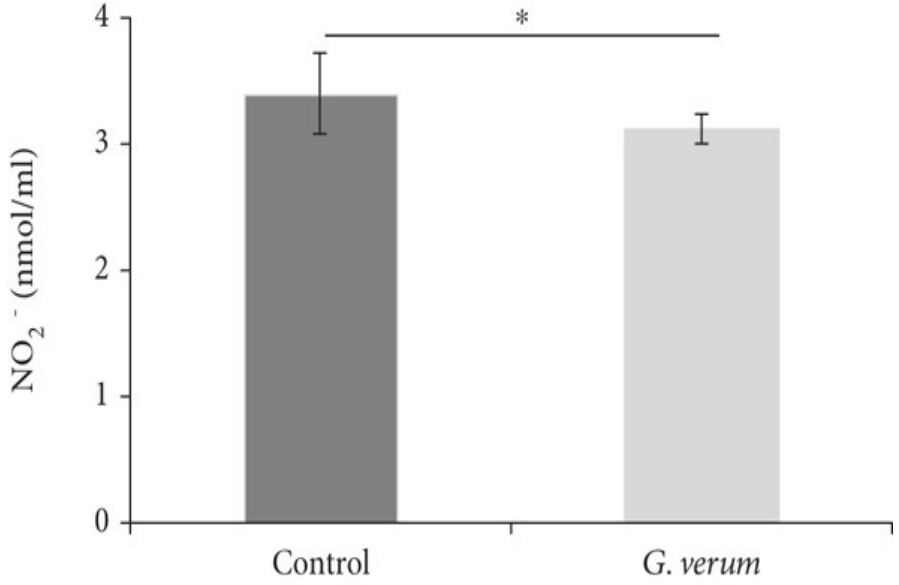
* control vs GVE

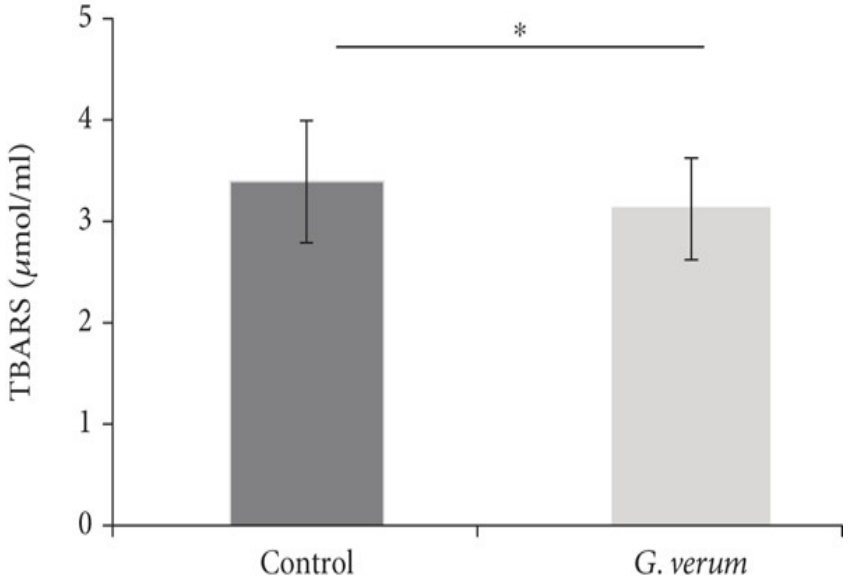
Systemic redox state

Pro-oxidants

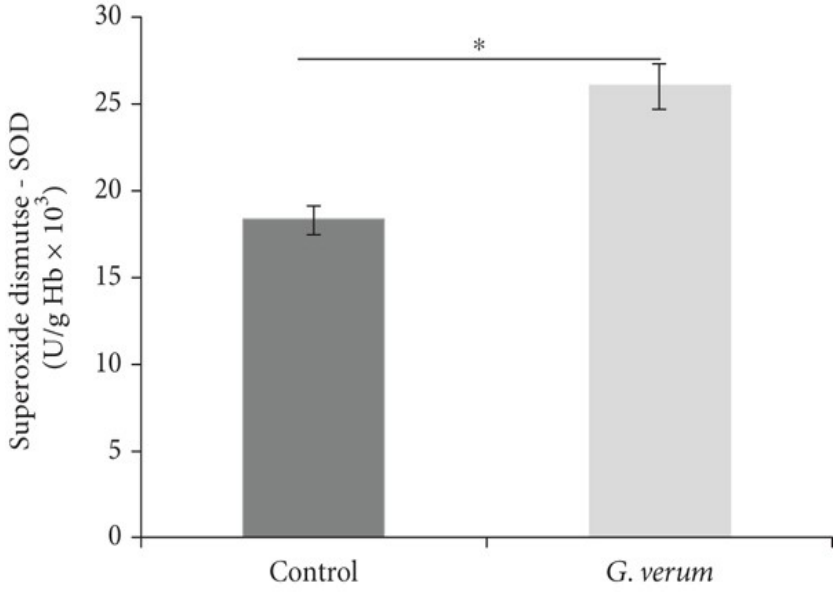


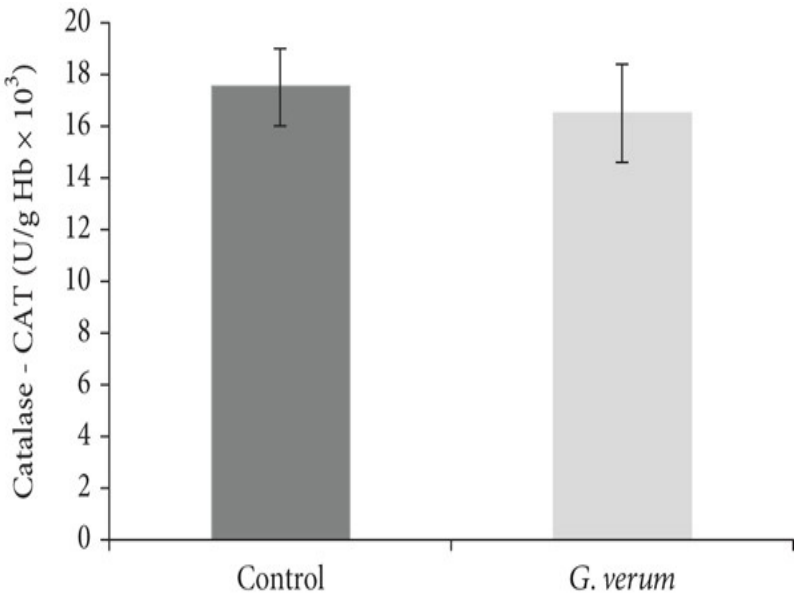


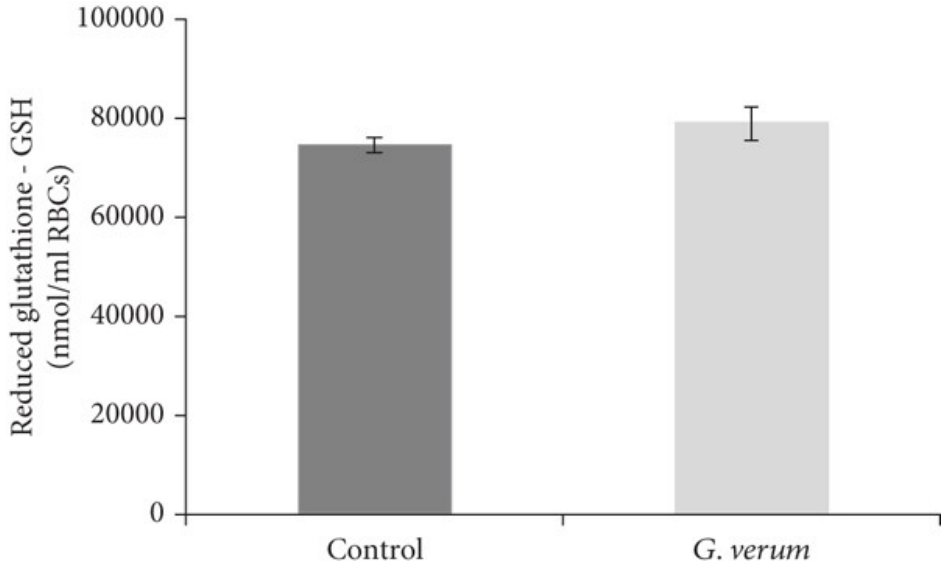




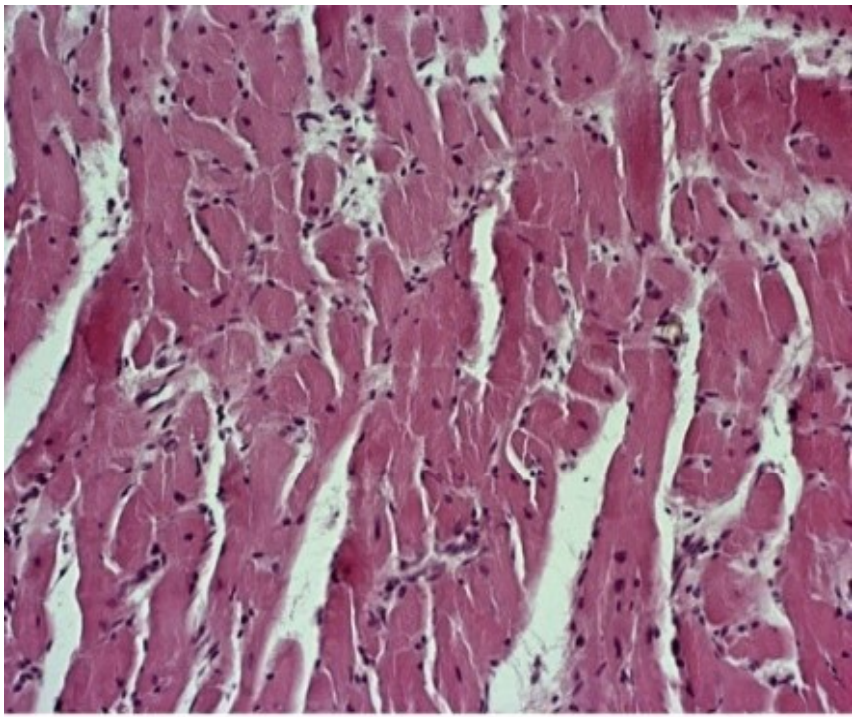
Antioxidants



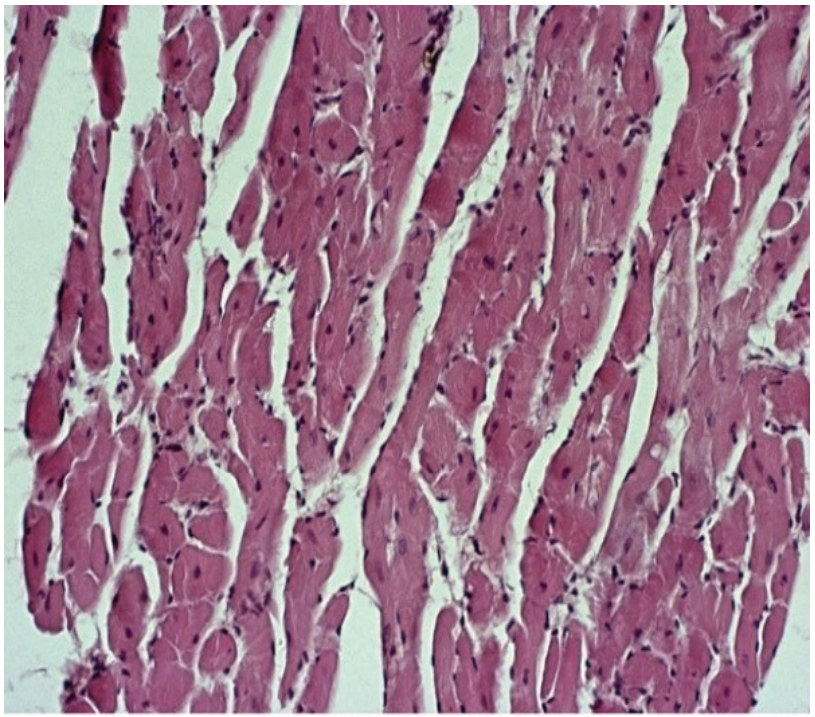




Histological changes in myocardium



Control



G. verum

- In the control group, degenerative changes were more prominent involving zonal necrosis of higher number of cardiomyocytes, with hypereosinophilia, fragmentation of the fibers, and loss of nucleus.
- Focal necrosis of lower number of cardiomyocytes or unicellular was found in the *G. verum* group

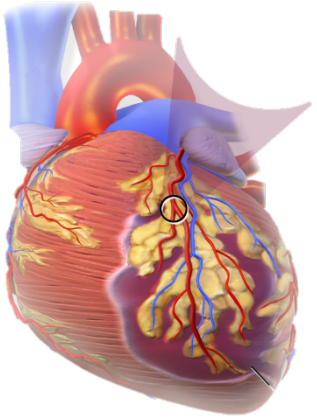
Take home conclusions

- *G. verum* extract led to the improvement of functional recovery of cardiac contractility, systolic and diastolic function when applied before ischemia.

