

Rutin (quercetin-3-rutinoside) modulates hemostatic disturbances and reactive species levels in experimental *Bothrops jararaca* envenomation

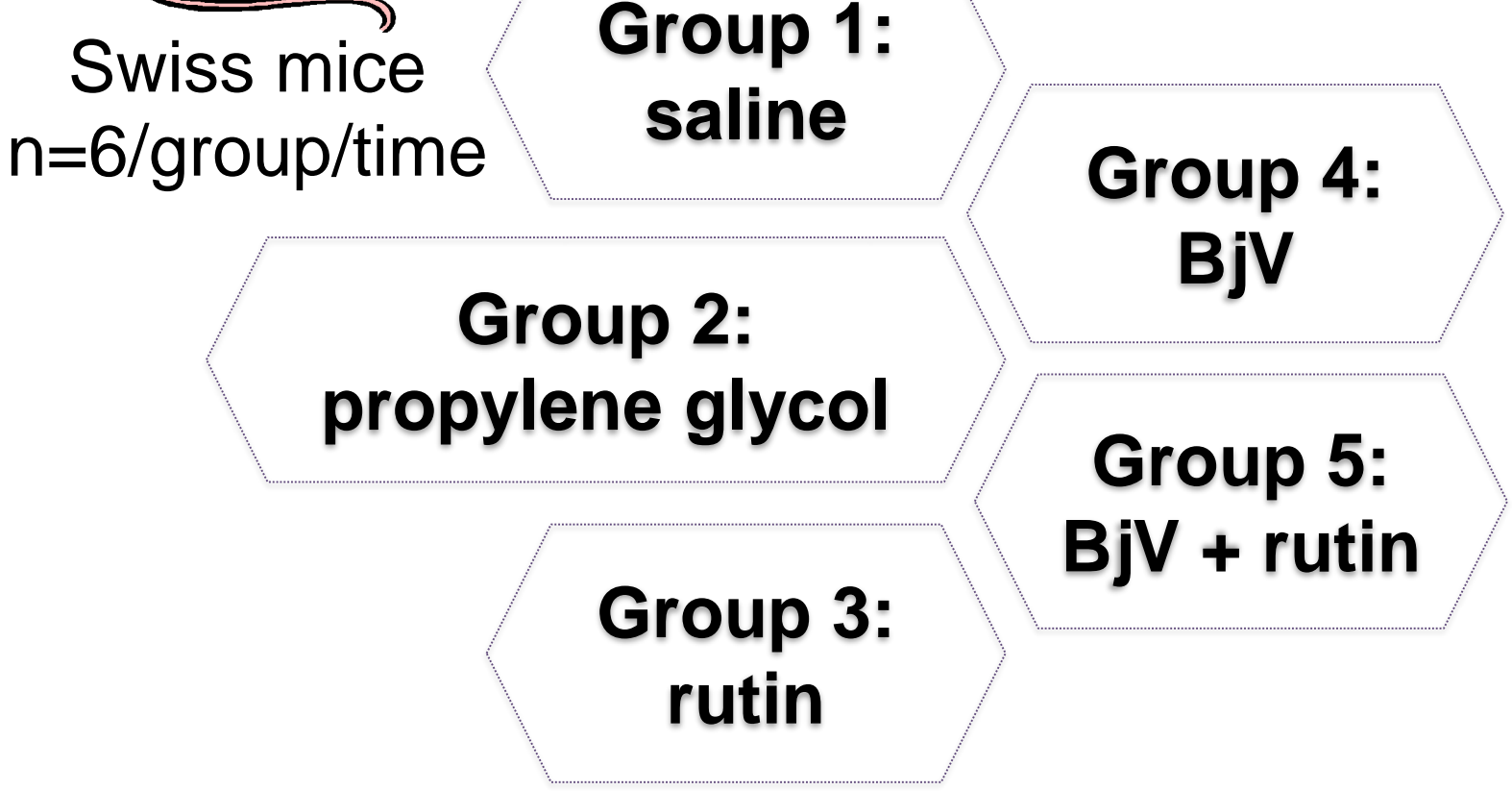
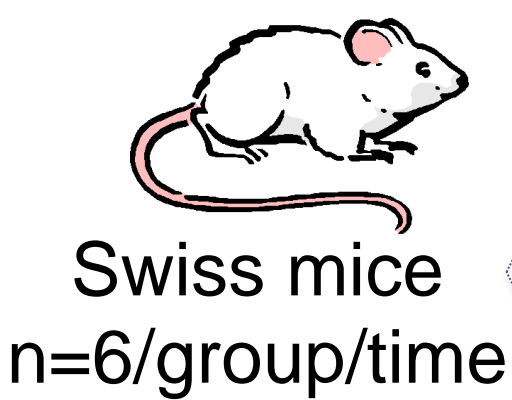
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INTRODUCTION

