

Oxidative phosphorylation of rat liver mitochondria with intoxication of haloxyfop-r-methyl and indoxacarb pesticides

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Abstract. In this work, the effect of sophoroflavonoid (SFL) and flavonoids of narcissus on respiration and oxidative phosphorylation processes of liver mitochondria of rats poisoned by haloxyfop-R-methyl and indoxacarb pesticides was studied in in vivo experiments depending on the dynamics of 10, 20, 30, 40 days. Experimental animals were divided into IV model groups according to intoxication with haloxyfop-R-methyl and indoxacarb pesticides and their correction with flavonoids. According to the results obtained, it was found that in the presence of succinate substrate, the respiration rate of liver mitochondria of group II pesticide-poisoned animals decreased in the V_3 state compared to the control (group I), and the respiration rate increased in the V_4 state. Respiratory control index and ADF/O value were found to decrease. When group III animals poisoned by pesticides were treated with SFL (10 mg/kg) and group IV animals with narcissin flavonoids (10 mg/kg) once a day for 10 days, it was found that the metabolic processes of liver mitochondria recovered by 40% days.

1 Introduction

Nowadays, various pesticides are used in agriculture in the countries of the world to achieve high and efficient productivity. However, these chemical compounds have a negative impact on the environment and living organisms, causing biochemical and physiological changes in cells and tissues [1]. Improper use of pesticides in agriculture and increase in the authorized rate leads to the accumulation of sutemizuchi in the form of residues in liver tissues [2]. Also toxic hepatitis begins to develop as a result of pesticide exposure on liver hepatocytes. Liver mitochondria are a target for pesticides. Under the influence of pesticides, the energy status of liver mitochondria decreases and the activity of respiratory chain enzymes decreases. The study of the processes of metabolism and ATP synthesis of liver mitochondria in toxic hepatitis is an urgent problem. At the same time, during activation of compensatory

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mechanisms of the cell in response to harmful effects of toxic substances, it is relevant to study the state of the mitochondrial respiratory chain in the initial period of the pathological state development. Such information will help to develop adequate approaches to the treatment of toxic hepatitis. As a result of pathologic processes that develop in the liver under direct exposure to toxins, oxidative stress is manifested [3]. Oxidative stress leads to the formation of reactive oxygen species (ROS) and oxidation of molecular components of the cell [4]. In many pathological processes, electron transport along the respiratory chain is altered as a result of impaired hydrogen ion transport of liver mitochondrial membranes. As a result, the gradient of hydrogen ions in mitochondria is disturbed, membrane potential is reduced, which causes separation of OF processes and formation of free radicals. Many flavonoid compounds increase the activity of ATP synthetase by reducing the formation of free radicals from the mitochondrial respiratory chain. In studies conducted on many types of pesticides, a partial separation of the OF process in mitochondria is given [5]. Many types of polyphenols and flavonoids that reduce the toxic effect of pesticides on the respiratory chain of mitochondria have been identified. However, only some of their biological activities have been studied. The corrective effect of SFL and flavonoids of narcissus on disorders of mitochondrial energy metabolism under conditions of pesticide poisoning by haloxyfop-R-methyl and indoxacarb is practically not studied. To this end, in this work we studied in vivo experiments the corrective effect of SFL and flavonoids of narcissus on pesticide-induced mitochondrial respiration of rat liver and dysfunction of the OF process.

2 Materials and methods

2.1 Pesticide intoxication and pharmacotherapy

When determining the toxicity of pesticides, their lethal doses (LD50) are taken on the basis of milligrams and grams depending on the live weight of the animal [6]. Haloxyfop-R-methyl and indoxacarb, chosen for the experiment, are among the less toxic pesticides with low toxicity. As their LD50 dose is 623 mg/kg for haloxyfop-R-methyl and 5000 mg/kg for indoxacarb (www.pesticidy.ru>active_substance>haloxyfop-R-methyl; www.pesticidy.ru>active_substance>indoxacarb) [8-16].

