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## Correction Parameters of Endogenous Intoxication in Experimental Burn Disease at the Stage of Toxemia.

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### ABSTRACT

The effectiveness of melanin drug in the correction of endogenous intoxication indicators at experimental burn disease in toxemia phase was studied. The effectiveness of this drug in the correction of disorders in the endogenous intoxication system in cases of simulation of alkali burns in the esophagus of the 2nd degree at immature rats was established. The melanin demonstrates anti-toxic activity and is a promising tool for the prevention and treatment of burn effects.

**Keywords:** alkali esophageal burn, **syndrome of endogenous intoxication**, melanin

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## INTRODUCTION

Burns are one of the most topical and socially important problems of child injuries in Ukraine. It is tissue structures immaturity and imperfection of protective reactions at early age at children that are the causes of pathological post-burn disorders and lasting restoration of tissue structure [1, 6]. At burns the most of the victims die due to endogenous intoxication, which is one of the most important criteria that determine the severity of the human condition [2, 7].

Toxemia is the second stage of burn disease that begins after burn shock stage and lasts 8-9 days [1]. This is a difficult period, the treatment at this time is very diverse, however, the most current treatments are not always effective and timely. Even at minor chemical burns of the esophagus at children there is a high probability of risk for toxemia stage. At a clinical condition at toxemia the syndrome of endogenous intoxication (SEI) is on the first place that is due to the entry into blood of biologically active substances, toxins, microbes with characteristic phase flow - from elementary toxemia to endotoxemia [1]. Various origin toxic substances act at all levels of the body (systemic, organ, cellular and sub-cellular). Significant multiple pathological disturbances in the body take place - circulatory disorders, inhibition of the liver and kidney functions [2]. Most victims with burns die from endogenous intoxication, which is one of the most important criteria that determine the severity of the human condition. Most authors consider average weight molecules (AMW), oligopeptides (OP) and cytokines as a marker of endogenous intoxication [3]. Despite the large amount of research of these problems SEI subject matters upon conditions of chemical burns of the esophagus at children were not studied sufficiently, the most often mechanical transfer of regularities of burn disease at adults to the children clinic takes place.

In this relation, the possibility of using melanin drug at chemical burns is of great interest. It is a powerful antioxidant that prevents the degradation of collagen fibers and increases micro-circulation [4, 5]. The drug shows properties of gastro protector, melanin is used in the treatment of the effects of stress on the body [5].

Therefore, the aim of this study was to investigate markers of endogenous intoxication and some biochemical parameters in blood serum of immature rats upon the simulation of esophagus chemical burns of the 2nd degree when using melanin in various doses.

In our experiments we used nonlinear immature white rats (1 month) weighing 90-110g (according to 1-4 age children) in compliance with general ethical principles of experiments of animals, approved the first National Congress on Bioethics Ukraine and other international agreements and the national legislation in this area. The animals were kept on a standard vivarium diet. The animals were experimentally simulated with the alkali esophageal burn (AEB) with 20% (grade 2) solvent of NaOH [6]. The animals were divided into 3 groups: group 1st - AEB of 2<sup>nd</sup> grade, which was injected with melanin start from 2<sup>nd</sup> day of the experiment at a dose of 0,1 mg/kg for 14 days, group 2<sup>nd</sup> - AEB of 2<sup>nd</sup> grade, which was injected with melanin start from 2<sup>nd</sup> day of the experiment at a dose of 1mg/kg for 14 days, group 3<sup>rd</sup> - AEB of 2<sup>nd</sup> grade saline was injected in the appropriate dose and time. In our investigate used the melanin that produced yeast-like fungi *Nadsoniella nigra* strain X1 which obtained from cliffs Halindez island. The material selected for the research at 7<sup>th</sup> day, according to toxemia stage of burn disease [1]. The method of removing animals from the experiment was cervical dislocation. Biochemical parameters were measured in serum, which receiving of blood centrifugation at 2000g × 40 min. Indexes of the blood (total protein, albumin, AST, ALT, urea, creatinine, creatinine kinase, K<sup>+</sup>, Na<sup>+</sup>, Cl<sup>-</sup>, Ca<sup>2+</sup>) were determined by biochemical analyzer Humalyser 3000 using respective sets. Level of average weight molecules were measured by method Gabrielian with modifications [8]. Number of oligopeptides was estimated by the level of protein in the supernatant using the method of Bredford. Serum cytokines level (IL-4 and IL-10) were analyzed by ELISA using sets of reagents Biotrack ELISA System company «Healthcare» following the instructions. The statistical analyses of the obtained results was performed using the methods of variation statistics and correlation analysis using the computer program Excel. To determine the reliability of the differences between the two samples we used the Student test (t). Whereby differences P < 0.05 were deemed reliable.

