

UDC 616.682:618.2:632.954

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**To cite this article:** Moisieieva N., Vlasova O., Vakhnenko A., Zviagolska I., Miahkohlib A. (2024). Fitoterapevtychni aspekty honadoproteksii za toksychnoho vplyvu herbicydiv [Phytotherapeutic aspects of gonad protection under the toxic effect of herbicides]. *Fitoterapiia. Chasopys – Phytotherapy. Journal*, 1, 70–76, doi: <https://doi.org/10.32782/2522-9680-2024-1-70>

## PHYTOTHERAPEUTIC ASPECTS OF GONADOPROTECTION IN THE TOXIC INFLUENCE OF HERBICIDES

**Relevance.** In industrialized countries, there has been a concerning rise in uro-andrological issues among men, leading to a decline in fertility and overall health. This is a pressing medical and social issue. The causes are multifaceted, with toxicants being one contributing factor among many.

**The aim of this study** is to investigate the use of the phytotherapeutic complex as a gonadoprotector against the toxic effects of the herbicide clopyralid.

**Materials and methods.** The experiment was conducted on male Vistar rats, the toxicant was exposed for 56 days, which is due to the duration of spermatogenesis at a dose of 150 mg/kg of body weight, which was 0.33 LD<sub>50</sub> (LD<sub>50</sub> – 5000 mg/kg/day), in clinical groups,  $\alpha$ -tocopherol acetate (10 mg/kg/day), or a phytotherapeutic complex in a therapeutic dosage. Diene conjugates, spontaneous hemolysis of erythrocytes, activity of blood antioxidant enzymes were determined in blood plasma and testicular tissues: superoxide dismutase; catalase and ceruloplasmin, thiobarbituric acid-reactants in blood and testicular tissues. When assessing the quantitative and functional state of sperm, the total number, pathological forms of spermatozoa, and the kinesisgram were studied. The morphological state of the spermatogenic epithelium was studied.

**Results.** The data obtained indicate a significant toxic effect of herbicides, specifically clopyralid, on the functional capacity of the testes. This effect is due to the induction of VRPO lipids in the blood and tissues, which results in a decrease in the spermatogenesis index, a violation of the stages of sperm maturation, and ultimately, a significant reduction in the motility of spermatozoa. Additionally, there is an increase in the number of pathological forms of spermatozoa, which leads to a decrease in male fertility.

The attempt to neutralise the toxic effect of herbicides with  $\alpha$ -tocopherol acetate was partially successful. This is evidenced by the increase in the body's antioxidant defences. However, the influence on the state of the spermatogenic epithelium was clearly insufficient. The division of germ cells increased, but the quality of sperm did not change significantly. The data obtained demonstrate the potent antioxidant abilities of  $\alpha$ -tocopherol acetate. However, it has shown to have an insufficient positive effect on the tissues of the germinal epithelium.

The phytotherapeutic complex was used in conjunction with a herbicide (clopyralid) to increase antioxidant protection in the blood and testicular tissues. Additionally, it normalized the state of the spermatogenic epithelium, improved the quantitative and qualitative indicators of sperm, and restored sperm motility.

**Conclusions.** The study showed that the phytotherapeutic complex had a more significant protective effect on the testicles' morphology and sperm's functional readiness. Therefore, it is recommended to use the phytotherapeutic complex as a gonadoprotector to counteract the chronic toxic effects of herbicides.

**Key words:** herbicides, fertility dysfunction, gonadoprotection, phytotherapeutic complex.

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**Бібліографічний опис статті:** Моїсєєва Н., Власова О., Вахненко А., Звягольська І., Мягкохліб А. (2024). Фітотерапевтичні аспекти гонадопротекції за токсичного впливу гербіцидів. *Фітотерапія. Часопис*, 1, 70–76, doi: <https://doi.org/10.32782/2522-9680-2024-1-70>

**ФІТОТЕРАПЕВТИЧНІ АСПЕКТИ ГОНАДОПРОТЕКЦІЇ  
ЗА ТОКСИЧНОГО ВПЛИВУ ГЕРБІЦИДІВ**

**Актуальність.** Серед чоловічого населення, особливо в індустріально розвинутих країнах, відчувається неухильне, катастрофічне зростання уроандрологічних проблем, погіршення чоловічого здоров'я і, як наслідок, зниження фертильної здібності, і це є не тільки актуальна медична, але і соціальна проблема. Причин існує безліч, одна з яких є вплив різноманітних токсикантів.