- Additionally *G. verum* extract consumption was associated with the less production of pro-oxidants, which indicate alleviation of oxidative stress as one of possible mechanisms underlying cardioprotection during I/R injury.

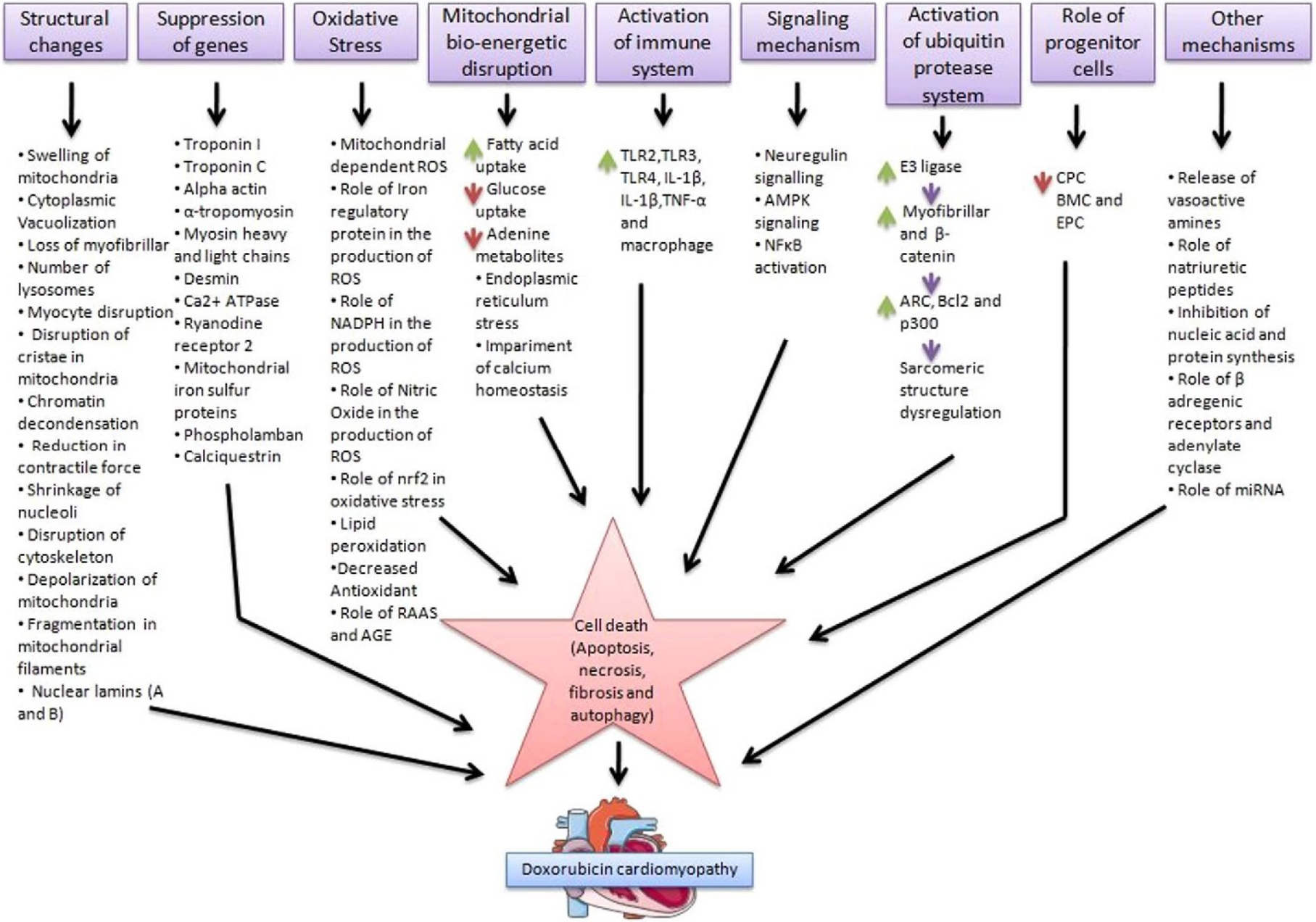
Second part of the investigation

Galium verum as a new tool for attenuation of doxorubicin-induced cardiotoxicity



Dox-induced cardiotoxicity

- Reports suggest that cardiotoxicity induced by anthracycline derivatives accounts for more than 30% of cardiotoxicity related to chemotherapy


Structural changes
Suppression of genes
Oxidative Stress
Mitochondrial bio-energetic disruption
Activation of immune system
Signaling mechanism
Activation of ubiquitin protease system
Role of progenitor cells
Other mechanisms
• Swelling of mitochondria
• Cytoplasmic Vacuolization
• Loss of myofibrillar
• Number of lysosomes
• Myocyte disruption
• Disruption of cristae in mitochondria
• Chromatin decondensation
• Reduction in contractile force
• Shrinkage of nucleoli
• Disruption of cytoskeleton
• Depolarization of mitochondria
• Fragmentation in mitochondrial filaments
• Nuclear lamins (A and B)
• Troponin I
• Troponin C
• Alpha actin
• α-tropomyosin
• Myosin heavy and light chains
• Desmin
• Ca2+ ATPase
• Ryanodine receptor 2
• Mitochondrial iron sulfur proteins
• Phospholamban
• Calciquestrin
• Mitochondrial dependent ROS
• Role of Iron regulatory protein in the production of ROS
• Role of NADPH in the production of ROS
• Role of Nitric Oxide in the production of ROS
• Role of nrf2 in oxidative stress
• Lipid peroxidation
•Decreased Antioxidant
• Role of RAAS and AGE
Fatty acid uptake
Glucose uptake
Adenine metabolites
• Endoplasmic reticulum stress
• Impariment of calcium homeostasis
TLR2,TLR3, TLR4, IL-1β, IL-1β,TNF-α and macrophage
• Neuregulin signalling
• AMPK signaling
• NFκB activation
E3 ligase
Myofibrillar and β-catenin
ARC, Bcl2 and p300
Sarcomeric structure dysregulation
CPC
BMC and EPC
• Release of vasoactive amines
• Role of natriuretic peptides
• Inhibition of nucleic acid and protein synthesis
• Role of β adregenic receptors and adenylate cyclase
• Role of miRNA
Cell death (Apoptosis, necrosis, fibrosis and autophagy)
Doxorubicin cardiomyopathy