Experimental animals were divided into separate model groups for pesticide intoxication with haloxyfop-Rmethyl and indoxacarb and their correction with flavonoids. Initially, male rats selected for haloxyfop-R-methyl pesticide intoxication were divided into groups:

- Group I healthy (control) (n=5);
- Group II haloxyfop-R-methyl (n=5-6);
- Group III haloxyfop-R-methyl+SFL (n=5-6);
- Group IV haloxyfop-R-methyl+narcissin (n=5-6):

Animals in groups II, III and IV of the experiment were poisoned with a single LD50 dose of 1/10 haloxyfop-R-methyl pesticide through a special probe. After administration of haloxyfop-R-methyl pesticide, SFL at a dose of 10 mg/kg of group III and flavonoid narcissin at a dose of 10 mg/kg were administered orally to animals for 10 days.

Rats poisoned with indoxacarb pesticide were also divided into groups;

- Group I of healthy (control) (n=5);
- Group II indoxacarb (n=5-6);
- Group III indoxacarb + SFL (n=5-6);
- Group IV indoxacarb + narcissin (n=5-6):

Animals of groups II, III and IV of the experiment were poisoned once with a dose of 1/10 LD50 through a special probe with indoxacarb pesticide. After administration of indoxacarb pesticide, experimental group III was administered SFL at a dose of 10 mg/kg

and group IV was administered flavonoid narcissin at a dose of 10.0 mg/kg orally once daily for 10 days. 10, 20, 30 and 40 days after administration of SFL and narcissus flavonoids to pesticide-poisoned rats, the recovery of metabolic processes of liver mitochondria was studied.

2.2 Mitochondria isolation from rat liver

Rat liver mitochondria were isolated by the method of differential centrifugation by W.C.Schneider [7]. Rats were put to sleep by decapitation, liver was removed and placed in buffer solution with pH 7.4 (sucrose-250 mM, Tris-HCl-10 mM, EDTA-1 mM). The liver tissue was weighed and passed through a micropress with 1 mm diameter holes for pulverization. The pulverized liver tissue was added to the cooled buffer solution at a 1:6 ratio and homogenized using a dedicated homogenizer (HG-15A). The obtained homogenate was centrifuged (temperature 0-2°C) and the centrifugation process was carried out in 2 stages. In the first stage, centrifugation took 7-8 minutes at 1500 g. Cellular debris and nuclear fractions were precipitated. In the next stage, the supernatant was poured into a centrifuge tube and centrifuged at 6000 g for 15 minutes. The supernatant was separated and the precipitated mitochondria were washed with EDTA-free buffer solution.

2.3 Identification of mitochondrial respiration and oxidative phosphorylation

The frequency of mitochondrial respiration and OF were determined by polarography (Strathkelvin 782 2-Channel oxygenometer, Germany). IM composition: 100 mM sucrose, 75 mM KCl, 2.5 mM KH_2PO_4 , 10 mM Tris-HCl and 10 mM succinate oxidation substrates were added at pH-7.4. Mitochondrial respiration frequency (V_3 / V_4) was determined according to the Chance method (Chance B. et al.1955). In addition, the ADP/O (P/O) ratio was used to evaluate the efficiency of mitochondrial ATP synthesis.

V_2 or V substrate is the respiration rate of mitochondria in the presence of substrates (in the absence of ADP);

V_3 - metabolic state activated by the addition of ADP;

V_4 - resting state, i.e., the respiration rate of mitochondria in the presence of substrates, the last state after all this added ADP has been converted to ATP;

Maximum respiration rate, respiratory control (RC) and ADP/O status (relative to 200 μM ADP) were calculated in the presence of V_{DNF} -oxidation and phosphorylation.