**Мета дослідження** – вивчити застосування фітотерапевтичного комплексу азупростат як гонадопротектора на тлі токсичного впливу гербіциду клопіралід.

**Матеріали та методи.** Експеримент проводився на щурах-самцях Vistar, токсикант впливав 56 днів, що обумовлено тривалістю сперматогенезу у дозі 150 мг/кг маси тіла, що склало 0,33 LD 50 (LD 50 – 5000 мг/кг/добу), в клінічних групах додатково призначались  $\alpha$ -токоферолу ацетат (10 мг/кг/добу), або фітотерапевтичний комплекс в терапевтичній дозировці. У плазмі крові та тканинах сім'яників визначали дієнові кон'югати, величину спонтанного гемолізу еритроцитів, активність антиоксидантних ферментів крові: супероксиддисмутази; каталази та церулоплазміну, у крові та тканинах сім'яників – ТБК-реактанти. При оцінці кількісного та функціонального стану сперми вивчали загальну кількість, патологічні форми сперматозоїдів та кінезисграму. Вивчали морфологічний стан сперматогеного епітелію.

**Результати дослідження.** Отримані дані свідчать про виражену токсичну дію гербіцидів (клопіраліду) на функціональну здатність сім'яників, що було обумовлено індукцією ВРПО ліпідів в крові та тканинах, і викликало зниження індексу сперматогенезу, порушення стадій визрівання спермієв, а в підсумку значне порушення рухливості сперматозоїдів, зростала кількість патологічних форм сперматозоїдів, що викликало падіння фертильності у самців.

*Спроба нівелювати токсичну дію гербіцидів  $\alpha$ -токоферолу ацетатом частково вдалась, про що свідчить підвищення антиоксидантного захисту організму. Але явно недостатній вплив на стан сперматогенного епітелію, зріс поділ статевих клітин та суттєво не змінилася якість спермій. Отримані дані демонструють потужні здібності  $\alpha$ -токоферолу ацетату як антиоксиданту, але явно недостатній позитивний вплив на тканини гермінативного епітелію.*

**Висновки.** Застосування фітотерапевтичного комплексу на тлі токсичного впливу гербіциду (клопіраліду) сприяло не тільки значному підвищенню антиоксидантного захисту в крові та тканинах сім'яників, а й нормалізації стану сперматогенного епітелію, кількісних і якісних показників сперми, сприяло відновленню рухливості сперматозоїдів. Морфологічні зміни у сім'яниках та функціональна готовність сперми продемонстрували більш виражену гонадопротективну дію фітотерапевтичного комплексу. Це дає підставу доцільності застосування фітотерапевтичного комплексу азупростат як гонадопротектор для нівелювання хронічного токсичного впливу гербіцидів.