The content of the total protein and albumin in blood serum of rats upon the simulation of AEB of the 2<sup>nd</sup> degree was investigated, which decreased by 36.7 % and by 37 % compared with control the values, respectively. Biochemical indicators of AST, ALT were investigated, which may characterize functional changes

in the liver and these indicators show growth by 24 % and 102 % respectively on the 7<sup>th</sup> day of the experiment. Upon condition of the administration of antioxidant drug melanin in different dosages during simulation of AEB the following changes take place: the indicator of the total protein at melanin administration with dose 0.1 mg/kg and 1 mg/kg increased by 51.7 % and 54.1 %, the albumin concentration increased by 22.5 % and 25 %, AST enzyme activity decreased by 10,8% and 19,5%, ALT decreased by 18% and 33,4%, respectively, compared with those in rats with AEB that have not received the drug (table 1).

During the study of blood serum of immature rats upon the simulation of chemical burns in the esophagus of the 2<sup>nd</sup> degree at toxemia stage we observed significant violations of nitrogen metabolism. There was increase of such indicators as urea, creatinine, creatinine kinase by 28,2 %, 65 %, 83 %, respectively, compared with the control values. When melanin administration in doses of 0,1mg/kg and 1mg/kg urea concentration decreased by 13% and 24,4%, creatinine concentration decreased by 34 % and 36 %, creatinine kinase activity decreased by 18 % and 14 % respectively (table 1).

**Table 1. Biochemical parameters of blood serum of rats under experimental AEB grade 2<sup>nd</sup> in the stage of toxemia under conditions administration of melanin (M±n, n=10)**

Index	Control	AEB	AEB+melanin 0,1mg/1kg	AEB+melanin 1 mg/1kg
Total protein, (g/L)	65,01±0,1	41,2±1*	62,5±0,56*.#	63,5±0,89*.#
Albumin, (g/L)	37,5±2,5	23,6±1,12*	28,9±2,4*.#	29,5±1,5*.#
AST, (U/L)	54,8±1,1	67,8±2,2*	60,5±1,56*.#	54,6±1,41#
ALT, (U/L)	37,5±0,1	75,8±0,98*	62,3±1,44*.#	50,5±1,34*.#
Urea, (µmol/L)	36,2±0,21	46,4±0,38*	40,3±1,8*.#	35,1±1,3#
Creatinine, (µmol/L)	88,0±3,9	145,01±2,1*	95,6±2,6*.#	93,4±1,3*.#
Creatininekinase (U/L)	191,4±0,9	350,8±1,35*	288,9±2,03*.#	303,6±0,22*.#
K <sup>+</sup> , (µmol/L)	5,0±0,3	8,0±0,5*	6,6±0,98*.#	6,1±0,88*.#
Na <sup>+</sup> , (µmol/L)	156,2±0,5	148±0,7*	150,9±1,6*.#	149,4±1,9*
Cl <sup>-</sup> , (mg/dl)	110±0,4	101,8±0,9*	105,6±1,6*.#	103,8±2,3*
Ca <sup>2+</sup> , (µmol/L)	10,6±0,1	8,6±0,9*	11,8±0,9*.#	10,9±0,4#

