

THE MECHANISM OF ANTIULCER ACTION OF MELANIN

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It is known that pigment melanin, as well as many others natural polyphenols, protect gastric mucosa from injuries evoked by stress, serotonine, aspirine and improve the ulcer healing in rats. However, the exact mechanisms responsible for this phenomenon are still poorly understood.

- The aim of the present study is to investigate the mechanism of antiulcer action of melanin producers of which is a strain of black yeast *Nadsoniella nigra X-1*.

THE METHODS

The investigation was carried out on white rats, males. The damages of gastric mucosa were induced by combination of cold (for 2h) and immobilization stress (for 24h). Several groups of rats were used to study the mechanism of melanin action on the gastric lesions induced by stress: 1) control group without exposure to stress; 2) stress applied alone; 3) rats exposed to stress and pre-treated with melanin (5 mg/kg i.g.); 4) rats exposed to stress and pre-treated with melanin in combination with inhibitor of PPAR γ GW9662 (1 mg/kg i.p.); 5) rats exposed to stress and pretreated with GW9662 alone. The square of gastric injuries (gastric ulcers, erosions and haemorrhages) was measured. The level of NO in blood was measured by Griess reagent system. Expression of eNOS was detected by immunoblotting, using specific antibody.

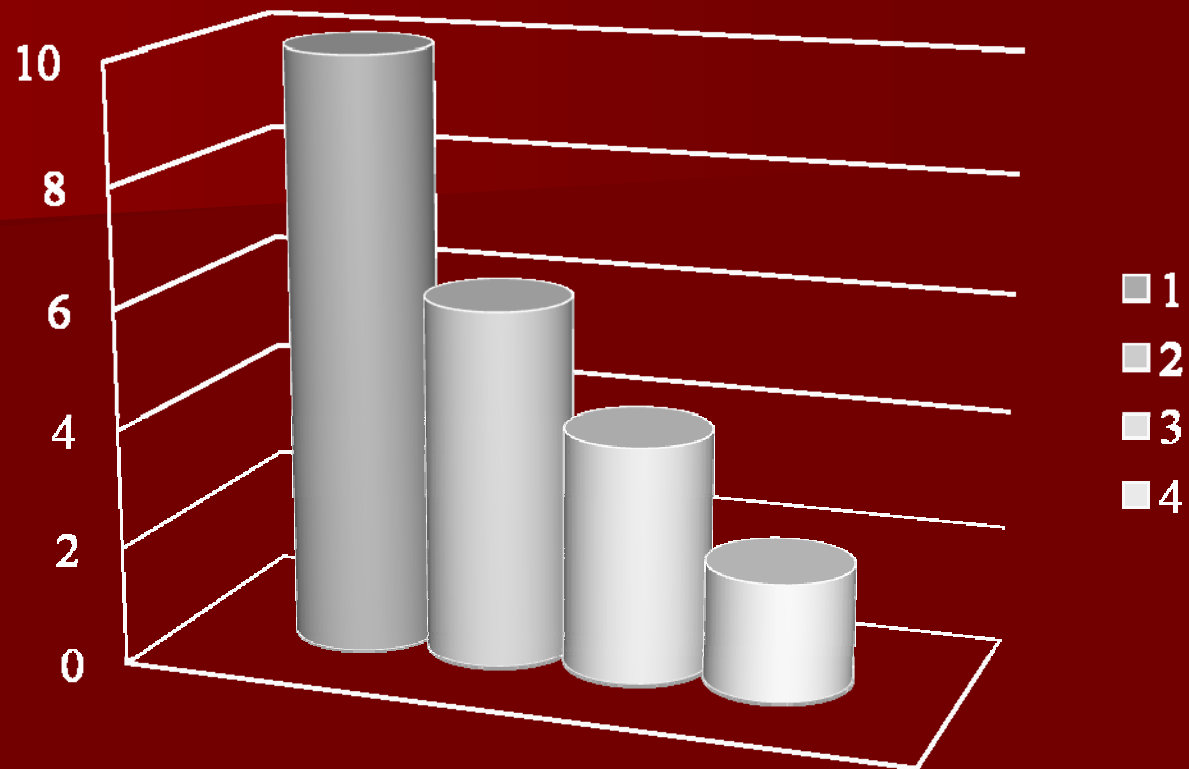


Fig. 1 Number of animals with gastric ulcer in different experimental groups :

1 group, control (stress + vehicle);

2 group, stress + 5 mg/kg synthetic melanin "Sigma";

3 group, stress + 5 mg/kg melanin from *Nadsoniella nigra* var. *hesuelica*;

4 group, stress + 5 mg/kg melanin from *Nadsoniella nigra*, X-1 strain;

n = 10 in each group.