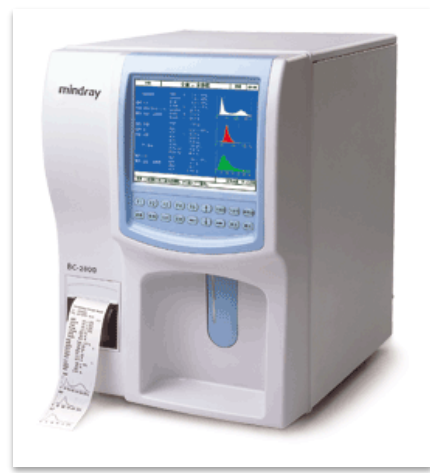
- Snakebites are a major public health problem worldwide;
- Patients bitten by *Bothrops* snakes manifest bleeding disorders, thrombocytopenia, consumptive coagulopathy, increased plasma tissue factor (TF) and oxidative/nitrosative stress (ONS);
- ONS is not neutralized by specific antivenom and may induce additional hemostatic disturbances;
- It is important to search for new complementary therapies, such as rutin, a natural antioxidant that also inhibits protein disulfide isomerase (PDI), which is a enzyme required for the thrombus formation *in vivo*;
- AIMS: to investigate the potential of rutin against ONS and hematological/hemostatic disturbances evoked in mice injected with *B. jararaca* venom (BjV).

MATERIALS AND METHODS



After 3, 6 and 24 h → Collection of blood and tissues

Complete blood cell counts



- Plasma**
- ROS/RNS levels
Fluorescence assay
 - Fibrinogen levels
 - Total antioxidant capacity
Colorimetric assay



BjV: 1.6 mg/kg b.w., s.c.
Rutin: 14.4 mg/kg b.w., s.c.
Pre-incubation: 30 min at 37°C

Ethics statement: approved by CEUA-IB 4388061115, CEUA-FMUSP 188/15.

Statistical analysis:
Shapiro-Wilk normality test
One/Two-way ANOVA and Tukey test

In vitro - BjV and BjV + rutin

Activity of venom proteins

- Snake venom metalloproteinases (SVMP)
- Snake venom serineproteases (SVSP)
 - L-amino acid oxidases (LAO)
 - Phospholipases A₂ (PLA₂)

Tissues

- Protein expression
Western blotting
- TF activity
Coagulant assay



REFERENCES

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Strapazzon JdO, Parisotto EB, Moratelli AM, Garlet TR, Bastos J, Zimmermann IR, et al. Systemic oxidative stress in victims of *Bothrops* snakebites. Journal of Applied Biomedicine. 2014.
Yamashita KM, Alves AF, Barbaro KC, Santoro ML. *Bothrops jararaca* venom metalloproteinases are essential for coagulopathy and increase plasma tissue factor levels during envenomation. PLoS Negl Trop Dis. 2014;8(5):e2814.