Second aim of the study

To assess the influence of 14-day application of *G. verum* methanol extract on cardiac dysfunction induced by DOX in rats

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Treatment with *G. verum* extract

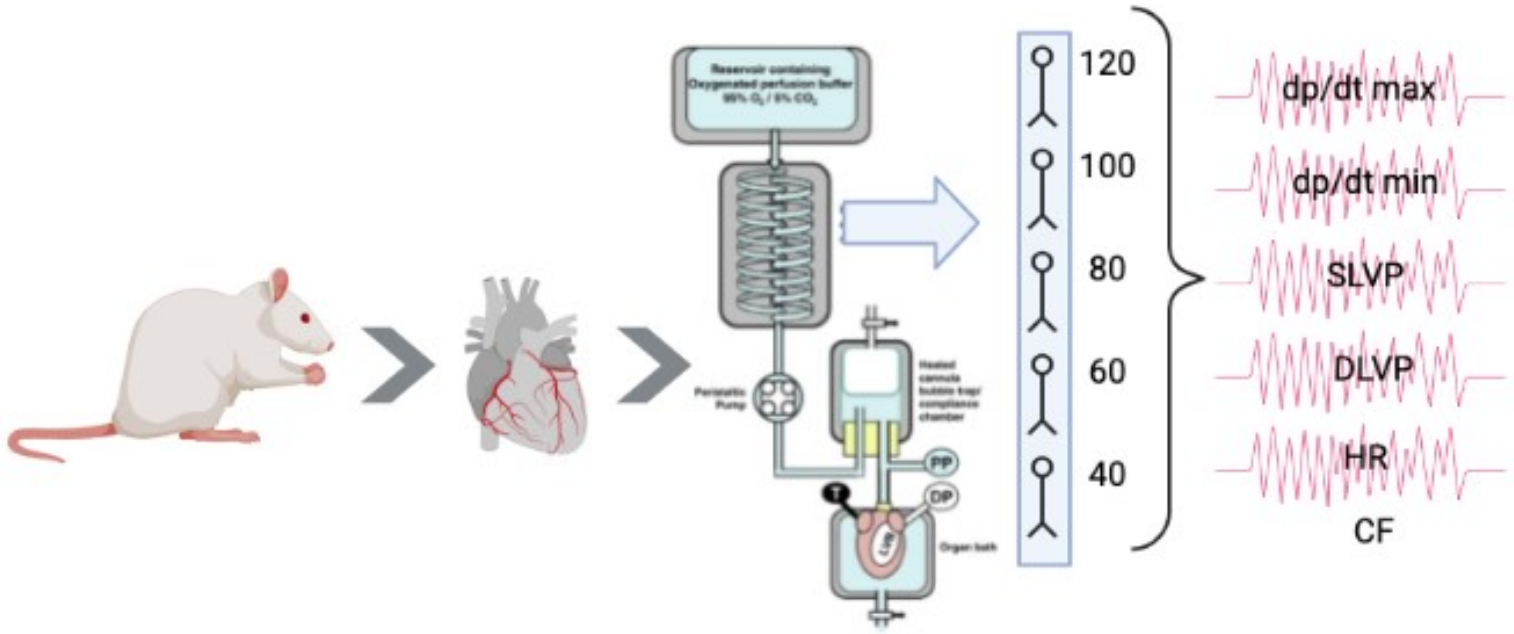
Rats (n=30)

1. CTRL — healthy untreated rats
2. DOX — rats treated with single DOX injection
3. GVE + DOX — rats treated with 50 mg/kg *G. verum* extract for 2 weeks after 2 weeks, rats receive single DOX injection

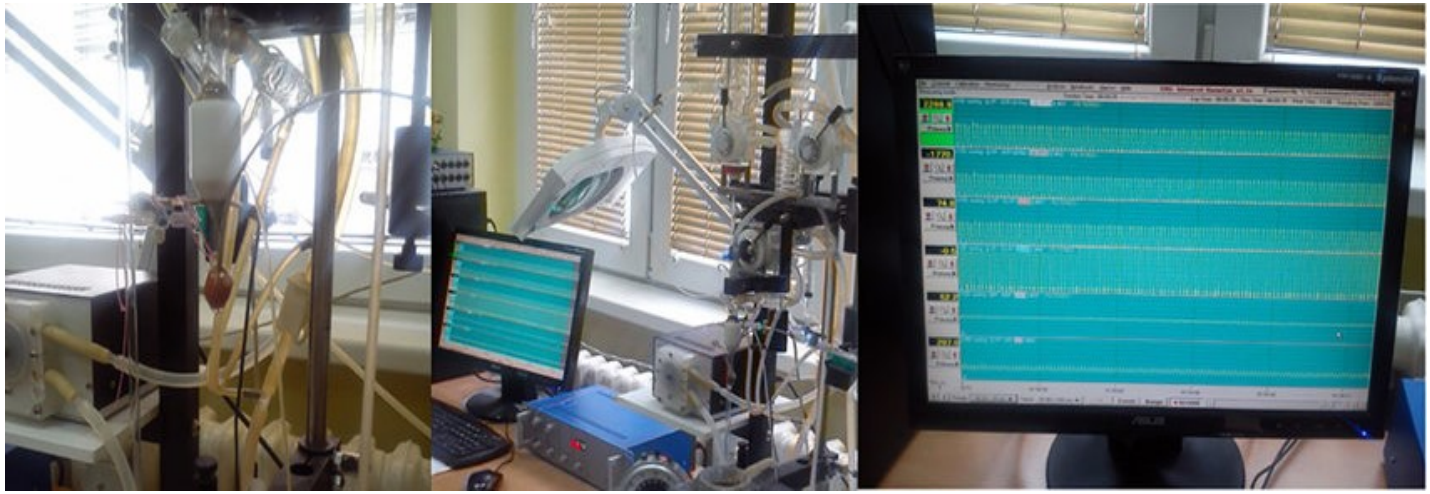
- 72 h following dox injection rats were sacrificed by cervical dislocation.