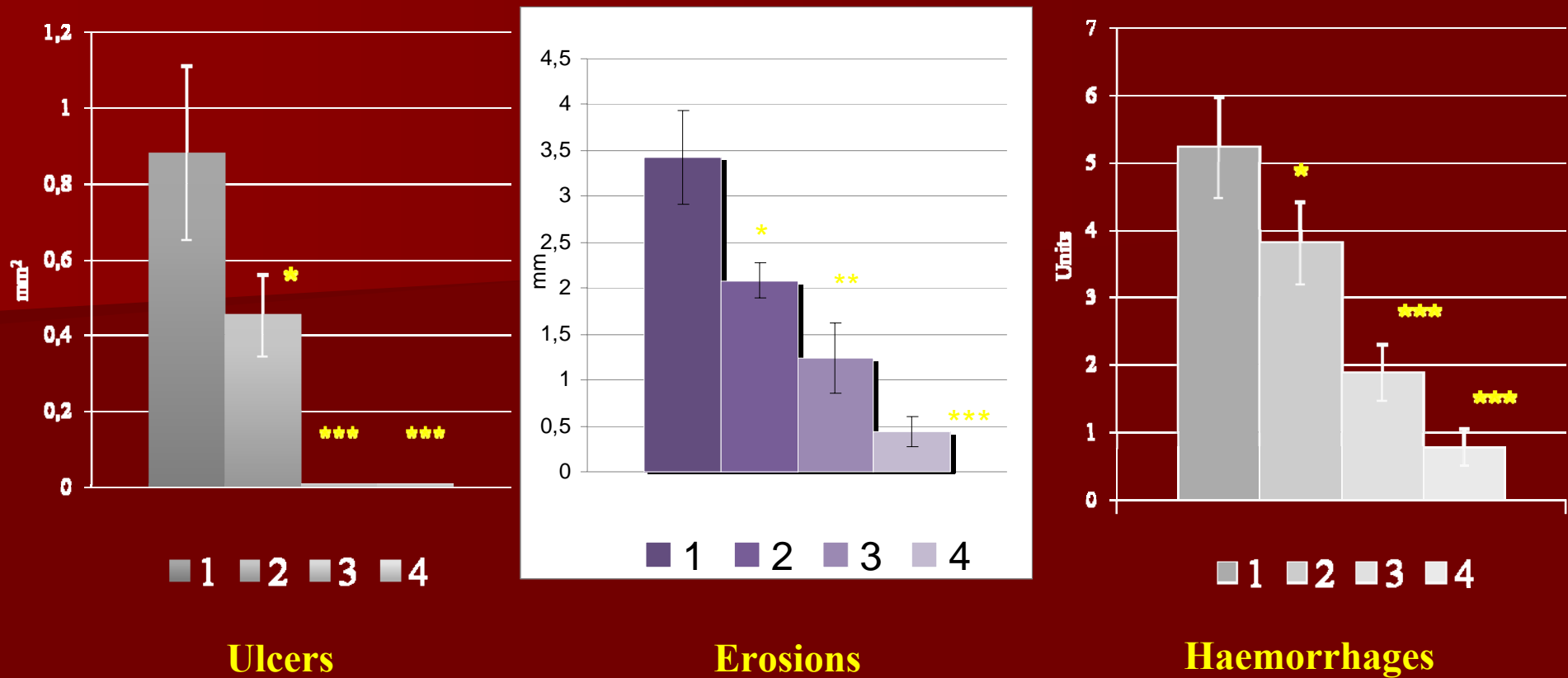


Fig. 2. Influence of melanin of various origins on gastric mucosal lesions in rats caused by "social" stress:

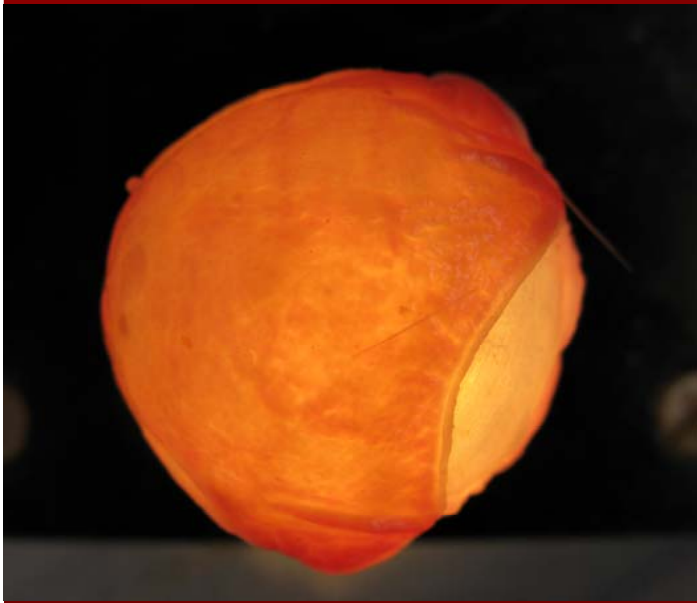
1 group, control (stress + vehicle);