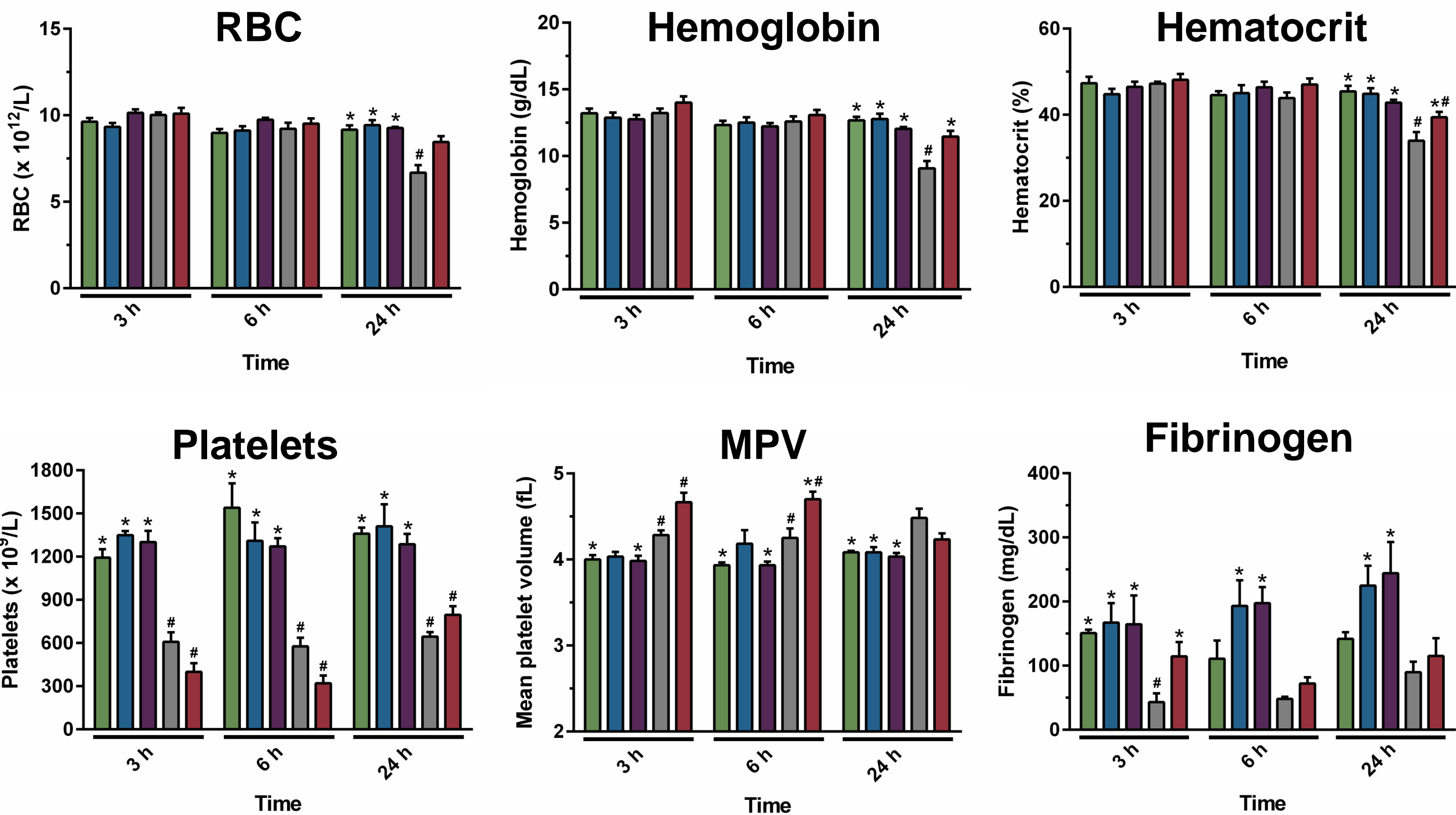
RESULTS

Venom proteins	Activity (%)	
	BjV	BjV + rutin
SVMP	100	94
SVSP	100	98
LAO	100	90
PLA ₂	100	98