Functional parameters of the heart- Cardiodynamic parameters

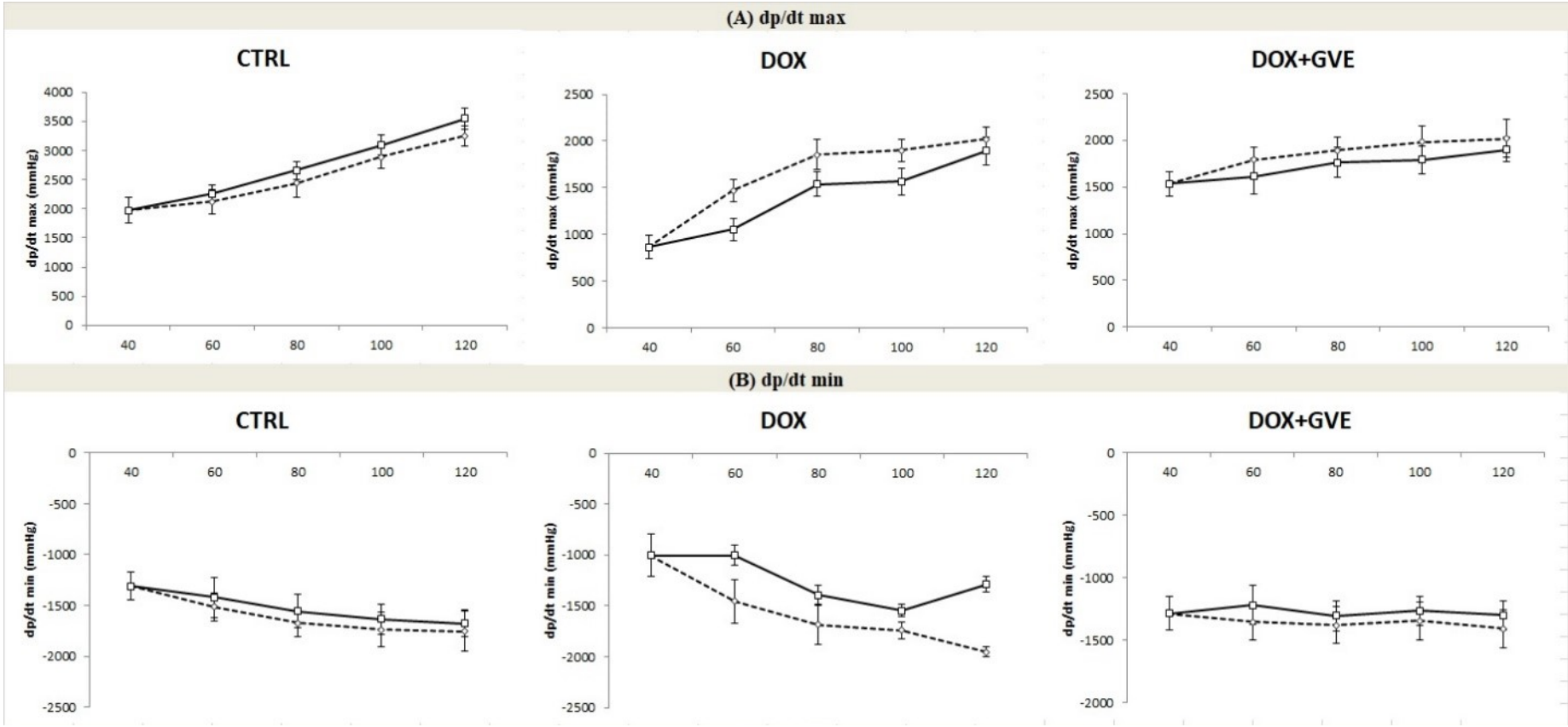
- After urgent thoracotomy, hearts were rapidly excised, the aortas were cannulated and retrogradely perfused according to *Langendorff* technique
- Hearts were exposed to gradually increased perfusion pressures – PCP1 and PCP2