2 group, stress + 5 mg/kg synthetic melanin "Sigma";

3 group, stress + 5 mg/kg melanin from *Nadsoniella nigra* var. *hesuelica*;

4 group, stress + 5 mg/kg melanin from *Nadsoniella nigra*, X-1 strain;

n = 10 in each group, (*M*±*m*). * - *p*<0,05; ** - *p*<0,01; *** - *p*<0,001 – versus to control



Control



stress



stress+
melanin

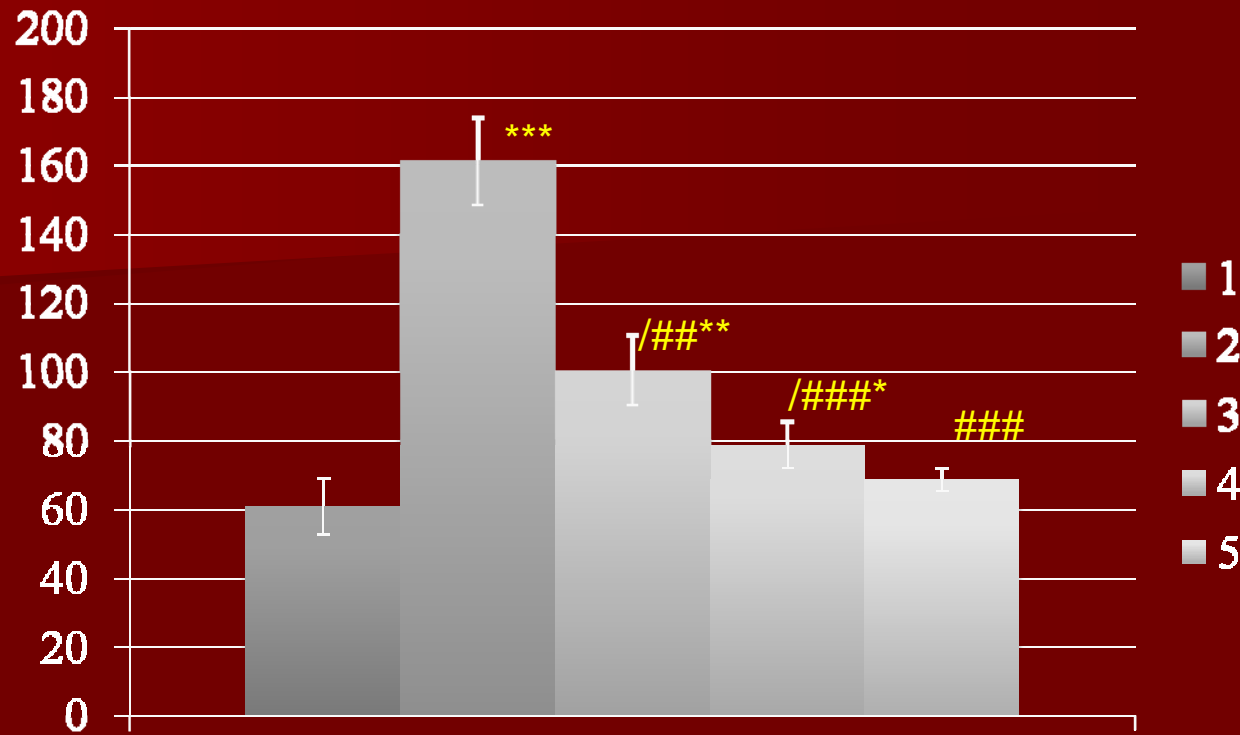


Fig. 3. Influence of melanin of various origins on blood MDA level (µmol/ml) in rats exposed to "social" stress:

1 group, control (+ vehicle);

2 group stress (+ vehicle);

3 group, stress + 5 mg/kg synthetic melanin "Sigma";

4 group, stress + 5 mg/kg melanin from *Nadsoniella nigra* var. *hesuelica*;

5 group, stress + 5 mg/kg melanin from *Nadsoniella nigra*, X-1 strain;

n = 10 in each group, (*M*±*m*). * - *p*<0,05; ** - *p*<0,01; *** - *p*<0,001 – versus to control. # - *p*<0,01; ### - *p*<0,001 – versus to stress.