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The ratio of the amount of oxygen consumed by the time of complete synthesis of all ADP in a polarograph cell containing mitochondria to the IM in ATP was measured. The amount of oxygen in 0.5 ml of incubation medium was 250 ng atoms. Experiments were performed at 25°C under constant stirring, and mitochondria were added at a concentration of 1 mg/ml protein. When succinate was used as an oxidation substrate, rotenone was added to the medium to prevent the accumulation of oxaloacetate, a competitive inhibitor of succinate oxidation.

2.4 Statistical processing of the obtained results

Origin 6.1 (Microsoft, USA) was carried out using a computer program. In experiments, mitochondrial metabolism was carried out as a percentage of the maximum, by way of calculating the arithmetic mean of 5-6 different experiments. The difference between the values of control, pesticide poisoning and pesticide+experiment was calculated using t-criterion. Where values * $P < 0.05$ and ** $P < 0.01$ represent statistical significance.

3 Results, discussion and conclusion

According to the results obtained, the respiration rate of liver mitochondria of group II haloxyfop-R-methyl poisoned liver in the presence of succinate substrate in V₃ state compared to control (group I) was 126.1±6.4% at 10, 20, 20, 30 and 40 days, respectively. 142.1±6.8%; It was found to be decreased by 107.0±6.2% and 25.7±2.2% (Table 1).

Table 1. Dynamic effects of SFL and narcissin flavonoids on respiration and OF process of liver mitochondria of rats poisoned with haloxyfop-R-methyl after 10, 20, 30 and 40 days.

Days	Experimental groups	Breathing rate, %				RC	ADP/O
		V ₂	V ₃	V ₄	DNF		
10	Control	100±3,4	449,4±25,8	93,7±4,2	428,2±20,5	4,8±0,31	1,78±0,18
	Haloxypop-R-methyl	100±5,6	323,3±15,1**	153,3±9,3**	394,0±23,7**	2,1±0,20*	1,12±0,10*
	Haloxypop-R-methyl +SFL	100±4,1	405,4±14,1*	126,3±6,8*	610,2±38,2**	3,2±0,31*	1,47±0,17*
	Haloxypop-R-methyl +Narcissin	100±6,4	412,3±25,4**	108,6±7,9	527,7±23,7**	3,8±0,27*	1,59±0,18*
20	Control	100±5,3	470,8±26,8	100±6,3	431,6±28,0	4,7±0,29	1,82±0,19
	Haloxypop-R-methyl	100±4,9	328,7±23,1**	182,7±8,2**	288,0±22,1**	1,80±0,15**	1,22±0,13*
	Haloxypop-R-methyl +SFL	100±6,5	372,7±34,6*	98,9±6,7	330,6±24,1**	3,76±0,27*	1,54±0,15*
	Haloxypop-R-methyl +Narcissin	100±5,4	363,0±20,5*	94,8±5,3	371,1±24,9*	3,83±0,30*	1,65±0,18*
30	Control	100±6,1	449,4±30,4	89,0±7,2	425,6±36,5	5,02±0,41	1,85±0,18
	Haloxypop-R-methyl(II)	100±4,9*	342,4±23,9**	168,5±11,5**	316,9±22,9**	2,03±0,15**	1,19±0,14*
	Haloxypop-R-methyl +SFL	100±5,2	431,5±34,7*	112,5±9,2	380,3±24,1*	3,82±0,22*	1,42±0,16*
	Haloxypop-R-methyl +Narcissin	100±6,7	342,4±18,5*	98,3±9,6	374,7±5,9*	3,91±0,28*	1,68±0,20*
40	Control	100±5,7	444,8±26,3	88,6±5,5	427,2±25,8	5,06±0,44	1,76±0,19
	Haloxypop-R-methyl	100±3,6*	419,1±33,2*	141,0±9,1*	366,4±21,8*	2,97±0,18**	1,32±0,16*
	Haloxypop-R-methyl +SFL	100±6,7	433,9±26,8*	109,8±7,8	401,1±23,2**	3,95±0,28*	1,53±0,16*
	Haloxypop-R-methyl +Narcissin	100±6,6*	439,7±26,2**	110,6±9,9	410,0±26,8**	3,98±0,25*	1,66±0,17*

Note: (In all cases *P<0.05; **P<0.01, confidence levels of indicators of groups III and IV are obtained relative to group II; n=4-5).