**Ключові слова:** гербіциди, фертильна дисфункція, гонадопротектори, фітотерапевтичний комплекс.

**Relevance.** In the rapidly changing modern world, a paradoxical trend is emerging where nations are 'aging' while diseases are 'rejuvenating'. According to the World Health Organization (WHO), 8-12% of married couples of young reproductive age are infertile globally. The critical level is 15% (WHO, 2023; Starc, 2019). Additionally, the male factor is the main or concomitant cause in almost 50% of cases (Farber, 2019; Polis, 2017)). Infertility is not only a pressing medical issue but also a social problem. In industrialized countries, there is a concerning increase in uro-andrological problems among men, leading to a decline in their overall health and quality of life. Male fertility is closely linked to quality of life, and abnormalities in the spermatogram can limit fertility even when erectile function is preserved. The complexity of this fact can cause confusion, especially in those experiencing psycho-emotional exhaustion, excessive mistrust, and anxiety disorders. This confusion can lead to the development of neurotic or depressive states, which can negatively affect the hormonal state and contribute to infertility in men. Thus, the causes of male infertility can be localized at different levels (Barratt, 2017; Choy, 2018). The germinal epithelium is one of the links that, when damaged, can lead to a general violation of spermatogenesis. Normal functioning of gonadal cell membranes plays an important role in allowing spermatozoa to mature. One mechanism by which toxic substances can cause damage is through the disruption of redox processes, specifically an increase in free radical peroxidation (FRP) of lipids. This occurs primarily in the membranes of cells in the spermatogenic epithelium, leading to their destruction throughout the reproductive period (Farber N.J., 2019).

In the current situation in our country, which is facing a long-term economic crisis, restrictions due to the global pandemic, and a full-scale military invasion, there has been a decline in the overall quality of agriculture. Many agricultural lands are experiencing an increase in soil contamination, with 60-70% of the cultivated areas showing a higher degree of contamination. Controlling the number of weeds in agricultural land is becoming an urgent issue due to the significant losses in agriculture caused by weed

clogging. The chemical and biological industry offers a wide range of opportunities to protect cultivated plants and reduce weed pollution, particularly through the use of herbicides. However, it is important to consider the ecological acceptability of herbicide use and the rehabilitation of soils contaminated by toxic chemical residues.

The range of herbicides actively used in agriculture has expanded considerably in recent years. One of the widely used herbicides, as a mono-chemical or tank mixture, are derivatives of pyridinecarboxylic acid – 3,6-dichloro-2-pyridinecarboxylic acid (clopyralid) and its salts: triethylamine, triisopropylamine, monoethylamine (Martins-Gomes, 2022; Sun, 2021). which are post-emergent herbicides with high activity on weeds resistant to aryloxyalkancarboxylic acids and their derivatives: plants of the comfrey, umbrella, buckwheat and legume families; and limited activity on cereals, plants of the crucifer family, flax and sugar beet. The half-life in soil is 49-73 days, in dry summer up to 1 year. Biotransformation often occurs by microbiological means, where the effect of hydrolysis and photolysis is insignificant. In addition, clopyralid can migrate in soils and leach into groundwater, which is related to its high solubility in water and low ability to bind to soil. During a growing season, the drug is not degraded in plants, which is why, for example, its residues (0.02-0.67 mg/kg) are found in cereals. 2 months after treatment, the substance is found in straw at levels of 0.04-3.3 mg/kg, and residues are also found in bran (Starc, 2019).

There is a belief that herbicides have little interaction with what we eat. However, this is a misconception because herbicides poison neighbouring plants and the environment through groundwater and soil, harming both nature and human health (Stevanus, 2021).

The generalized mechanism of the impressive effect of herbicides is the imbalance of the processes of lipid RPO, and the most vulnerable are the membranes of cells, especially cells of tissues with a high level of proliferation, in particular, the germinal epithelium (Hashim, 2022). Destruction of the membranes of Sertoli cells, spermatogonia and Leydig cells occurs throughout the reproductive period. Damage to Leydig cells leads

to a pronounced gonadotoxic effect, which is caused by a violation of steroidogenesis, and as a result – to a violation of spermatogenesis (Farber, 2019, Alamgir, 2023; Thu Ha Pham, 2019; Mehrpour, 2014).

The striking effect of the imbalance of lipids in VRPO, which leads to a violation of the integrity of biomembranes and the development of a cytolytic syndrome in the germinal epithelium, has prompted attempts to use antioxidants from various groups as gonadoprotectors in the case of toxic damage to the testis.