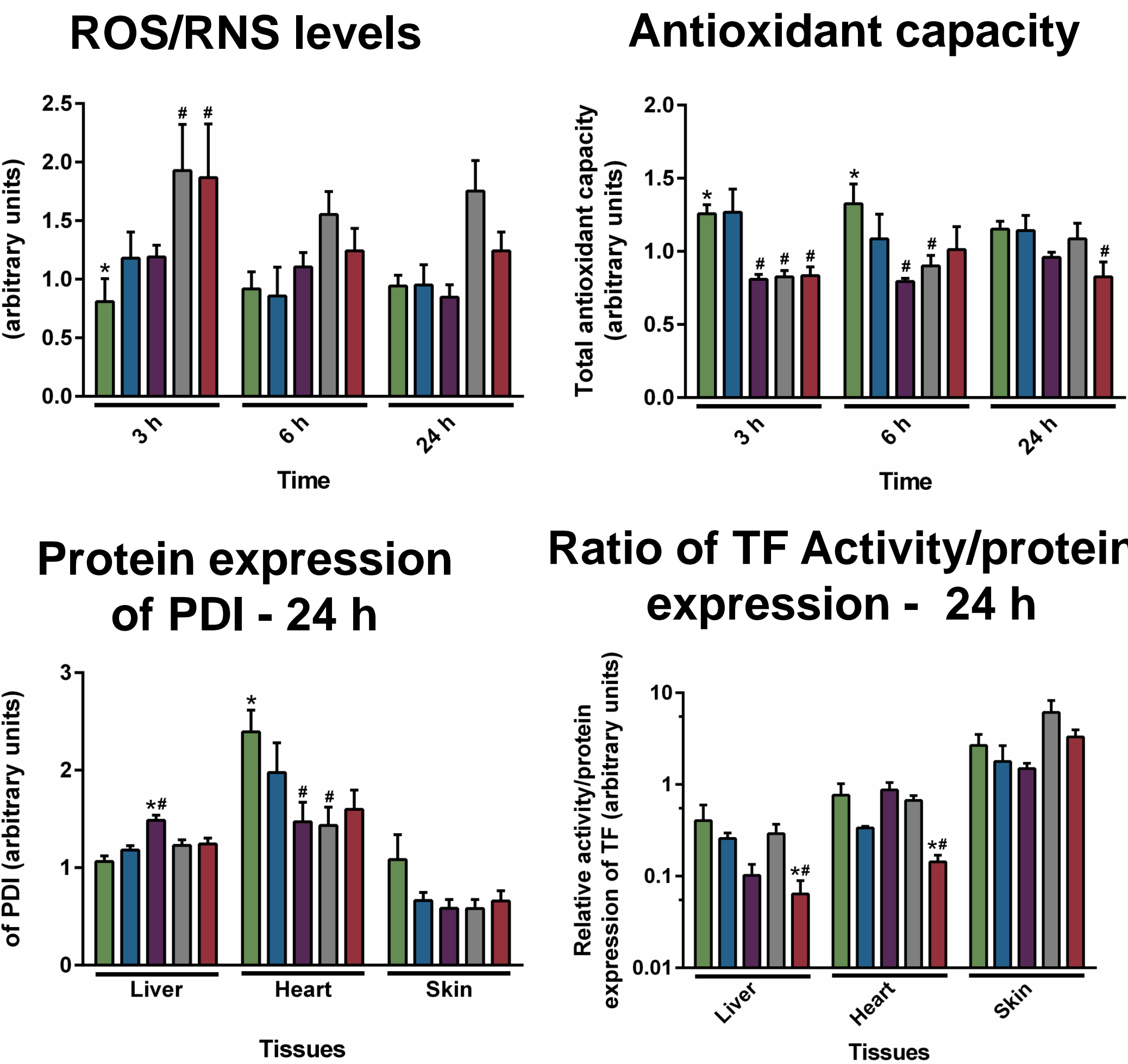
✓ **Rutin does not inhibit venom proteins in vitro.**

All data expressed as mean ± SEM.
p < 0.05 when compared to saline control group.
* p < 0.05 when compared to BjV group.