We have investigated that the level of potassium at AEB of the 2<sup>nd</sup> degree increased by 60 % compared with the control values, the administration of melanin in doses of 0.1 and 1 mg/kg resulted in a decrease of this index by 17.5 % and 25 %. The level of sodium, chloride and calcium decreased by 6 %, 8 % and 19 % respectively, compared with the control values upon the simulation of burn. With the administration of the melanin drug in doses of 0.1 and 1 mg/kg we observed a slight increase in the concentration of sodium and chloride, the concentration of Ca<sup>2+</sup> was increased by 37 % and 27 % respectively, compared with those in rats with AEB that did not receive the drug (table 1).

The study of burn of esophageal established elevated level of specific toxins such as oligopeptides by 34.8 % compared with the control values. Upon the administration of melanin in doses of 0.1 and 1 mg/kg the concentration of OP decreased by 42 % and 52 % respectively (table 2). The analysis of the made research showed that the AEB causes an increase in the level of AMW by 17 % compared with the control value. Upon the administration of the research drug doses 0.1 mg/kg and 1 mg/kg AMW content decreased by 22 % and 26 % respectively, compared with those in rats with burns who did not receive the drug (table 2). We found that upon the simulation of AEB of the 2<sup>nd</sup> degree on the 7<sup>th</sup> day a slight increase in the level of IL-10 occurred. The administration of melanin slightly affected the results. (table 2). During our research it was found that upon the simulation of LEB of the 2<sup>nd</sup> degree there is a significant reduction in the level of IL-4 by 11 % compared with the control value (table 2). With the administration of the drug in doses of 0.1 mg/kg and 1 mg/kg IL-4 level increased by 6 % and 9 %, respectively, compared with the rats with AEB.

**Table 2. Level of markers endogenous intoxication in blood serum of rats under experimental AEB grade 2<sup>nd</sup> in the stage of toxemia under conditions administration of melanin (M±n, n=10)**

Index	Control	AEB	AEB+melanin 0,1mg/1kg	AEB+melanin 1mg/1kg
Oligopeptides, (mg/ml)	0,23±0,02	0,31±0,05*	0,18±0,02*.#	0,15±0,02*.#
AMW (standard unit)	0,24±0,01	0,28±0,01*	0,22±0,02#	0,21±0,01**
IL-4 (standard unit)	0,089±0,001	0,079±0,001*	0,084±0,002*.#	0,086±0,001**
IL-10 (standard unit)	0,078±0,006	0,073±0,0028	0,076±0,005	0,074±0,001*

The analysis of the research showed that the AEB of the 2<sup>nd</sup> degree results in burn disease development that results in a violation of metabolism, the drug administration helped the normalization of certain metabolic processes.

In the study of blood serum of immature rats upon the simulation of chemical burns in the esophagus of the 2<sup>nd</sup> degree at toxemia stage we observed significant violations of nitrogen metabolism. There was increase of such indicators as urea, creatinine, creatinine kinase compared with the control values at the administration of melanin the indicator decrease was observed. The resulting changes indicate normalization of nitrogen metabolism [4].

There was discovered violation of water-electrolyte balance, which is characterized by significant extra-renal fluid LEB. The burn disease is characterized by increasing levels of potassium in the bloodstream [9, 10]. We have investigated that the level of potassium in AEB of the 2<sup>nd</sup> degree increased compared with the control values, the administration of melanin in doses of 0.1 and 1 mg/kg resulted in the decrease of this indicator. Sodium maintains the existing balance between constantly ion electrolytes, with water it enters the cells causing edema, hyper-hydration comes first in cell area of the burn and then outside. At the same time moving of sodium ions in the extracellular space of the lesion causing a large amount of fluid accumulation increased swelling and movement of sodium ions in intact cells [1, 9, 11]. All this leads to a reduction in sodium content, which we showed. The level of sodium, chloride and calcium decreased compared with the control values upon the burn simulation, the melanin administration in doses of 0.1 and 1 mg/ kg caused increase of the indicators. The established changes upon the simulation of AEB of the 2<sup>nd</sup> degree at toxemia stage show considerable violations of water-salt, nitrogen metabolism and biochemical indicators of liver function, the administration of melanin contributed to the normalization of these indicators.

