International Journal of
Biological Engineering and Agriculture

ISSN: 2833-5376
Volume 2 | No 3 | March -2023

Rat Liver Mitochondrial Damage under Pesticide Galoxyfop-R-Methyl -Induced Intoxication: Protection by Soforoflavonoside and Narcissin Flavonoids

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Abstract: **Relevance of the research**: Effective use of chemical and various other factors in plant protection is of great importance now. Most of the chemical agents used to protect plants from pests and diseases are distinguished by their general effectiveness. These effective chemicals can be used to control all types of crops, soil and waterborne pests, disease-spreading parasites, weeds, and defoliation. At the same time, the effects of these toxic chemicals are harmful not only for insects, microbes that cause plant diseases, but also for warm-blooded animals and the human body. These pesticides enter the human body in different ways and accumulate as residues [1;10;12].

Keywords: liver, mitochondria, haloxyphop-R-methyl, soforaflavonolonoside (SFL), narcissin, lipid peroxidation (LPO), mPTP (mitochondrial permeability transition pore), ATP-dependent potassium channel (KATP-channel).

Residual amounts of pesticides accumulated in organs and tissues can later cause various pathologies [4]. Active reactive oxygen species (ROS) generated as a result of oxidative stress in cell organelles, including mitochondria, when poisoned with pesticides lead to increased LPO process in the inner and outer membrane. This causes various pathological conditions in tissues and organs [2;16;13;9]. In biomembranes, LPO is a free-radical biochemical molecular process by which unsaturated lipids and free fatty acids, which are part of biological membrane phospholipids, are oxidized. This situation was highlighted by the formation of H_2O_2 as a result of the oxidation of Fe^{2+} with molecular oxygen [6]. The liver is an important organ in the metabolism of pesticides [2;14]. Therefore, it is of great importance to study the formation of free radicals in the liver mitochondria damage of rats exposed to pesticides and the changes in mitochondria that may occur as a result of this, and the elimination of these changes with the help of natural compounds isolated from plants.

The effects of pesticides at the cellular level are very dangerous, as they produce ROS in the metabolism and disrupt the homeostatic mechanism of the cell. They interact with membrane lipids, and their lipids and proteins disrupt the physiological balance between substrates and electron carriers, especially in mitochondria [15]. Mitochondrial membrane is highly permeable to Ca^{2+} ions,

which is ensured by PTP. In various pathological conditions, the transition of the mitochondria from the high permeability pore conformation to the open state is reported [5;11].

Protecting the liver from the toxic effects of pesticides and developing effective pharmacological methods are important issues. However, no studies have been conducted on the effect of the haloxyfop-R-methyl pesticide, which is currently widely used in agriculture, on the LPO process in the liver mitochondrial membrane, on the change in the conformational state of mPTP, and on the conductance of mitoK_{ATP}-channel, and their correction with plant substances. For this purpose, in our experiments, the correcting effect of SFL and narcissin flavonoid for 10, 20, 30 and 40 days on the disturbances in the liver mitochondrial membrane of rats intoxicated with haloxyfop-R-methyl was studied depending on the dynamics.

The purpose of the study: to study the dynamics of the correcting effect of SFL and narcissin flavonoids on Fe^{2+}/citrate-induced LPO, mPTP permeability, and mito K_{ATP}-channel activity in liver mitochondria of rats poisoned with galoxyfop-R-methyl for 10, 20, 30, and 40 days.

Materials and methods: Male white rats weighing 180-200 g were used for the experiment. Male rats selected for intoxication of experimental animals with haloxyfop-R-methyl were divided into groups:

Group I, healthy (control);

Group II; galaxiphop-R-methyl

Group III; galaxiphop-R-methyl+ SFL

IV group; galaxiphop-R-methyl+narcissin

Animals of the II, III and IV groups of the experiment were poisoned once with LD_{50} 1/10 dose of haloxyfop-R-methyl herbicide per os. After administration of galoxyfop-R-methyl, experimental group III was given SFL 10 mg/kg, and group IV animals were given narcissin flavonoid at a dose of 10.0 mg/kg per os once a day for 10 days.

After 10, 20, 30 and 40 days after administration of soforaflavonolonoside and narcissin flavonoids to herbicide-poisoned rats, their liver mitochondria were separated by differential centrifugation W.C.Schneider method.

The Fe^{2+}/citrate system was used to study the process of LPO in the mitochondrial membrane. Under the influence of this system, the loss of the functional state of the mitochondria membrane and the change in the size of the organelle as a result of membrane LPO was determined photometrically at 25°C by constant mixing with the following IM. Incubation medium (IM) (mM): KSI – 125, KCl – 65, HEPES –10, pH 7.2; The amount of mitochondria is 0.5 mg/ml; Mitochondria were incubated for 2 minutes in a medium containing 2 mM citrate before adding 50 μM Fe^{2+} [7].

