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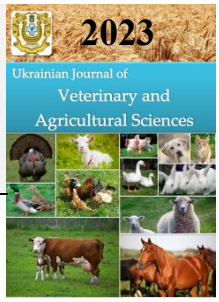

Research on the embryotoxic effect and carcinogenicity of the drug "BTF plus" – a means for normalizing metabolic processes in animals and poultry

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Abstract

Laboratory studies were conducted to determine the embryotoxic effect and carcinogenicity of the veterinary drug "BTF plus" on white rats and white mice. The drug "BTF plus" is a complex vitamin-mineral drug based on butophosphane, L-carnitine, and cyanocobalamin, which is used to normalize and correct metabolic processes in animals and poultry. The drug is used for various types of animals and poultry as a stimulating, tonic and general strengthening agent for obstetric pathologies (complicated childbirth, postpartum complications, paresis, eclampsia, sexual cycle disorders); metabolic disorders caused by irrational feeding, malnutrition, asthenic syndrome, etc.; anemia with helminthiasis; secondary anemias, as an additional means in the treatment of magnesium and calcium deficiency; to increase muscle activity, with significant loads, overstrain and exhaustion in animals; to increase the body's resistance to various pathogens; to stimulate growth, development and live weight gain in young animals and poultry; as an additional means in the treatment of diseases caused by various factors (infectious and non-infectious origin). The drug "BTF plus", under the conditions of subcutaneous administration to pregnant female rats in doses (based on the absolute weight of the drug) of 200.0 and 2000.0 mg/kg of body weight, does not cause death and pathological changes in embryos do not have an embryotoxic and teratogenic effect since indicators of total, preimplantation, and postimplantation embryonic lethality in rats of the experimental groups had no significant differences compared to indicators in control and also did not show changes in the weight of the placenta, fetuses, and their cranio-caudal size. The drug "BTF plus", under conditions of 5-day subcutaneous administration to white mice in doses (based on the absolute weight of the drug) of 200.0 and 2000.0 mg/kg of body weight, does not show a carcinogenic effect (during microscopic studies, the proportion of polychromatophilic erythrocytes was not probable deviations between themselves and was 0.117-0.133%, which is within the normal range of up to 0.2 %). Further studies will be the next stage of pre-registration tests aimed at studying the ecotoxicity of "BTF plus", which is a mandatory material of the "Safety and residue studies" section of the dossier for this drug.

Keywords: "BTF plus"; rats; mice; embryotoxic effect; carcinogenicity; preimplantation lethality; postimplantation lethality.

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1. Introduction

An effective fight against non-communicable diseases of various etiologies of animals in Ukraine is possible with the use of highly effective and affordable accompanying medicines (Bashchenko et al., 2020; Katsaraba et al., 2021; Gutyj et al., 2022; Rivis et al., 2022; Slivinska et al., 2022; Koreneva et al., 2023).

Therefore, today, the development of tonic, stimulating, and strengthening agents, which are an integral part of systemic therapy and possess significant efficiency and environmental safety, has remained relevant (Sachuk, 2019; Sachuk et al., 2019, 2023). Thus, "DAVIE" LLC was offered a new drug – "BTF plus". One milliliter of the drug contains active substances: butaphosphan – 100 mg, L-carnitine – 100 mg, vitamin B_{12} – 0.05 mg. Excipients: water for injections, butyl alcohol – up to 1 ml.

The drug "BTF plus" is a complex drug used to correct and normalize metabolic processes in animals and poultry.

Butaphosphane is a derivative of phosphonic acid. It has a tonic effect, is an adaptogen and a stimulator of metabolic processes, increases the body's resistance to a complex of

negative factors, and promotes the growth and development of animals (Martyshuk et al., 2020, 2022).

L-carnitine is an amino acid that transports fatty acids through the mitochondrial membrane and is an essential factor in maintaining a certain level of coenzyme A (coenzyme A) in all types of cells. L-carnitine has a pronounced anabolic effect: it stimulates the synthesis of muscle tissue proteins, mobilizes lipids from the fat depot (liver, muscles, adipose tissue), and promotes growth and development. L-carnitine improves appetite and the digestive tract's secretory function, promoting the assimilation of feed nutrients. L-carnitine reduces the intensity of apoptosis of all types of cells and increases the intensity of the supply of organic acids (acetic, propionic, lactic, etc.) and ketone bodies to the Krebs cycle, thereby preventing the development of acidosis and ketosis. Also, L-carnitine increases the tone of skeletal muscles, and the myocardium promotes rapid recovery after physical exertion (Pekala et al., 2011; Ferreira & McKenna, 2017; Kepka et al., 2020; Sahebnasagh et al., 2022).