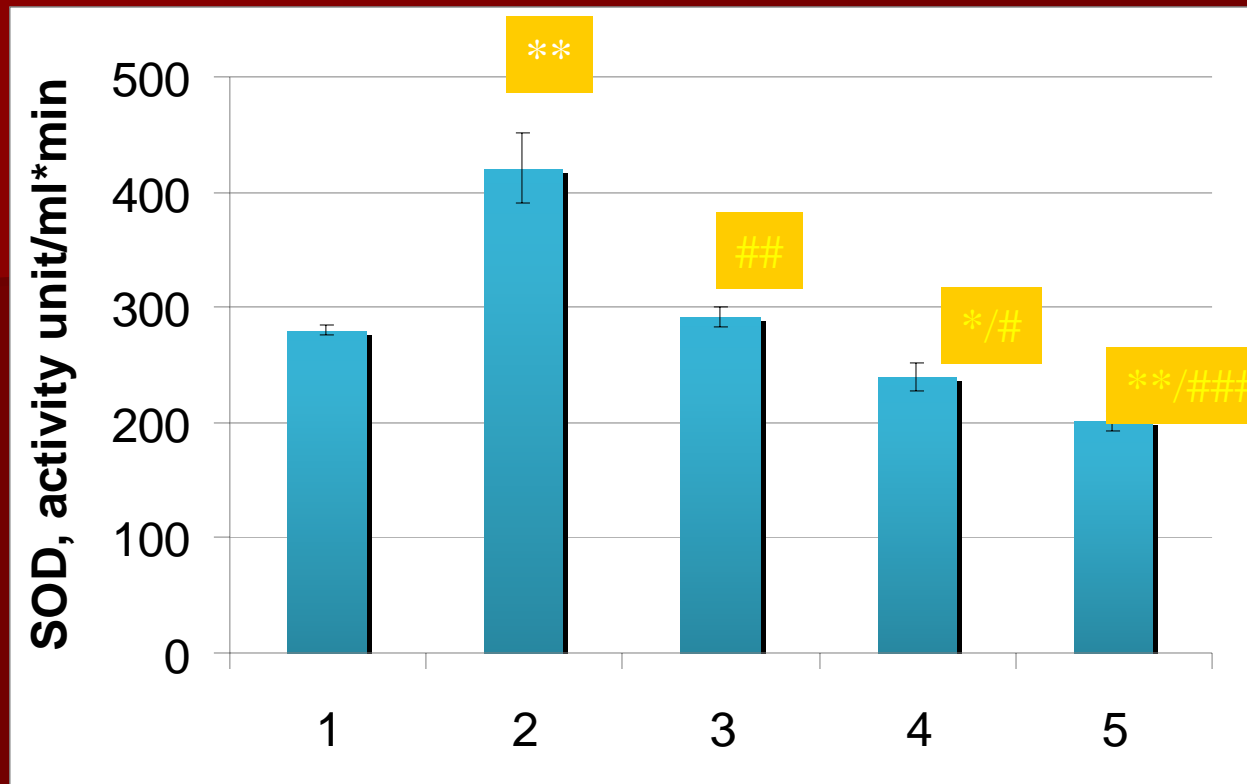


Fig. 4. Influence of melanin of various origins on SOD activity in the blood in rats exposed to "social" stress:

1 group, control (+ vehicle);

2 group stress (+ vehicle);

3 group, stress + 5 mg/kg synthetic melanin "Sigma";

4 group, stress + 5 mg/kg melanin from *Nadsoniella nigra* var. *hesuelica*;

5 group, stress + 5 mg/kg melanin from *Nadsoniella nigra*, X-1 strain;

n = 10 in each group, ($M \pm m$). * - $p < 0,05$; ** - $p < 0,01$; *** - $p < 0,001$ – versus to control.

- $p < 0,01$; ### - $p < 0,001$ – versus to stress.