Kinetics of mitochondrial swelling (0.3-0.4 mg/ml protein) was determined by spectrophotometer (spectrophotometer V-5000) at 540 nm in an open cell (volume 3 ml) with constant stirring of the mitochondrial suspension at 26°C. was determined. The following incubation medium was used to determine the permeability of PTP in mitochondria: 200 mM sucrose, 20 μM EGTA, 5 mM succinate, 2 μM rotenone, 1 μg/ml oligomycin, 20 mM Tris, 20 mM HEPES, and 1 mM KH_2PO_4, pH 7.4 [8].

MitoK_{ATP}-channel conductance (0.3-0.4 mg/ml protein) was determined by changes in optical density at a wavelength of 540 nm in 3 ml cells. IM as follows: 125 mM KCl, 10 mM HEPES, 5 mM succinate, 1 mM $MgCl_2$, 2.5 mM K_2HPO_4, 2.5 mM KH_2PO_4, 0.005 mM rotenone and 0.001 mM oligomycin, pH 7.4 [3].

In the experiments, the kinetics of mitochondrial decay was calculated as a percentage of the maximum, by calculating the arithmetic mean value of 5-6 different experiments. Statistical processing of the results and drawing of pictures were performed using the Origin 6.1 (USA) computer program. In this case, $P<0.05$ and $P<0.01$ values represent statistical reliability.

Research results and their discussion: In our experiment, we determined the dynamics-dependent effect of SFL and narcissin flavonoids on the LPO process induced by Fe^{2+}/citrate in the liver mitochondrial membrane of rats poisoned with haloxyfop-R-methyl pesticide for 10, 20, 30 and 40 days. According to the results of the experiment, in the presence of Fe^{2+}/citrate in the IM, the induced LPO process, that is, the rate of mitochondria swelling, was taken as 100% (Fig. 1). According to the obtained results, haloxyfop-R-methyl injected (group II) rats liver mitochondria under the influence of Fe^{2+}/citrate inducer is 2.6 compared to the control group, depending on the dynamics of 10, 20, 30 and 40 days; 5.7; A sharp increase of 7.7 and 5.8 times was noted. When group III rats intoxicated with galoxyfop-R-methyl were treated with SFL and group IV rats were treated with narcissine for 10 days, their liver mitochondrial membrane Fe^{2+}/citrate-induced LPO II values were re-evaluated depending on the dynamics of 10, 20, 30 and 40 days. recovery was determined (Fig.1).

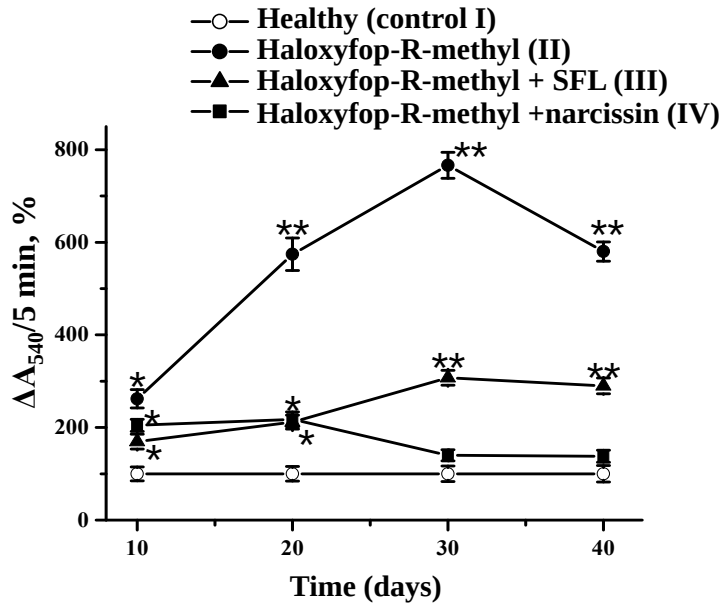

Fig. 1. Effects of SFL and Narcissine on Fe^{2+}/Citrate-Induced Peroxide Oxidation of Hepatic Mitochondrial Membrane Lipids of Galoxyfop-R-Methyl Intoxicated Rats (*P<0,05; *P<0,01; n=5-6).

In this case, the antitoxic property of narcissin flavonoid was found to be more effective than that of SFL flavonoid in a dynamic manner. An increase in the intensity of the mitochondrial membrane LPO under the influence of pesticides, in turn, leads to a violation of the functional activity of ion transport systems.