Cyanocobalamin (vitamin B_{12}) is a methylation factor cofactor of enzymes of hematopoiesis and metabolism of organic acids and has a lipotropic effect (Green & Miller, 2020; Kather et al., 2020; Bhowmik et al., 2021; Pardo-Cabello et al., 2023).

Thus, the complex effect of the active substances of the drug leads to an increase in the intensity of growth and development of animals, resistance, and productivity due to the stimulation of metabolic processes.

The drug "BTF plus" is used for all types of animals (horses and cattle, foals and calves; sheep and goats; lambs, goats; pigs; piglets; dogs, cats, fur animals, and rabbits) and birds as a tonic, stimulating and general strengthening agent. With: obstetric pathologies (complicated childbirth, postpartum complications, paresis, eclampsia, sexual cycle disorders); metabolic disorders caused by irrational feeding, malnutrition, asthenic syndrome, etc.; secondary anemias, anemias with helminthiasis; as an additional means in the treatment of calcium and magnesium deficiency; to increase muscle activity, with significant loads, overstrain and exhaustion in animals; to increase the body's resistance to various pathogens; to stimulate growth, development and live weight gain in young animals; as an additional means in the treatment of diseases caused by various factors (infectious and non-infectious origin).

Therefore, the research aimed to provide a toxicological (preclinical) evaluation of the veterinary medicinal product "BTF plus" by determining its embryotoxic effect and carcinogenicity in laboratory animals.

The goal of the work – is to carry out a toxicological evaluation of the veterinary drug "BTF plus", produced by "DEVIE" LLC (Lytin, Ukraine) under the conditions of a subacute toxicological experiment on the model of white rats and mice.

2. Materials and methods

A preclinical study of a complex vitamin-mineral preparation based on butophosphane, L-carnitine, and cyanocobalamin, which is used to normalize and correct metabolic processes in animals and poultry, was conducted based in the laboratory for quality control, safety, and registration of veterinary medicinal products and feed additives of LLC "DEVIE". The drug is used for various types of animals and poultry as a stimulating, tonic and general strengthening agent for obstetric pathologies (complicated childbirth, postpartum complications, paresis, eclampsia, sexual cycle disorders); metabolic disorders caused by irrational feeding, malnutrition, asthenic syndrome, etc.; anemia with helminthiasis; secondary anemias, as an additional means in the treatment of magnesium and calcium deficiency; to increase muscle activity, with significant loads, overstrain and exhaustion in animals; to increase the body's resistance to various pathogens; to stimulate growth, development and live weight gain in young animals and poultry; as an additional means in the treatment of diseases caused by various factors (infectious and non-infectious origin). Pharmacological studies were carried out in the volume determined according to the standard test method (Kotsiumbas et al., 2006; Vasylyev et al., 2021).

The research was carried out in the vivarium of "DAVIE" LLC. Premises with a total area of 50 m2, where a relatively small number of animals are kept for scientific purposes under the supervision of "DEVIE" LLC specialists. The diet includes all the necessary ingredients. Laboratory animals were housed in standard cages (8 cages) with a floor area of 40×60 cm, i.e., sufficient space for free movement, and two cages of 20×40 cm, where the space for movement was reduced by three times.

To determine the embryotoxicity, mutagenicity, and teratogenicity of the "BTF PLUS" drug, an experiment was conducted on female white rats with an initial weight of (230 ± 5) g, according to (Kotsiumbas et al., 2006).

Studies of the estrous cycle were conducted in females. The first day of pregnancy of females was determined by the presence of spermatozoa in vaginal smears.

According to the principle of analogs, using body weight as the main criterion, one control and two experimental groups of 30 pregnant animals each were formed, further divided into three subgroups of 10 animals each.

The drug "BTF PLUS" was injected subcutaneously in doses (by absolute weight) of 200.0 mg/kg (therapeutic) and 2000.0 mg/kg (10-fold) of body weight: animals of subgroups I – from the first to the 6th day of pregnancy (1st period – period of preimplantation development); II subgroups – from the 6th to the 16th day (the second period – the period of implantation and organogenesis); III subgroups – from the 16th to the 20th day of pregnancy (the third period – the period of fetal development), respectively.

Pregnant female rats of the control group were given water for injections at the indicated times.

Rats were weighed on the study's 1st, 5th, 12th, 16th, and 20th days. On the 20th day of pregnancy, under chloroform anesthesia, the females were euthanized by dislocation of the cervical vertebrae.