And given the fact that a person most often prefers herbal medicines, it would be very appropriate to use phytopreparations. Moreover, the combination of antioxidant compounds with different chemical structures seems to be the most promising (Farber, 2019). In the role of gonadoprotector, it is possible to use  $\alpha$ -tocopherol acetate, taking into account its known antioxidant effect, but the phytotherapeutic complex drug, which contains  $\alpha$ -tocopherol acetate, retinol palmitate, echinacea purpurea extract and  $\beta$ -sitosterol, looks more promising (Choy, 2018; Polis, 2017). Echinacea purpurea extract, which also has antioxidant properties, is often considered a prostate protector (Sharapova, 2021; Mao, 2021; Motamedi, 2018). And although  $\beta$ -sitosterol is not an antioxidant, its presence in this combination will help to normalise steroidogenesis as it is a known prostate protector (Macoska, 2023; Arivarasu, 2023).

**The aim of the study** was to use the phytotherapeutic complex, with the inclusion of natural antioxidants, as a gonadoprotector to study its effect on the parameters of VRPO of lipids in the blood and tissues of the germinal epithelium, morphological changes in the testicles of males (rats) and functional indicators in the spermogram against the background of toxic exposure herbicide clopyralid.

#### Research materials and methods

Male rats of the Vistar line weighing 190-205 g were used in all experimental studies. The control group received daily clopyralid at a dose of 150 mg/kg of body weight, which was 0.33 LD50 (LD50 – 5000 mg/kg/day). The duration of exposure to the toxicant was 56 days, which was dictated by the duration of spermatogenesis in rats and the maturation time of spermatozoa in the epididymis. Two experimental groups were formed to study the effects of a toxicant. In the first group, animals were administered  $\alpha$ -tocopherol acetate (10 mg/kg/day), while in the second group, a phytotherapeutic complex was administered in a therapeutic dosage.

To investigate changes in lipid peroxidation in blood plasma and testicular tissue, we measured diene conjugates (DC) and TBC reactants in both blood and testicular tissue. We also examined the extent of spontaneous haemolysis of erythrocytes, as well

as the activity of antioxidant enzymes in the blood, including superoxide dismutase (SOD), catalase, and ceruloplasmin.

When assessing the quantitative and functional state of the spermatozoa, the viability of the spermatozoa was studied and pathological forms of the spermatozoa were detected by means of an eosin test. A morphological study of the state of the spermatogenic epithelium was also carried out.

Statistical analysis was conducted using standard software packages, including 'MS Excel' and 'Statistica for Windows, Release 6.0'.

**Results and discussion.** Upon analysis of the results, it was discovered that the animals exposed to the toxicant experienced a 13% decrease in body mass, significant baldness, reduced general activity, and a 15% decrease in testicular mass. However, their mass coefficients remained within the normal range. The blood biochemical data revealed a 1.6-fold increase in DC levels ( $P<0.01$ ) and a 3.6-fold increase in TBK reactants ( $P<0.01$ ), indicating an elevation in VLDL lipids in the blood. A comparable pattern was observed in the testicular tissues, where TBK reactants were recorded at a significantly higher level, exceeding the norm by 1.5 times ( $P<0.01$ ). The activity of antioxidant enzymes decreased, with catalase capacity decreasing by 2.5 times ( $P<0.05$ ) and ceruloplasmin by 1.4 times ( $P<0.01$ ). However, SOD activity increased by 1.9 times compared to the norm ( $P<0.001$ ). The analysis of sperm indicators revealed a 36% decrease in the total number of spermatozoa due to an increase in the absolute number of dead and pathological forms of spermatozoa ( $P<0.01$ ). The number of dead spermatozoa increased by 1.8 times and the number of pathological forms increased by 2.4 times compared to the intact group ( $P<0.001$ ). There was a significant decrease in sperm motility, with the number of spermatozoa exhibiting normokinesis decreasing by 3.3 times ( $P<0.001$ ), hypokinesis by 2.9 times ( $P<0.001$ ), akinesis by 3.1 times ( $P<0.001$ ), and dyskinesis by 10 times ( $P<0.001$ ). The detected disorders were complemented by changes in the morphological picture of the testicular tissues. A decrease in the number of normal spermatogonia by 23% ( $P<0.02$ ), an increase in the number of tubules with exfoliated epithelium by 4.4 times ( $P<0.05$ ), and an increase in the number of tubules with atrophic or damaged epithelium by 2 times ( $P<0.02$ ) were noted. The tissues of the testes showed dystrophic and degenerative changes, along with compensatory hyperplasia. The vessels of the microcirculatory channel exhibited dystrophic changes, which further intensified the degenerative changes in the spermatogenic epithelium. The Sertoli