In the next experiment, the effects of SFL and narcissin flavonoids on the permeability of liver mitochondria PTP of rats intoxicated with haloxyfop-R-methyl pesticide were investigated in a dynamic manner. According to the obtained results, the liver mitochondria of healthy group I experimental animals, taken as a control, did not have a dynamic state during 40 days. However, it was found that the number of liver mitochondria increased by 59.0±3.5% in 10 days, and by 65.7±4.6% in 20 days compared to the control group of group II animals treated with haloxyfop-R-methyl pesticide. In 30- and 40-day-old rats treated with galoxyfop-R-methyl pesticide, the number of liver mitochondria increased by 70.1±4.1% and 60.5±4.5%, respectively, compared to the control. Thus, 20 days after administration of haloxyfop-R-methyl pesticide to animals, a stable dynamic state was maintained in liver mitochondria suppression (Fig. 2).

Continuing the experiments, group III rats injected with haloxyfop-R-methyl pesticide were treated with SFL flavonoid (10 mg/kg) for 10 days. After that, rat liver mitochondrial permeability studies were conducted on days 10, 20, 30 and 40 of the experiments. According to the obtained results, it was found that liver mitochondria of group III animals were inhibited by 24.0±2.1% in 10 days, and by 30.7±3.2% in 20 days compared to pathological group (II). On the 30th and 40th days of the

experiment, group III rats poisoned with haloxyfop-R-methyl and injected with SFL flavonoid showed an increase in liver mitochondria by 46.8±4.5% and 45.0±3.0% compared to group II (Fig. 2). SFL flavonoid inhibited mPTP permeability of rat liver mitochondria in a 40-day dynamics-dependent manner. It proved that the inhibitory effect of SFL was more effective on days 30 and 40 than on days 10 and 20.

Continuing the experiments, group IV rats injected with haloxyfop-R-methyl pesticide were treated with narcissin flavonoid (10 mg/kg) for 10 days. After that, experiments were conducted on days 10, 20, 30 and 40, and mitochondria were isolated from the liver of rats. According to the obtained results, it was found that the number of liver mitochondria of group IV animals was inhibited by 18.5±1.5% in 10 days and by 17.9±2.2% in 20 days compared to group II.

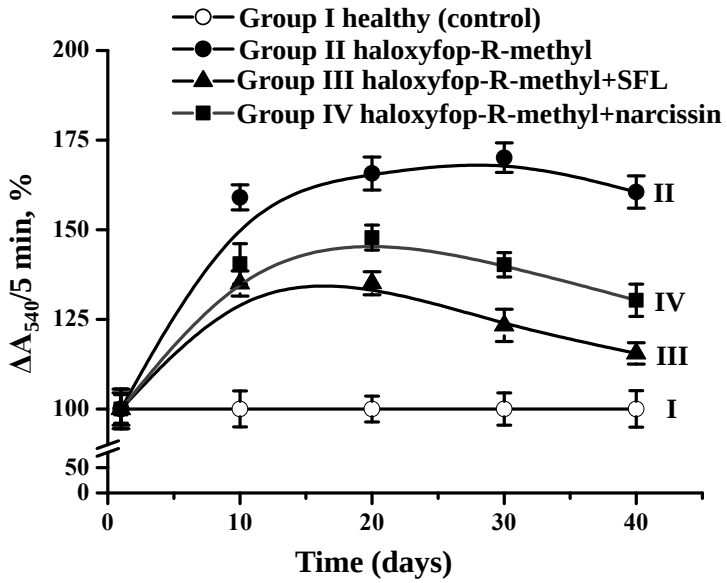

Fig. 2. Effects of SFL and narcissin flavonoids on the permeability of liver mitochondria PTP of rats intoxicated with the pesticide galoxyfop-R-methyl (40-day dependent dynamics)
(*P<0,05; **P<0,01; n=5-6).

On the 30th and 40th days of the experiment, it was noted that the number of liver mitochondria of group IV rats, intoxicated and corrected with narcissin flavonoid, was reduced by 29.9±3.4% and 30.2±2.5% compared to group II indicators (Fig. 2). Narcissin flavonoid was also found to effectively inhibit mitochondrial swelling at 30 and 40 days compared to 10 and 20 days.