A laparotomy was performed, studying the condition of the internal organs of the females. The horns of the uterus with the ovaries were separated and transferred to a Petri dish with an isotonic solution of sodium chloride.

With the help of a binocular magnifier, a thorough examination of the condition of the right and left ovaries was carried out, and the number of corpora lutea of pregnancy in them was counted. In the horns of the uterus, the number of implantation sites and the number of live and dead fetuses were determined.

The placenta was weighed and measured, and its condition was described. Live fetuses were weighed, and the cranio-caudal distance was measured.

After visual inspection, approximately half of the fetuses were placed in Buen's fluid to determine the condition of the internal organs. After fixation, the fruits were cut, 9 – with frontal cuts, and the state of the internal organs was examined with the help of a binocular magnifying glass.

Research on the condition of the internal organs of the fetus was carried out according to the method of J. Wilson. In 50% of the fetuses, the state of the bone system was examined according to the Dawson method.

Experiments on animals were carried out following the requirements of Article 26 of the Law of Ukraine No. 3447-15, "On the Protection of Animals from Cruelty", as amended on October 16, 2012, and Directive 86/609/E EC.

The number of fetuses with developmental anomalies, expressed as a percentage of the total number of live fetuses, was considered an indicator of the teratogenic effect of the drug.

Prediction of the carcinogenic effect, with subcutaneous administration of the drug BTF PLUS, was conducted using the micronucleus test (a method of assessing genotoxicity by detecting micronuclei in mammalian bone marrow cells). The test was carried out by the recommendations. Male and female white non-linear mice with a body weight of 22.0 ± 1.0 g were taken as an experimental model. The animals were kept in standard vivarium conditions at a temperature of 18–21 ºC and humidity of 55–65 %, with artificial lighting and free access to water and feed.

In the first experiment series, the drug "BTF plus" in doses of 200.0 mg/kg and 2000.0 mg/kg of body weight was administered subcutaneously once only to male mice (n = 6) with fixation of cellular material 24 hours after administration. In the second series, the tested drug in similar doses was administered subcutaneously to male and female mice (n = 6) every day for five days. Fixation of cellular material was carried out 24 hours after the last injection.

To experiment according to the principle of analogs, two control groups (positive and negative control) of 6 mice each were also formed. The negative control was water for injections. Cyclophosphamide at a dose of 20 mg/kg of body weight was used as a positive control for single administration (oral).

Cytogenetic preparation was prepared according to methodical instructions. The obtained preparations (two glasses from each animal) were subjected to microscopic cytogenetic analysis.

Two thousand polychromatophilic erythrocytes from each animal were analyzed, and the ratio of regular and polychromatophilic erythrocytes was determined by counting 500 erythrocytes. The criterion for a positive result was a reproducible and/or dose-dependent significant increase in the number of polychromatophilic erythrocytes (PCE) from the micronucleus in at least one of the groups compared to the control group. The obtained positive result indicates that the substance induces chromosomal damage and disruption of the mitotic apparatus of cells in experimental animals.

Statistical processing was carried out following generally accepted recommendations. The share of PCE from all erythrocytes typically should not exceed 0.2 %.

3. Results and discussion

It was established that during the entire pregnancy period in female rats of the control and experimental groups, the drug "BTF plus" did not affect the general clinical condition of the animals. Feed and water consumption; behavioral reactions corresponded to the general indicators of the physiological norm. During the entire pregnancy period, no differences were found in the dynamics of the body weight of rats in the experimental groups compared to the intact control (Table 1).

Table 1

Dynamics of the body weight of female rats during pregnancy when studying the embryotoxic, mutagenic, and teratogenic effects of the drug "BTF plus" (M ± m; n = 90)

Group	Day of introduction	Observation period/day/body weight of rats, g				
		1 Day	5 Day	12 Day	16 Day	20 Day
CONTROL	1–6	228.3 ± 2.35	232.1 ± 2.27	294.7 ± 1.46	332.9 ± 1.17	338.9 ± 1.27
	6–16	229.7 ± 1.63	234.7 ± 1.12	296.4 ± 2.24	334.4 ± 2.45	339.5 ± 2.12
	16–20	228.1 ± 2.53	232.5 ± 2.21	295.2 ± 2.85	333.2 ± 1.92	338.0 ± 2.34
I Experiment 200.0 mg/kg	1–6	226.7 ± 1.23	235.2 ± 2.13	295.5 ± 2.38	334.8 ± 2.71	339.4 ± 2.15
	6–16	228.2 ± 2.81	232.7 ± 2.27	296.3 ± 2.14	330.2 ± 1.25	336.5 ± 1.39
	16–20	227.6 ± 2.44	233.2 ± 1.38	294.4 ± 2.06	336.4 ± 2.13	341.7 ± 2.34
II Experiment 2000.0 mg/kg	1–6	226.7 ± 2.62	230.6 ± 2.21	296.8 ± 1.24	334.6 ± 1.44	339.6 ± 2.15
	6–16	229.4 ± 2.76	234.4 ± 1.12	296.1 ± 1.71	336.5 ± 2.26	341.4 ± 2.73
	16–20	229.2 ± 1.29	234.7 ± 2.46	295.5 ± 2.57	332.1 ± 1.19	338.7 ± 1.11