cells (follicular) were swollen, significantly larger than normal cells, and contained a small nucleus. The Leydig cells showed pathological changes and were significantly reduced in number. The study observed destruction of the seminiferous tubules, damage to the spermatogenic epithelium (especially in the final stages of spermatogenesis), and necrosis of the seminiferous tubules. These changes significantly affect male fertility, resulting in a decrease in the index of spermatogenesis, functional capacity of spermatozoa, an increase in pathological forms of spermatozoa, and impaired motility.

The analysis of the parameters of the group of animals that received alpha-tocopherol acetate against the toxic effect of the herbicide revealed a decrease in RPPO lipids in the blood and testicular tissues. The concentration of DK in blood serum decreased by 23% ( $P < 0.05$ ) against the background of a 2.5-fold decrease in the concentration of TBC reactants ( $P < 0.05$ ), which corresponds to the norm. Additionally, a 1.5-fold reduction in spontaneous erythrocyte haemolysis was observed ( $P < 0.05$ ). Enzyme systems underwent significant changes in the experimental group compared to the control group. Blood SOD activity decreased by 29% ( $P < 0.05$ ), while catalase activity increased by 2.5 times ( $P < 0.01$ ). Additionally, ceruloplasmin concentration increased by 44% ( $P < 0.01$ ). The concentration of TBC reactants in testicular tissue was reduced by 27% ( $P < 0.001$ ).

A similar pattern was observed in the analysis of pathological forms of spermatozoa, with a 22% decrease in their number ( $P < 0.02$ ), although it did not reach the normal range ( $P < 0.001$ ). The kinesisgram results also showed a similar trend: spermatozoa with normokinesis increased by 2.9 times ( $P < 0.001$ ), but did not reach normal values ( $P < 0.05$ ), while those with hypokinesis significantly decreased by 2 times ( $P < 0.001$ ), although their concentration was 45% lower than the norm ( $P < 0$ ). The amount of akinesis was two times lower than the control group ( $P < 0.001$ ), but still 58% higher than the norm ( $P < 0.02$ ). The amount decreased significantly by 2.3 times with dyskinesis ( $P < 0.01$ ), but still exceeded the norm by 4.4 times ( $P < 0.001$ ). The examination of the morphological parameters of the germinal epithelium showed that the number of normal spermatogonia increased by 1.3 times. Additionally, the number of tubules with the 12th stage of meiosis was 2.4 times higher than that of the control group ( $P < 0.05$ ), which is within the normal range. Furthermore, the number of tubules with atrophic or damaged epithelium decreased by 2 times, which is also within the normal range. The germinal epithelium's morphological characteristics indicate the recovery of first and second-order spermatogonia, a significant decrease in empty tubules, almost normalized connective tissue membranes, and a spermatogenesis index that corresponds to the norm. The obtained results demonstrate the powerful abilities of

$\alpha$ -tocopherol acetate as an antioxidant, which confirm a significant increase in the overall antioxidant protection of the body, but a clearly insufficient positive effect on the tissues of the germinal epithelium.