One of the mitochondrial factors controlling the metabolic and functional activity of the cell is mitoK_{ATP}-channel. Currently, the biophysical properties of mitoK_{ATP}-channel and its physiological significance are well studied. However, literature data on changes in the functional activity of the mitoK_{ATP}-channel in liver intoxication-related damage are rare. This phase of our in vivo experiment consisted in correcting rats poisoned with haloxyfop-R-methyl pesticides with SFL and narcissin flavonoids and studying their effect on liver mitoK_{ATP}-channel functional activity. The effects of SFL and narcissin flavonoids on hepatic mitoK_{ATP}-channel conductance in rats treated with galoxyfop-R-methyl are presented in fig. 3.

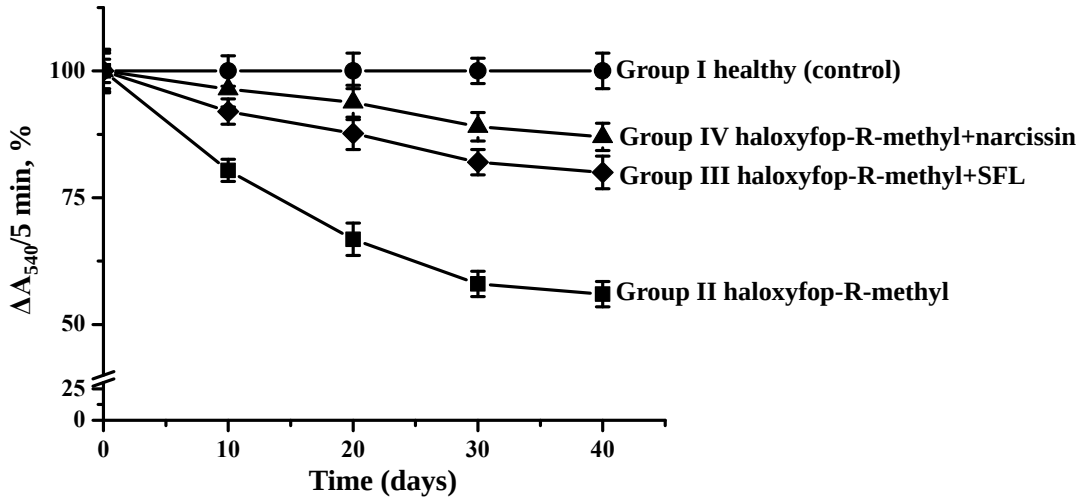

Fig. 3. Effects of SFL and narcissin flavonoids on ATP-dependent potassium channel activity in liver mitochondria of rats intoxicated with the pesticide galoxyfop-R-methyl (40-day dynamics dependent) (*P<0,05; **P<0,01; n=5-6).

According to the obtained results, the conductance of liver mitoK$_{ATP}$-channel in group II rats treated with haloxyfop-R-methyl was 19.6±1.1 in the presence of 200 μM ATP in IM on days 10, 20, 30 and 40, respectively, compared to the control (group I). 33.2±2.2%; It was found that it was inhibited by 42±3.2% and 44±3.2%. When galoxyfop-R-methyl administered group III rats were administered SFL orally at a dose of 10 mg/kg for 10 days, their liver mitoK$_{ATP}$-channel permeability was 11.6±0.8% compared to group II values on days 10, 20, 30, and 40, respectively; 20.9±1.2%; It was found to be activated by 24±2.1% and 24±1.3% (Fig. 3).

The activity of the liver mitoK$_{ATP}$-channel of rats treated with 10 mg/kg dose of narcissin for 10 days was 16±1.0%, respectively, compared to the values of the II group; 30±2.2%; It was noted that it increased by 31±2.5% and 31.1±2.2%.

Inhibition of the mitoK$_{ATP}$-channel of the liver under the influence of galoxyfop-R-methyl pesticides is expressed by a sharp decrease in the flow of potassium ions from the membrane. A decrease in the potassium ion cycle can cause a decrease in the mitochondrial membrane potential and a change in the size of the matrix. Under the influence of selected plant compounds SFL and narcissin, the toxicity of pesticides can be reduced and, as a result, the flow of K^+ ions in the membrane of liver mitochondria can be restored.

Conclusions. Thus, when the pesticide haloxyfop-R-methyl is administered to rats, it leads to the development of destructive processes in the metabolism of liver cells. These destructive changes reach the level of the organelle and cause the acceleration of the LPO process in the mitochondrial membrane and, as a result, the disruption of membrane permeability. As a result of peroxidation of the lipoprotein parts of the mitochondrial membrane, the permeability changes sharply, that is, the high permeability pore changes to an open conformational state, the permeability of the mitoK$_{ATP}$-channel is inhibited. Plant compounds, SFL and narcissin flavonoids restore liver mitochondrial membrane damage developed under the effect of galoxyfop-R-methyl pesticide. In this case, its effective effect was shown 30 and 40 days after poisoning.

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