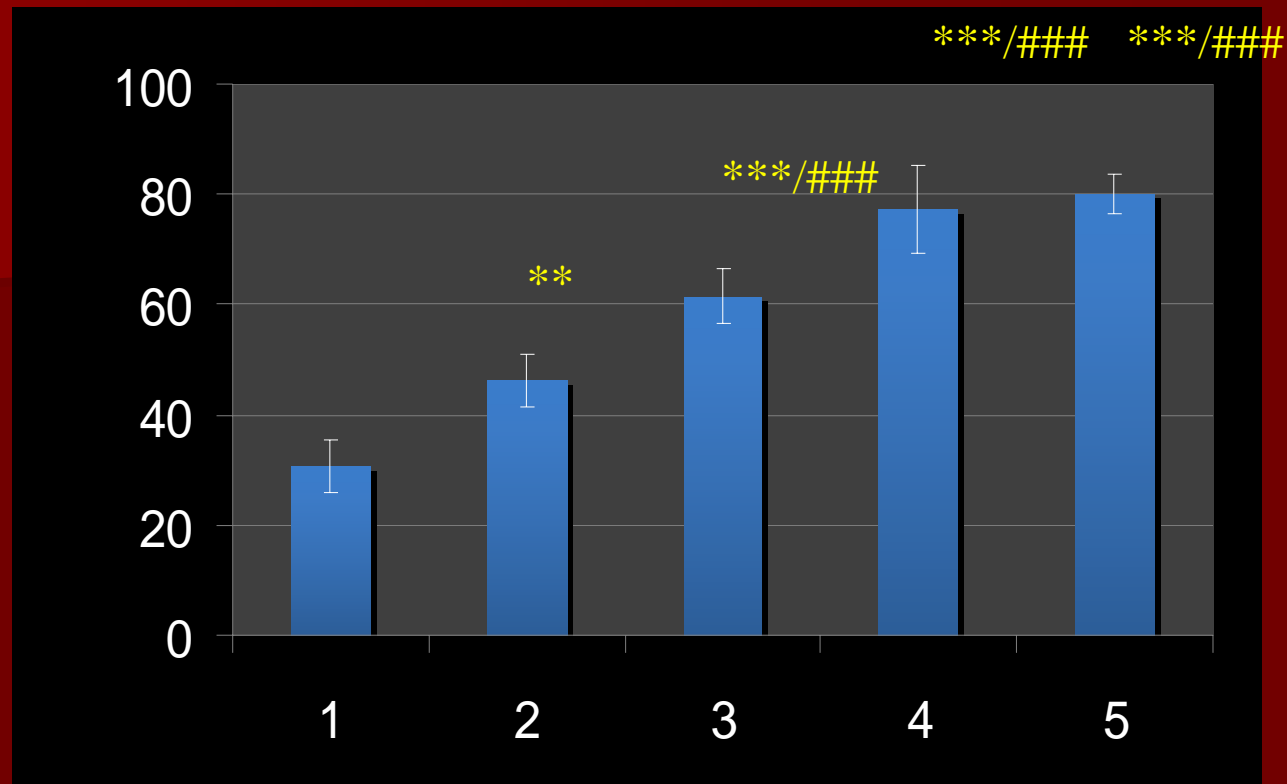


Fig. 5. Influence of melanin of various origins on catalase activity ($\mu\text{m H}_2\text{O}_2/\text{ml}\cdot\text{hour}$) in the blood of rats exposed to "social" stress:

1 group, control (+ vehicle);

2 group stress (+ vehicle);

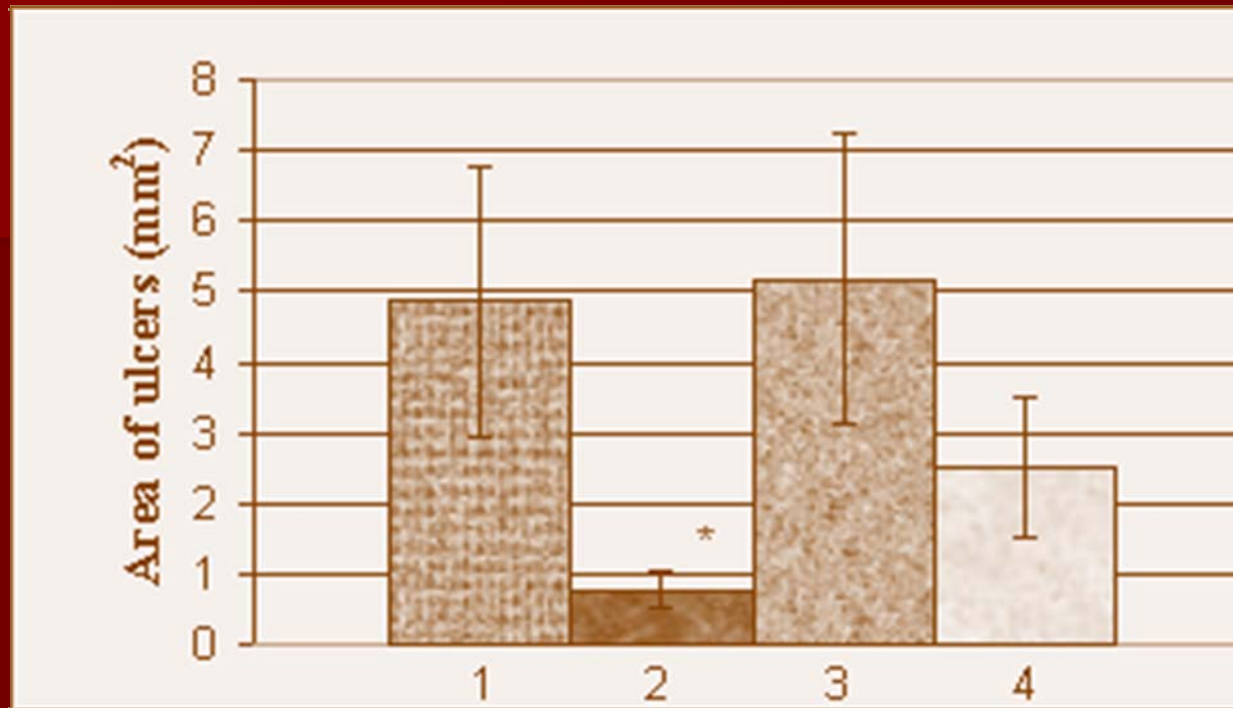
3 group, stress + 5 mg/kg synthetic melanin "Sigma";