Results for cardiodynamic parameters

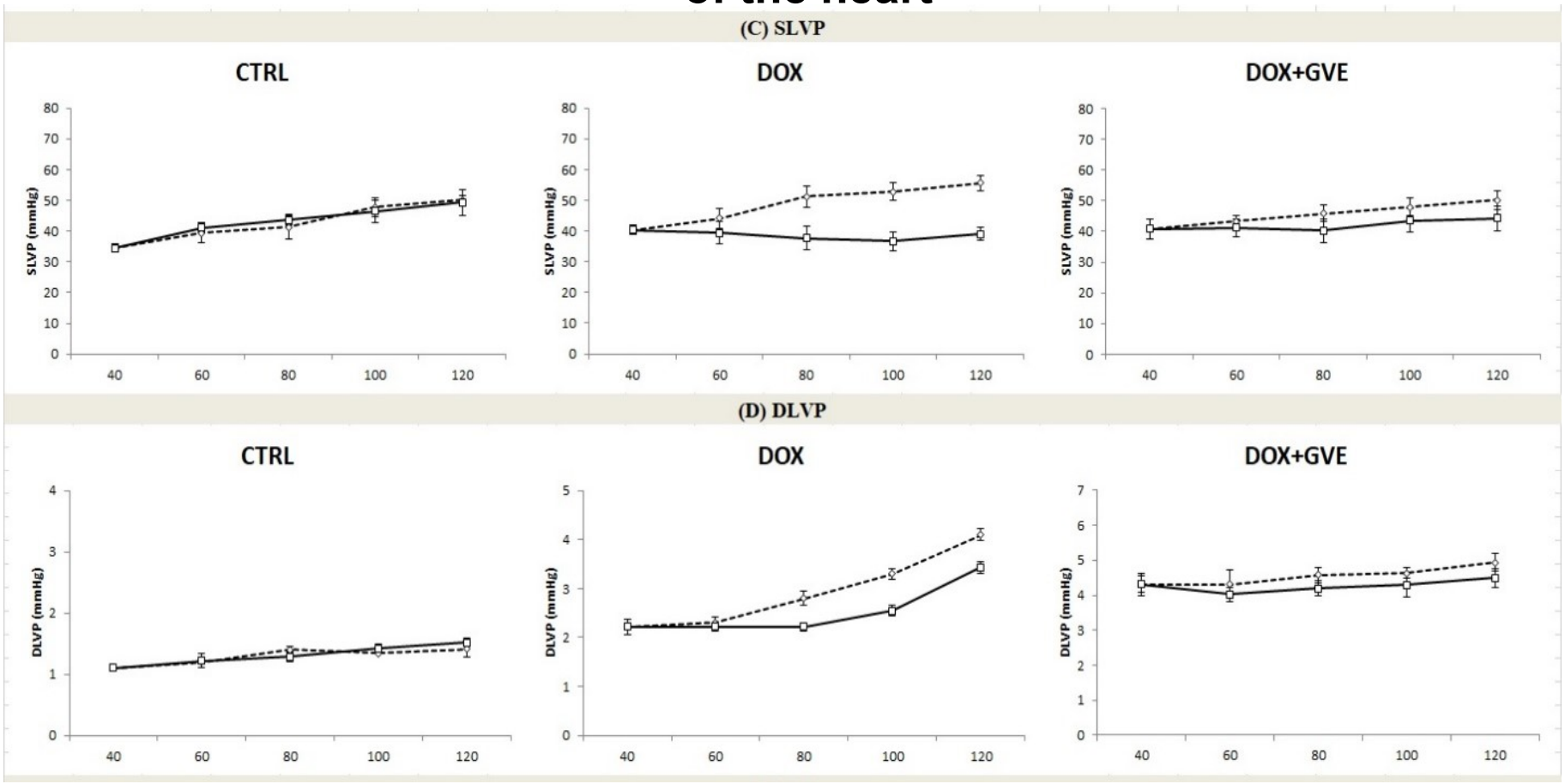


Values of dp/dt max (A) and dp/dt min (B) – cardiac contractility and relaxation



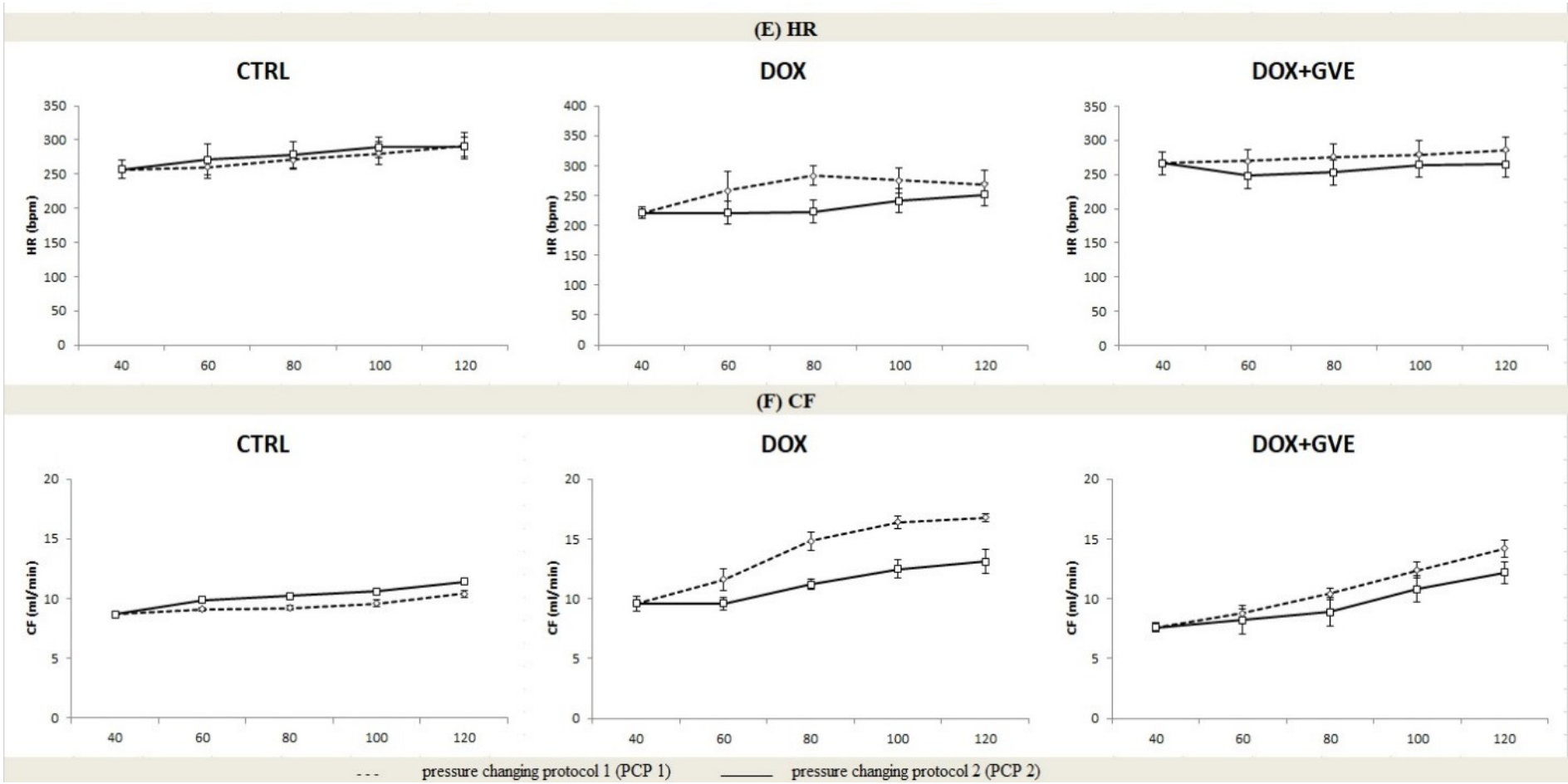
CPP	CTRL	DOX	DOX + GVE	CPP	CTRL	DOX	DOX + GVE
	dp/dt max				dp/dt min		
60	6,20	-28,55	-9,81	60	- 5,08	-31,15	-8,13
80	7,24	-17,78	-7,55	80	-5,91	-17,28	-6,85
100	6,98	-13,71	-9,26	100	-5,31	-12,23	-8,34
120	6,23	-9,77	-6,98	120	-3,18	-26,03	-5,36