Indicators of total, preimplantation, and postimplantation embryonic lethality in rats of experimental groups had no significant differences compared to indicators in controls. Also, no teratogenic effect on embryos was detected, and the weight of the placenta, fetuses, and their cranio-caudal size were at the level of the control group (Table 2).

Therefore, the drug "BTF plus" (solution for injections), under the conditions of subcutaneous administration to pregnant female rats in doses of 200.0 (therapeutic) and 2000.0 mg/kg (tenfold) of body weight, does not cause death or pathological changes of embryos, does not have an embryotoxic and teratogenic effect.

Thus, (Table 3) in mice from the negative control group, which received water for injections subcutaneously, the proportion of polychromatophilic erythrocytes was 0.1–0.117 %, within the normal range. In mice from the positive control group receiving cyclophosphamide at 20 mg/kg of body weight, the proportion of polychromatophilic erythrocytes was 0.667–0.717 %.

In mice of both sexes, which were subcutaneously injected with "BTF plus" for five days in a therapeutic dose (200.0 mg/kg of body weight) and tenfold (2000.0 mg/kg of body weight), the proportion of polychromatophilic erythrocytes had no probable deviations between themselves and was 0.117–0.133 %, which was within the normal range.

Table 2
Indicators of embryotoxic and teratogenic effect of the drug "BTF plus" after subcutaneous administration to pregnant female rats (M ± m; n = 90)

Indexes	Research results								
	Control			I group (200.0 mg/kg), day of introduction			II group (2000.0 mg/kg), day of introduction		
	1–6	6–16	16–20	1–6	6–16	16–20	1–6	6–16	16–20
Number of pregnant females	10	10	10	10	10	10	10	10	10
The number of corpora lutea	10,50 ± 0,37	10.30 ± 0.37	10.20 ± 0.36	10.60 ± 0.52	11.10 ± 0.31	11.20 ± 0.57	10.70 ± 0.26	10.00 ± 0.37	10.30 ± 0.37
Number of live fruits	9,50 ± 0,37	9.30 ± 0.30	9.30 ± 0.34	9.70 ± 0.45	10.20 ± 0.39	10.10 ± 0.58	9.70 ± 0.26	9.00 ± 0.33	9.30 ± 0.21
Number of dead and resorbed	0,10 ± 0,10	0.10 ± 0.10	0.20 ± 0.13	0.10 ± 0.10	0.10 ± 0.10	0.10 ± 0.10	0.10 ± 0.10	0.10 ± 0.10	0.10 ± 0.10
Total embryonic mortality, %	9.64 ± 0.38	9.49 ± 1.38	8.81 ± 1.04	8.12 ± 2.14	8.21 ± 1.64	9.98 ± 0.88	9.39 ± 0.22	9.89 ± 1.53	9.24 ± 2.02
Preimplantation mortality (%)	10.48 ± 0.77	10.40 ± 1.63	10.81 ± 1.84	8.89 ± 2.26	9.21 ± 2.03	11.09 ± 1.51	10.30 ± 0.90	10.89 ± 2.36	10.24 ± 2.29
Postimplantation mortality (%)	0.83 ± 0.51	0.91 ± 0.56	2.00 ± 0.82	0.77 ± 0.47	1.00 ± 0.61	1.11 ± 0.68	0.91 ± 0.56	1.11 ± 0.68	1.00 ± 0.61
Fruit weight (g)	2.77 ± 0.039	2.81 ± 0.058	2.78 ± 0.063	2.83 ± 0.024	2.88 ± 0.032	2.82 ± 0.029	2.78 ± 0.038	2.79 ± 0.039	2.81 ± 0.032
Cranio-caudal size (mm)	31.62 ± 0.30	31.46 ± 0.31	31.45 ± 0.34	31.52 ± 0.34	31.55 ± 0.40	31.48 ± 0.32	31.38 ± 0.44	31.48 ± 0.45	31.40 ± 0.45
Placenta weight (g)	0.50 ± 0.013	0.49 ± 0.022	0.49 ± 0.013	0.50 ± 0.015	0.50 ± 0.020	0.49 ± 0.019	0.50 ± 0.019	0.50 ± 0.020	0.49 ± 0.010
External examination of fruits									
the number of examined fruits, of them with developmental anomalies (abs.; %)	96 (0; 0)	94 (0; 0)	95 (0; 0)	98 (0; 0)	103 (0; 0)	102 (0; 0)	98 (0; 0)	91 (0; 0)	94 (0; 0)
The state of the bone system									
the number of examined fruits, of them with developmental anomalies (abs.; %)	48 (0;0)	47 (0;0)	47 (0;0)	49 (0;0)	51 (0;0)	51 (0;0)	49 (0;0)	45 (0;0)	47 (0;0)
The state of internal organs									
the number of examined fruits, of them with developmental anomalies (abs.; %)	96 (0; 0)	94 (0; 0)	95 (0; 0)	98 (0; 0)	103 (0; 0)	102 (0; 0)	98 (0; 0)	91 (0; 0)	94 (0; 0)