The respiration frequency in the V₄ state was 59.6±4.1% respectively; 82.7±8.2%; It was found to increase by 79.5±4.1% and 52.4±3.1%. Haloxypop-R-methyl caused a dramatic increase in mitochondrial respiration rate in V₄ state, in turn by 56.2±4.1% of RC by Chance at 10, 20, 30 and 40 days, respectively; 61.7±4.8%; 59.5±3.7% and 41.3±3.8% and ADP/O value 37.7±2.1%; 33.0±2.9%; It was found to decrease by 35.7±2.1% and 25.0±2.1% (Table 1).

During 10-40 days in the presence of DNF dissociated from OF, the respiration rate of mitochondria with succinate substrate decreased compared with the control in the presence of haloxyfop-R-methyl. There was a recovery of indices of groups III and IV, which received pharmacotherapy with SFL and narcissus flavonoids, compared to the pathological group. In the state of haloxyfop-R-methyl pesticide poisoning, the decrease of RC and ADP/O values

of liver mitochondria in comparison with the control indicates the separation of their OF processes.

When group III animals poisoned with haloxyfop-R-methyl were treated with SFL (10 mg/kg) and group IV animals with flavonoids narcissin (10 mg/kg) once a day for 10 days, metabolic processes of liver mitochondria were restored by day 40. At the same time, a dynamic decrease in mitochondrial respiration rate was observed in the presence of SFL and narcissin after 10-40 days in the presence of V₃. At the 40th day, compared with group II, SFL and narcissin increased by 14.0±1.4% and 20.6±2.1%, respectively, and 31.2±1.9% and 30.4±2.4 in V₄. They were found to decrease by %. Liver mitochondria of animals of groups III and IV were restored by 32.9±2.5% and 34.0±2.7% in comparison with group II under the influence of SFL and narcissine on the 40th day of RC, and the value of ADP/O was also 15.6±1.0 Increased by 1% and 25.7±1.6%. In the experiment performed with DNF, under the influence of SFL and narcissine, the mitochondrial process of OF was partially restored by the 40th day of RC (Table 1).

In our next experiment, when another pesticide was used together with indoxacarb, the following results were recorded. According to the results obtained, in the presence of succinate substrate, the respiration rate of liver mitochondria of group II rats receiving indoxacarb was 136.1±8.2% compared to the control (group I) on the 10th, 20th, 30th and 10th day 40 respectively; 179.9±15.5%; It was found to be decreased by 141.1±10.2% and 114.4±8.4% (Table 2). ATP synthesis was completed, i.e., respiration rate in V₄ state was 70.9±3.8% respectively; 62.6±4.4%; It was found to increase by 77.9±4.8% and 30.5±2.1%. The sharp increase in mitochondrial respiration rate in V₄ state under the influence of indoxacarb, in turn, increased the RC by Chance at 10, 20, 30 and 40 days by 59.7±4.4%; 64.2±5.1%; 39.9±3.3% and 42.2±3.5%, respectively, ADP/O value 37.5±2.1%; 30.9±2.0%; It was found to decrease by 32.1±1.9% and 23.4±1.7% (Table 2).

In the presence of DNF in IM after 10-40 days, a decrease in mitochondrial respiration rate in the presence of succinate substrate was observed compared to the control. When SFL pharmacotherapy was administered to group III animals poisoned with indoxacarb and group IV animals with narcissin flavonoids once a day for 10 days, an increase in mitochondrial respiration rate in the V₃ state was observed depending on the dynamics after 10-40 days. The respiration rate of mitochondria isolated from 40-day liver of animals of III and IV corrected group increased by 45,6±3,8% and 92,5±5,6%, respectively, and respiration in V₄ state under the action of SFL and narcissin in comparison with group II was found to decrease the acquisition rate by 19,9±1,1% and 10,2±0,8% (Table 2).