The study analysed the effects of a phytotherapeutic complex drug on a group exposed to herbicide intoxication. The results showed a significant decrease in DC concentration by 34% ( $P < 0.01$ ) and a three-fold decrease in TBC-reactants ( $P < 0.01$ ), which returned to normal levels. The blood enzyme systems demonstrated normalization of SOD activity and spontaneous hemolysis of erythrocytes. Catalase activity increased by 2.5 times ( $P < 0.02$ ), and ceruloplasmin increased by 1.4 times compared to the control group ( $P < 0.01$ ). In the testicular tissues, the content of TBC-reactants significantly decreased by 1.5 times ( $P < 0.01$ ). A confirmation of the positive effect of the phytotherapeutic complex is the reliable establishment of the lost weight of testicles by 17.5% ( $P < 0.05$ ), which was observed in the control group. The analysis of spermatogram data showed a significant increase in the total number of spermatozoa by 58% compared to the control ( $P < 0.01$ ), a decrease in the number of dead spermatozoa by 1.8 times ( $P < 0.001$ ), and a decrease in pathological forms of spermatozoa by 2 times ( $P < 0.001$ ). The study found that the phytotherapeutic complex had a significant effect on sperm motility. Normokinesis increased by 3.2 times compared to the control group ( $P < 0.001$ ), while hypokinesis was 2.6 times lower than control values ( $P < 0.001$ ). Akinesis was also 2.9 times lower than control ( $P < 0.001$ ), and dyskinesia was 6 times lower than in the control group ( $P < 0.01$ ). The kinesisgram indicators of this group were similar to those of the intact group. The results of the morphological studies indicate a significant improvement in the state of the germinal epithelium. The spermatogenesis index was normalized ( $P < 0.01$ ), and the number of normal spermatogonia increased by 31% ( $P < 0.05$ ) compared to the control. Additionally, the number of tubules with the 12th stage of mitosis increased by 2.5 times ( $P < 0.01$ ), while the number of tubules with exfoliated epithelium decreased by 3.4 times ( $P < 0.01$ ), and the number of tubules with atrophic and damaged epithelium decreased by 1.9 times ( $P < 0.02$ ). The histological picture of the spermatogenic epithelium was not significantly different from that of the intact group.

## Conclusions

**The results of the experiment indicate a significant toxic effect of herbicides, specifically clopyralid, on the functional capacity of the germinal epithelium. This effect was observed through the induction of VRPO lipids in blood and tissues, as well as a decrease in the body's antioxidant defense. These findings were confirmed**

through biochemical and morphological changes in the testicles. Overall, these alterations resulted in a decrease in the spermatogenesis index, disrupted maturation stages, and consequently, significantly impaired sperm motility. Additionally, there was an increase in the number of pathological forms of spermatozoa, leading to a reduction in fertilization ability, ultimately resulting in decreased male fertility.

The attempt to mitigate the toxic effects of herbicides using  $\alpha$ -tocopherol acetate was partially successful. This is supported by the inhibition of the processes of RPPO of blood lipids, an increase in the body's antioxidant capacity, and the normalization of the activity of antioxidant enzymes. However, the impact on the state of the spermatogenic epithelium was insufficient. The number of tubules in the maturation stage remained unchanged, and there was no significant alteration in the number of dead or pathological forms of spermatozoa. It is worth noting that  $\alpha$ -tocopherol acetate stimulated the division of germ cells, but did not have a significant effect on the quality of sperm. The results indicate that  $\alpha$ -tocopherol acetate is a powerful antioxidant, significantly increasing overall antioxidant protection

of the body. However, its positive effect on the tissues of the germinal epithelium is clearly insufficient.

The phytotherapeutic complex was used in conjunction with a herbicide (clopyralid) to significantly reduce VLDL lipid levels in both blood and testicular tissues. Additionally, it normalized the state of the spermatogenic epithelium, improved quantitative indicators of sperm, and reduced pathological forms of spermatozoa. Under the influence of the phytotherapeutic complex, the quality of sperm improved, and the spermatogenic epithelium was positively affected in conditions of intoxication, leading to the restoration of sperm motility. Morphological changes in the testicles and functional readiness of the sperm were observed. The results showed that the most significant changes occurred due to clopyralid intoxication. However, when corrected with a phytotherapeutic complex, a more pronounced gonad protective effect was observed.

The experimental results suggest that the phytotherapeutic complex can be used as a gonadoprotector to mitigate the chronic toxic effects of pyridinecarboxylic acid derivatives (herbicides) on male mammalian fertility.

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*Стаття надійшла до редакції 28.12.2023*

*Стаття прийнята до друку 24.01.2024*

**Конфлікт інтересів:** відсутній.

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