Values of SLVP (C) and DLVP (D) – systolic and diastolic capacity of the heart



CPP	CTRL	DOX	DOX + GVE	CPP	CTRL	DOX	DOX + GVE
SLVP				DLVP			
60	4,57	-11,85	-5,03	60	1,67	-3,48	-6,46
80	5,33	-35,77	-12,23	80	-5,86	-20,71	-8,05
100	-2,93	-43,88	-9,41	100	3,97	-22,73	-7,34
120	-1,60	-42,93	-13,12	120	3,80	-16,10	-9,11

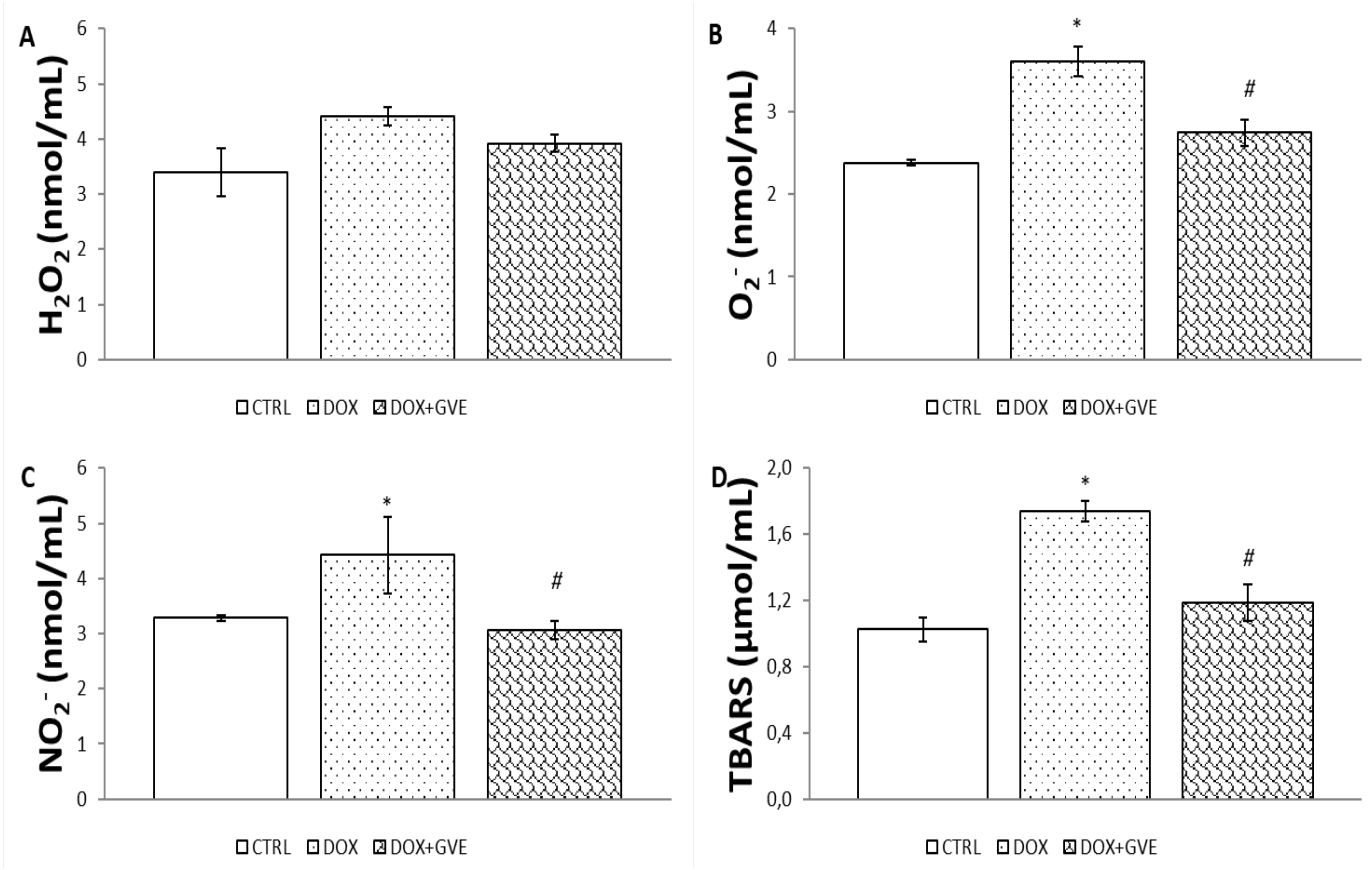
Values of HR (E) and CF (F)



CPP	CTRL	DOX	DOX + GVE	CPP	CTRL	DOX	DOX + GVE
	HR				CF		
60	4,27	-14,43	-7,92	60	8,79	-17,24	-6,82
80	2,58	-21,46	-8,20	80	10,87	-24,32	-13,62
100	3,21	-12,42	-5,44	100	10,42	-23,78	-12,90
120	-0,68	-6,41	-6,19	120	9,62	-22,02	-13,08