At intoxication toxins are distinguish by different nature: specific, nonspecific, mediators of immune responses and others. Specific toxins that come from burn wounds are in their origin denatured proteins, fragments of protein molecules and enzymes complexes with their inhibitors [1, 2, 12]. The study of burn of esophageal established elevated levels of specific toxins such as oligopeptides, the administration of melanin helped to reduce these indicators.

Nonspecific toxins include AMW formed by proteolytic cleavage of plasma proteins, biogenic amines, kinin system components, prostaglandins, lipid endoperoxides, hydrolases of cell origin and others. The level of these substances is screening of azotemia, an indicator of the degree of intoxication, uremia, and generally characterizes renal function condition [1, 13]. The analysis of the fulfilled research showed that the AEB causes an increase in the level of AMW, upon the administration of the experimental drug the reduction of the level occurred.

Mediators of immune responses include anti-inflammatory interleukins - IL-4, IL-10, they are antagonists of inflammatory cytokines and have the ability to inhibit their biological activity, in particular they inhibit the proliferation and T-cell response to antigens, block the production of IL-1, TNF- $\alpha$ , nitric oxide and prostaglandin, thus reducing inflammatory manifestations [14]. We found that upon the simulation of AEB of

the 2<sup>nd</sup> degree a slight increase in the level of IL-10 occurred. The administration of melanin slightly affected the results.

The researchers include increased concentration of IL-6 [15] in criteria for adverse outcome at sepsis, one of the factors increasing IL-6 at burn injury is reduction of the concentration of IL-4, so the level of IL-4 is also considered as a prognostic factor of complications [12, 16, 17]. During our research it was found that upon the simulation of AEB of the 2<sup>nd</sup> degree is a significant reduction in the level of IL-4 compared with the control values, the administration of the drug in doses boosted the level of IL-4. The received data show that the administration of the drug reduces the risk of septic complications.

We have shown that toxic substances of various origin act at all levels of the body. In the simulation of esophageal alkali burns of the 2<sup>nd</sup> degree at rats multiple pathological disturbances in the body occur. Upon the administration of melanin we observed normalization of metabolic processes, indicators of water-salt and nitrogen metabolism.

Thus, anti-toxic mechanisms affect the severity of SEI manifestation. At the normal operation of the protective mechanisms the body is able to resist toxins. In our studies it was shown that immature rats upon the simulation of AEB of the 2<sup>nd</sup> degree protective mechanisms (anti-toxic and regulatory system) cannot cope with it and in the body there is increase in the content of endogenous toxins that against the background of the dysfunction of the immune system leads to lower body resistance. The administration of melanin is effective in correction of endogenous intoxication indicators at experimental burn disease at the toxemia stage. So melanin manifests antitoxin properties and is a promising tool to prevent and treat the effects of burn disease.

It was studied that at simulation of esophageal alkali burns of the 2<sup>nd</sup> degree at immature rats syndrome of endogenous intoxication occurred. The increase of toxins of different nature: specific, non-specific and mediators of immune responses those are associated with a high risk of septic complications after burns. It was established that toxic substances of various origin act at all levels of the body. In our studies it was shown that melanin upon the simulation of AEB of the 2<sup>nd</sup> degree enhances the protective mechanisms, which contributed to reduction of endogenous toxins, resulting in the body resistance increase. It was established that the administration of melanin is effective in correction of endogenous intoxication indicators at experimental burn disease at the toxemia stage.

**Abbreviation:** AEB – alkali esophageal burn; AMW - average weight molecules; OP – oligopeptides; SEI - syndrome endogenous intoxication; TNF- $\alpha$  - tumor necrosis factor

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