4 group, stress + 5 mg/kg melanin from *Nadsoniella nigra* var. *hesuelica*;

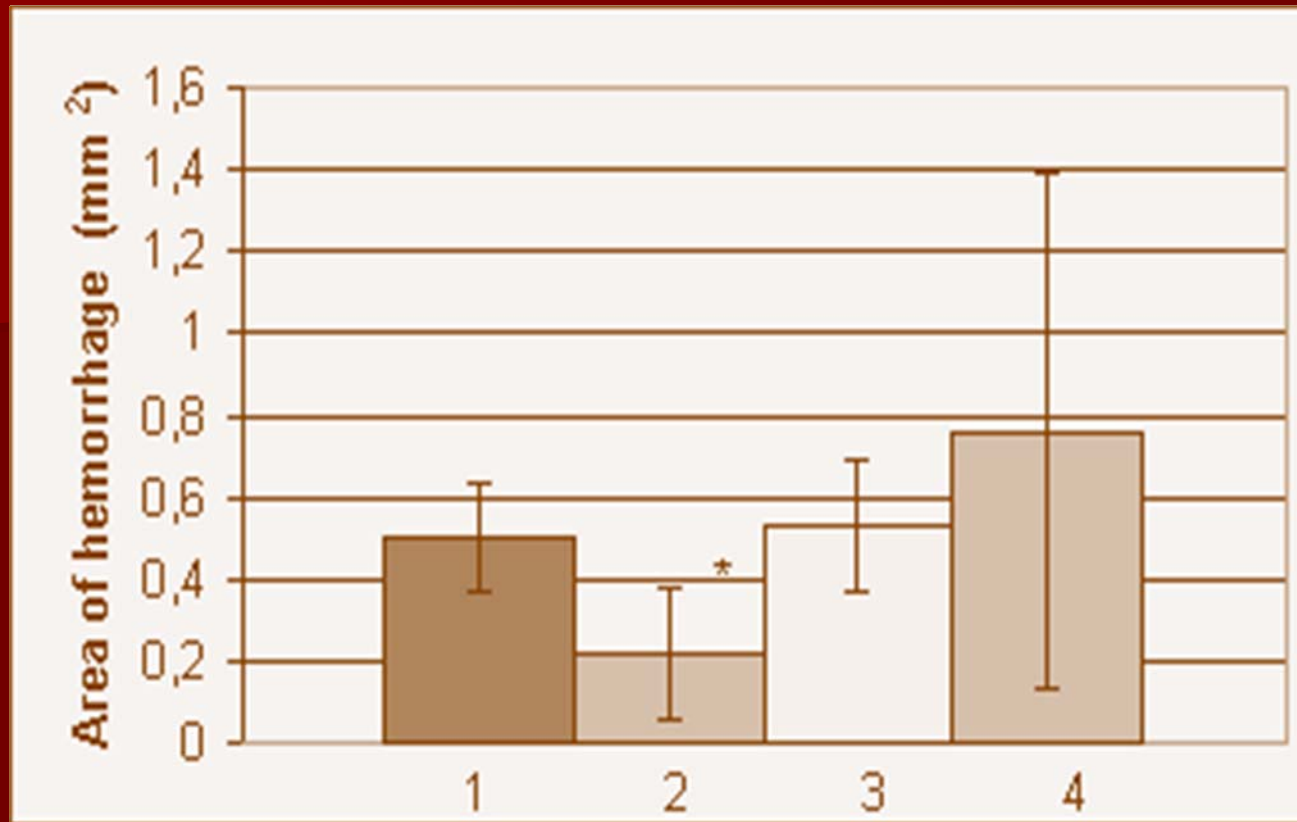
5 group, stress + 5 mg/kg melanin from *Nadsoniella nigra*, X-1 strain;

$n = 10$ in each group, ($M \pm m$). * - $p < 0,05$; ** - $p < 0,01$; *** - $p < 0,001$ – versus to control.

- $p < 0,01$; ### - $p < 0,001$ – versus to stress.



- **Fig 6. The influence of melanin (5 mg/kg, per os) on area of ulcers in stomach of rats evoked by stress in conditions of action of the blocker of PPAR_γ GW9662 (1 mg/kg, intraperitoneally), M±SD**
- 1. Stress (control), (n=15)
- 2. Stress + melanin, (n=16)
- 3. Stress + GW9662, (n=11)
- 4. Stress + melanin + GW9662, (n=13)
- * - p<0,05 – versus to control



- **Fig. 7. The influence of melanin (5 mg/kg, per os) on area of hemorrhages in stomach of rats evoked by stress in conditions of action of the blocker of PPAR_γ GW9662 (1 mg/kg, intraperitoneally), M±SD**
- **1. Stress (control), (n=15)**
- **2. Stress + melanin, (n=16)**
- **3. Stress + GW9662, (n=11)**
- **4. Stress + melanin + GW9662, (n=13)**
- *** - p<0,05 – versus to control**