Saline control
Propylene glycol control
Rutin control
BjV
BjV + Rutin



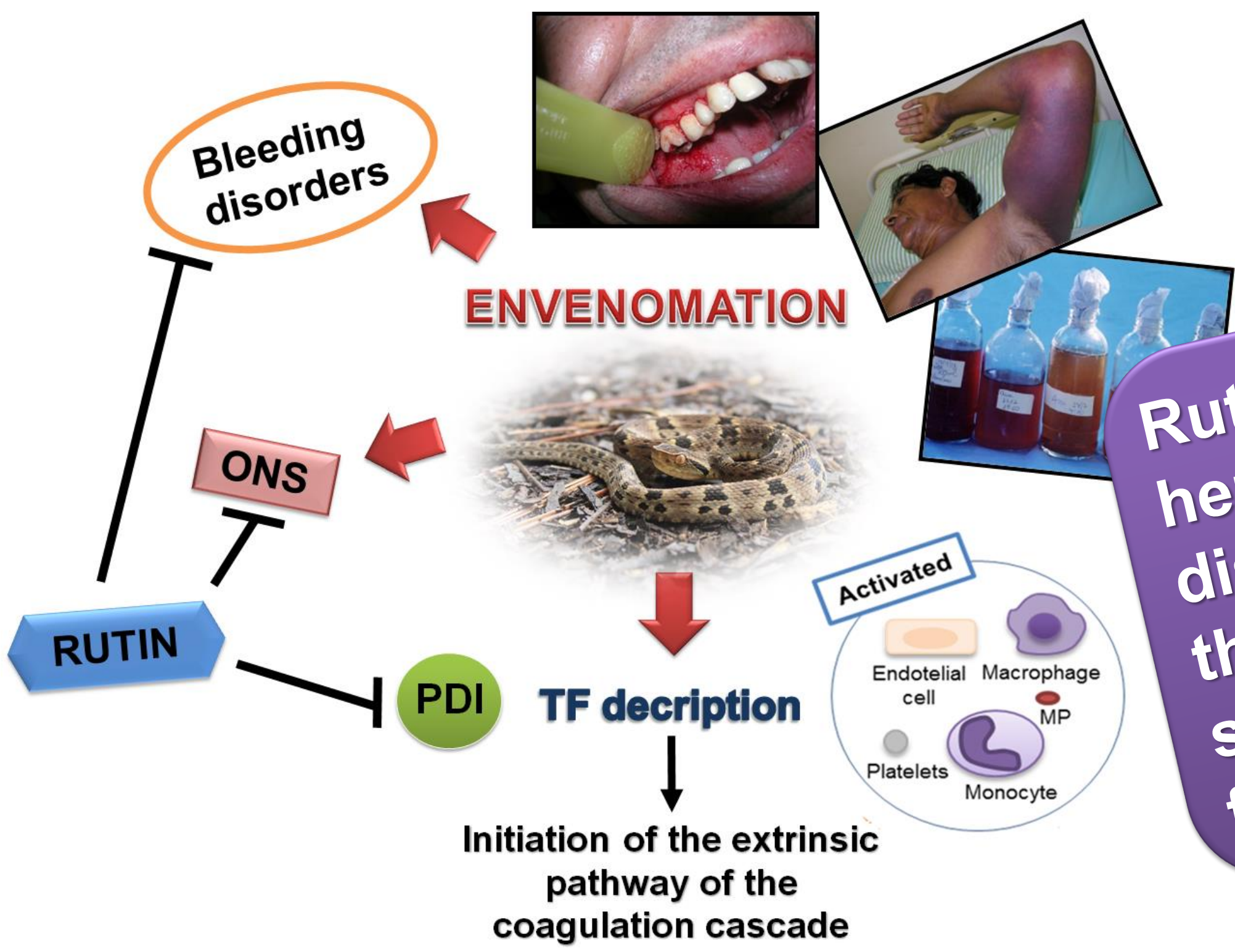
✓ **Envenomation induced important hemostatic disturbances, such as thrombocytopenia, hypofibrinogenemia and decrease in RBC and hemoglobin.**
✓ **Rutin inhibited the decrease of RBC, hemoglobin and fibrinogen.**



✓ **BjV induced ONS;**
✓ **Rutin decreased ROS/RNS over time.**

✓ **BjV decreased PDI (heart);**
✓ **Rutin modulated PDI expression in liver and heart;**
✓ **Rutin modulated TF activity in liver and heart;**

CONCLUSIONS



Rutin abbreviated the hemostatic and ONS disturbances, suggesting that it may be used as a supplementary treatment for snakebites.

Images credits: Hospital Vital Brazil and Frederico Alcântara.