Table 2. Dynamically dependent effects of SFL and flavonoids of narcissin on respiration and oxidative phosphorylation of liver mitochondria of indoxacarb-poisoned rats after 10, 20, 30 and 40 days.

Days	Experimental groups	Breathing rate, %				RC	ADP/O
		V ₂	V ₃	V ₄	DNF		
10	Control (I)	100±5,6	466,5±29,1	92,7±5,8	431,8±27,8	5,03±0,38	1,84±0,17
	Indoxacarb (II)	100±6,1	330,4±22,7* *	163,6±8,8* *	323,8±18,7*	2,02±0,14*	1,15±0,15*
	Indoxacarb + SFL (III)	100±5,8	378,8±25,2*	121,8±7,5*	364,2±25,5	3,11±0,21*	1,51±0,17*
	Indoxacarb +Narcissin (IV)	100±5,4	403,7±26,5*	116,7±8,5*	377,1±26,7*	3,11±0,19*	1,58±0,16*
20	Control (I)	100±6,0	458,3±28,7	91,6±5,4	425,7±25,8	5,0±0,32	1,75±0,17
	Indoxacarb (II)	100±6,7	278,4±15,5* *	154,2±8,4* *	287,0±18,4* *	1,79±0,12*	1,21±0,12*
	Indoxacarb + SFL (III)	100±5,8	430,0±26,8*	130,2±8,9*	397,4±23,7*	3,31±0,28*	1,56±0,14*
	Indoxacarb +Narcissin (IV)	100±5,6	384,5±23,8*	113,1±9,4*	363,7±21,4*	3,40±0,29*	1,60±0,13*
30	Control (I)	100±7,5	500,0±33,2	97,6±6,5	479,2±35,8	5,12±0,45	1,81±0,15

	Indoxacarb (II)	100±6,6	358,9±24,5* *	175,5±9,7* *	334,4±23,1*	2,04±0,12*	1,23±0,13*
	Indoxacarb + SFL (III)	100±6,2	432,5±26,1*	115,3±8,2*	405,7±24,8*	3,75±0,28*	1,58±0,14
	Indoxacarb +Narcissin (IV)	100±5,3	433,5±34,3*	111,4±8,2*	400,0±25,6*	3,88±0,30*	1,64±0,15*
40	Control (I)	100±7,1	483,5±7,5	96,0±6,3	469,8±6,2	5,03±0,39	1,84±0,12
	Indoxacarb (II)	100±5,4	369,1±3,8*	126,5±8,9*	339,5±5,1	2,91±0,18*	1,41±0,12*
	Indoxacarb + SFL (III)	100±7,6	414,7±4,5*	106,6±8,7	391,2±5,1*	3,91±0,23*	1,63±0,13*
	Indoxacarb +Narcissin (IV)	100±4,6	461,6±5,6*	116,3±6,8	432,7±5,4	3,96±0,19*	1,70±0,12* *

Note: (In all cases **P*<0.05; ***P*<0.01, confidence levels of indicators of groups III and IV are obtained relative to group II; n=4-5).

On the 40th day of the experiment liver mitochondria of animals of group III and IV were restored by 34.6±2.7% and 36.1±2.8%, respectively, compared to group II under the action of RC SFL and narcissin. There was also an increase in ADP/O value by 15.6±1.1% and 20.5±1.5%, respectively. In the experiment performed with DNF, under the influence of SFL and narcissin, the mitochondrial OF process was partially restored on the 40th day (Table 2).

Thus, it was found that in vivo experiments flavonoids SFL and narcissin can effectively influence the values of RC and ADP/O of liver mitochondria and restore their dysfunction under conditions of pesticide poisoning. It can be concluded that SFL and narcissin compounds correct the disturbance of energy metabolism processes of liver mitochondria caused by pesticides.

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