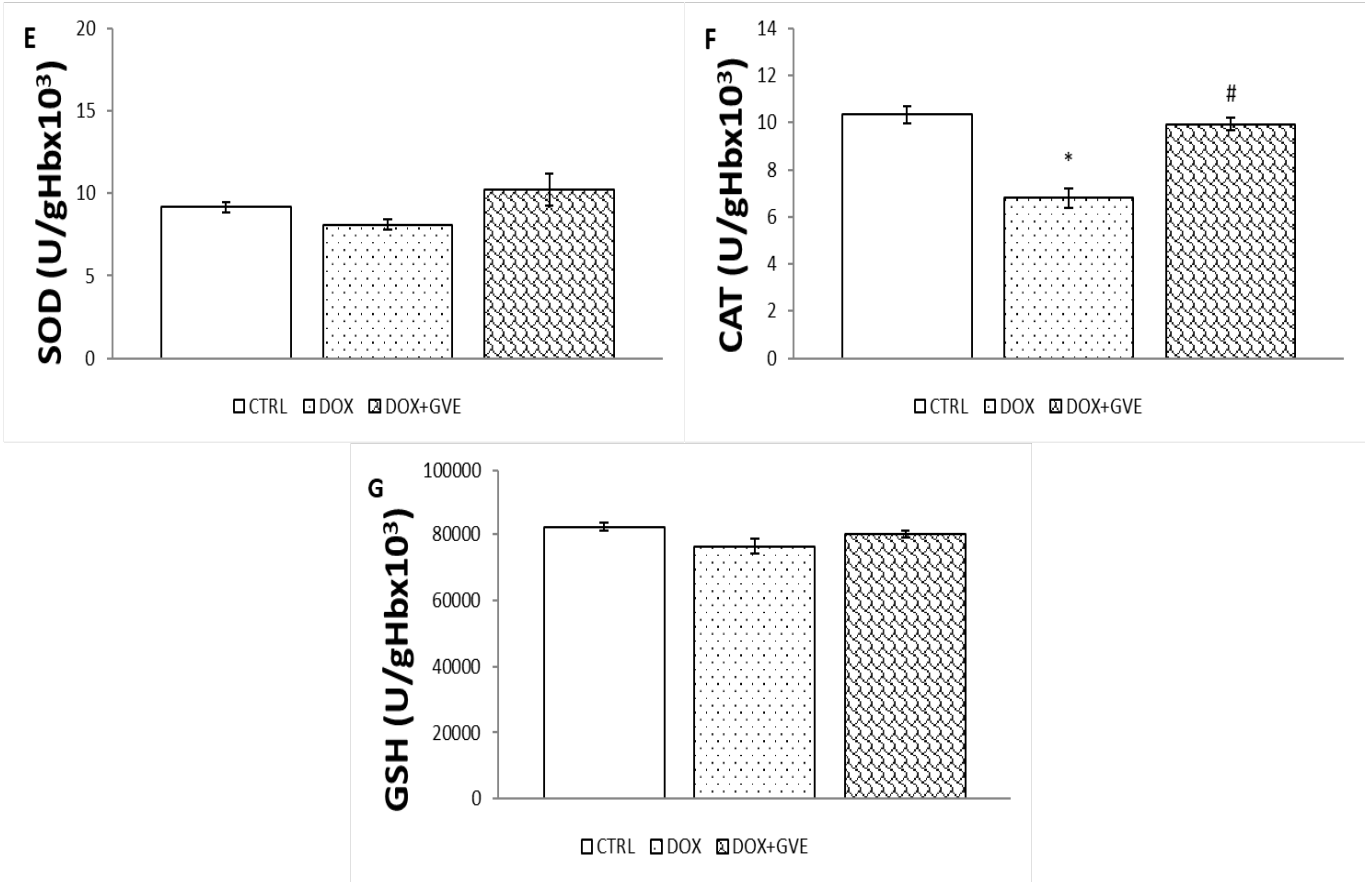
Systemic redox state

Pro-oxidants



*p < 0.05 compared to CTRL group; #p < 0.05 compared to DOX group.

Antioxidants

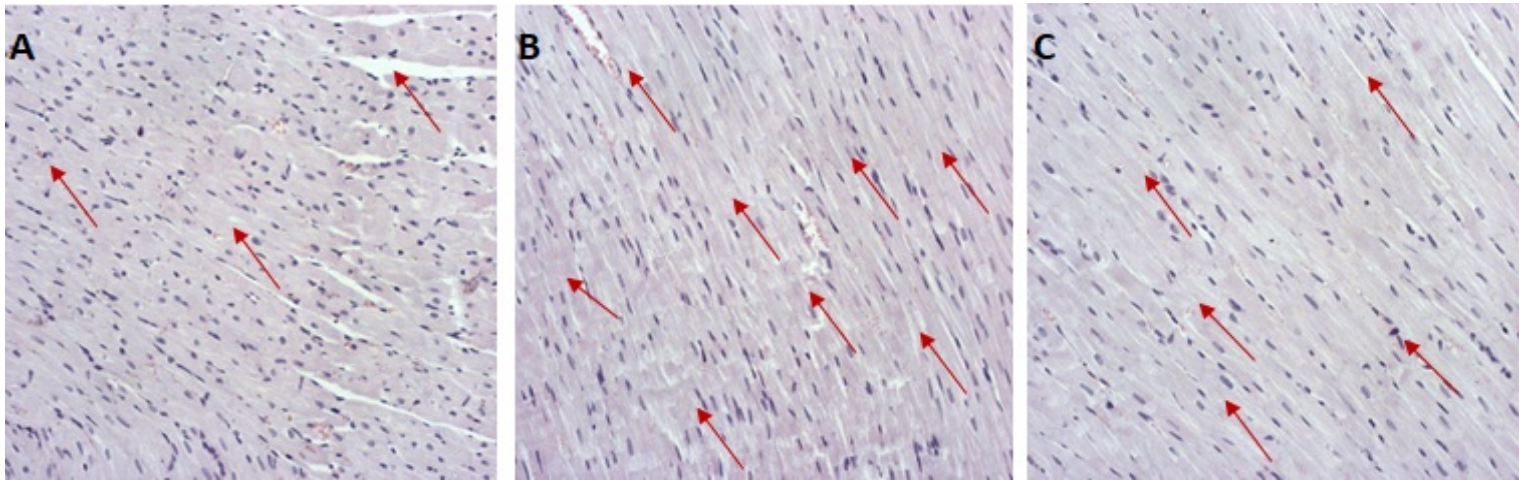


*p < 0.05 compared to CTRL group; #p < 0.05 compared to DOX group.

Histological changes in myocardium

Hematoxylin/eosin-staining

CTRL group **DOX group** **DOX + GVE group**



Degree of heart damage

Groups	Score
CTRL	0.8
DOX	3.5
DOX + GVE	2.5

No change=0 score; focal changes=1 score; Patchy changes=2 score; Confluent changes=3 score; Massive changes=4 score

CTRL: discrete histological lesions that vary from hyperemia and edema to degenerative changes and unicellular or partially focal necrosis of muscle cells.

DOX : degenerative changes and necrosis are significantly more pronounced, mostly confluent and massive, there are zonal necrosis of a larger number of muscle cells

DOX+GVE: patchy and confluent-type fields are observed more often.

Take home conclusions

- ✓ Consumption of *G. verum* attenuated oxidative stress and prevented the pathological injuries caused by DOX injection.

- ✓ In the light of these findings, *G. verum* extract can serve as effective add-on therapy in triggering cardioprotection in different pathologies.

Laboratory team