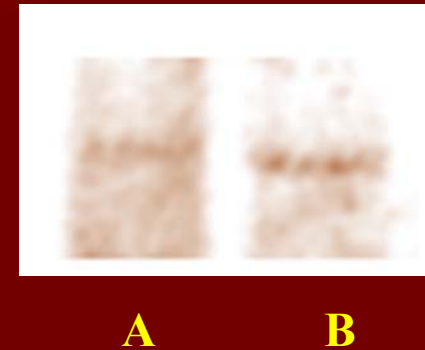
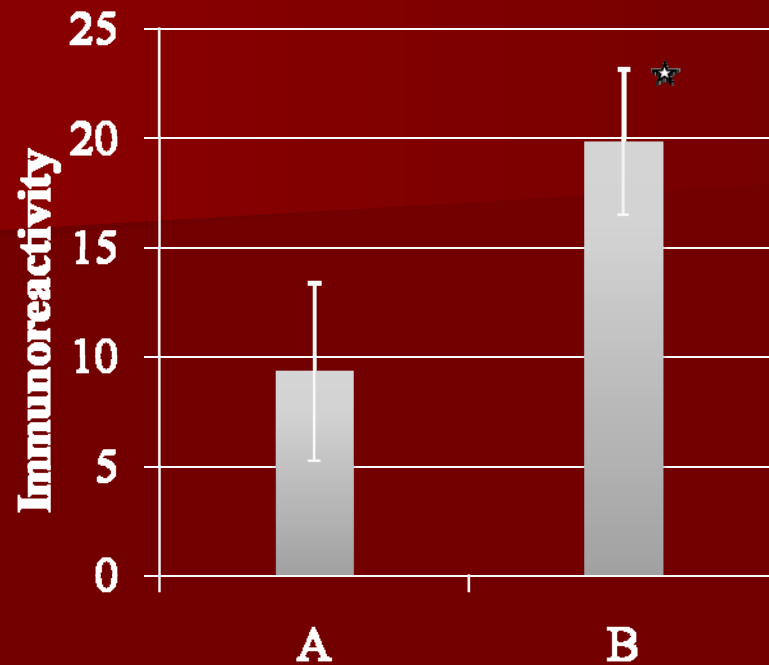
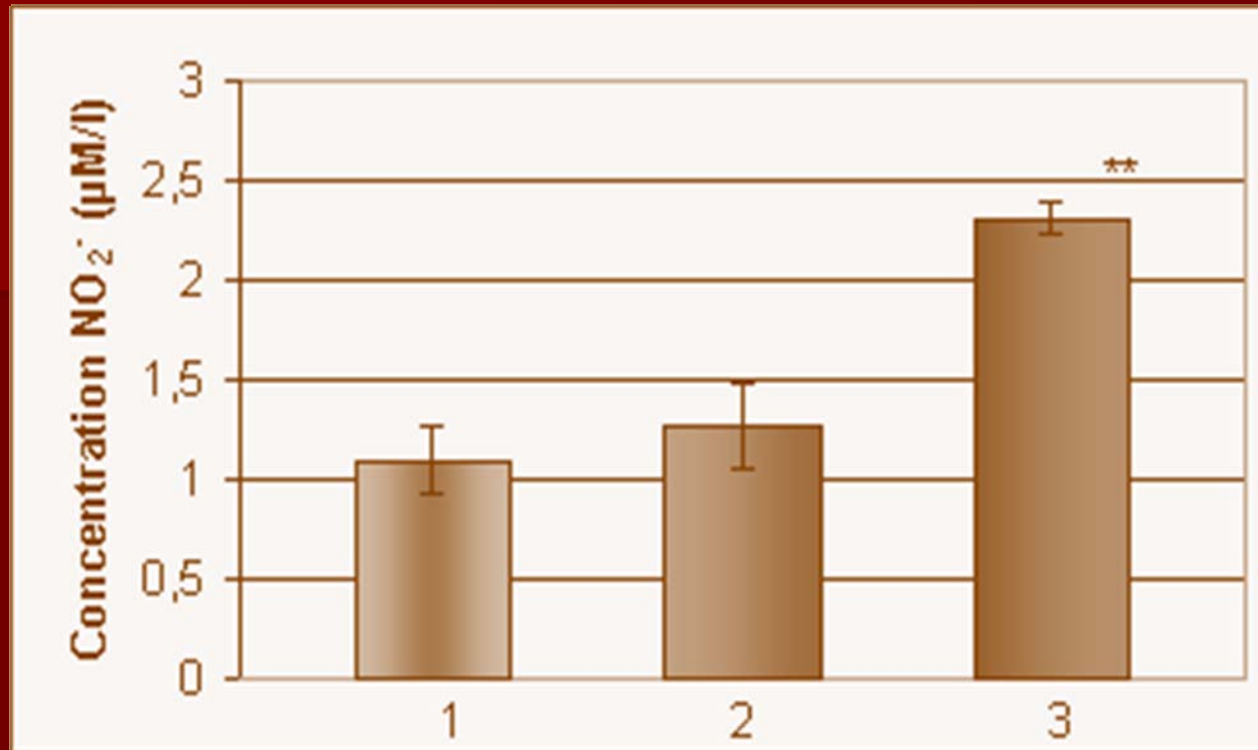


Fig.8. Immunoblots eNOS detection, prepared from whole cell lysates of rat stomach mucosa:

A – stress applied alone;

B – rats exposed to stress and pretreated with melanin (5 mg/kg i.g.).

* - $P < 0.05$, comparing to control



- **Fig.9. The influence of melanin (5 mg/kg, per os) on release of nitric oxide in the blood of rats after stress action, (M±m):**
- **1. control (n=5);**
- **2. stress (n=5);**
- **3. stress+melanin (n=5);**
- ****p<0,01 versus stress.**

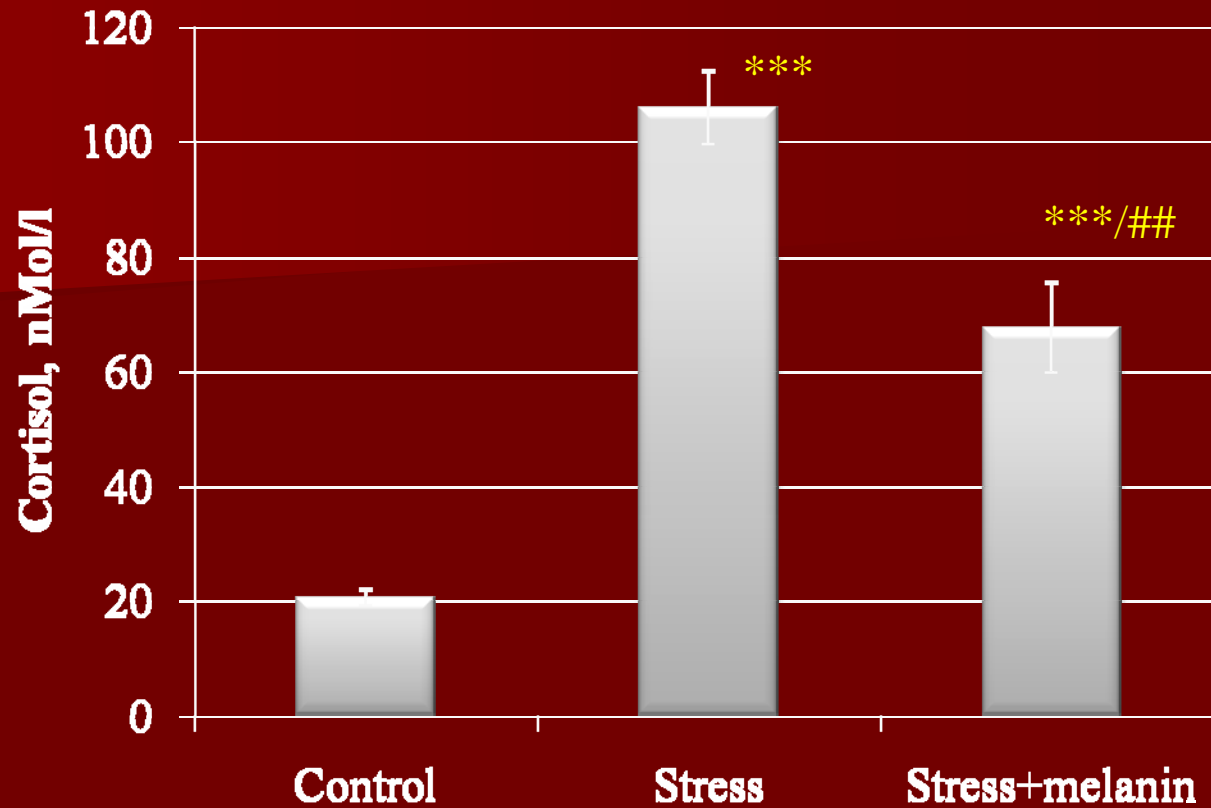


Fig.10. The influence of melanin (5 mg/kg, per os) on cortisol level in the blood of rats after stress action, (M±m):

- 1. control (n=6);
- 2. stress (n=6);
- 3. stress+melanin (n=6);
- ***p<0,001 versus control, ### p<0,01 versus stress.

CONCLUSION

- Melanin belongs to the drugs with antistress properties and antioxidant action.
- Potent gastroprotective activity of melanin against stress-induced lesions, like some other polyphenols, is mediated through PPAR γ and involves the increase in eNOS expression and NO generation.
- The other mechanism of antistress action of melanin includes the decrease of concentration in the blood of stress hormone cortisol.

Thank you for attention!



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