Table 3
Results of evaluation of the cytogenetic activity of the drug "BTF plus" in the micronucleus induction test in mammalian bone marrow cells

Group (drug, dose)	№ mouse	Number of PCE with micronuclei per 1000 PCE		The share of PCE from all erythrocytes, %
		For each mouse	For the group as a whole	
1	2	3	4	5
The first series of the experiment (single injection)				
Male mice, negative control (water for injection)	1	0	1.00 ± 0.63	0.1
	2	1		
	3	1		
	4	2		
	5	1		
	6	1		
Male mice, positive control (cyclophosphamide at a dose of 20 mg/kg of body weight)	1	7	6.67 ± 1.63	0.667
	2	8		
	3	5		
	4	9		
	5	6		
	6	5		
Male mice, "BTF plus", 200.0 mg/kg body weight	1	1	1.17 ± 0.41	0.117
	2	1		
	3	2		
	4	1		
	5	1		
	6	1		

continuation of table 3

1	2	3	4	5
Male mice, "BTF plus", 2000.0 mg/kg of body weight	1 2 3 4 5 6	2 1 1 1 1 1	1.17 ± 0.41	0.117
The second series of the experiment (subcutaneous administration for five days)				
Male mice, negative control (water for injection)	1 2 3 4 5 6	2 1 1 1 1 1	1.17 ± 0.41	0.117
Male mice, positive control (cyclophosphamide at a dose of 20 mg/kg of body weight)	1 2 3 4 5 6	7 7 7 9 6 5	6.83 ± 1.33	0.683
Male mice, "BTF plus", 200.0 mg/kg body weight	1 2 3 4 5 6	1 1 1 1 1 2	1.17 ± 0.41	0.117
Male mice, "BTF plus", 2000.0 mg/kg of body weight	1 2 3 4 5 6	1 1 1 2 1 1	1.17 ± 0.41	0.117
Female mice, negative control (water for injection)	1 2 3 4 5 6	1 0 1 1 1 2	1.00 ± 0.63	0.100
Female mice, positive control (cyclophosphamide in a dose of 20 mg/kg of body weight)	1 2 3 4 5 6	7 9 7 9 6 5	7.17 ± 1.60	0.717
Female mice, "BTF plus", 200.0 mg/kg body weight	1 2 3 4 5 6	1 1 1 1 1 2	1.17 ± 0.41	0.117
Female mice, "BTF plus", 2000.0 mg/kg of body weight	1 2 3 4 5 6	2 2 1 1 1 1	1.33 ± 0.52	0.133

Therefore, the drug "BTF plus", under the conditions of 5-day subcutaneous administration in doses of 200.0 and 2000.0 mg/kg of body weight, does not show a carcinogenic effect (during microscopic studies, the proportion of polychromatophilic erythrocytes did not have probable deviations from each other and was 0.117–0.133 %, which was within the normal range of 0.2 %).

4. Conclusions

1. The drug "BTF plus", under the conditions of subcutaneous administration to pregnant female rats in doses (based on the absolute weight of the drug) of 200.0 and 2000.0 mg/kg of body weight, does not cause death and pathological changes of embryos, does not cause embryotoxic and teratogenic effects actions, since the indicators of total, preimplantation, and postimplantation embryonic

lethality in rats of the experimental groups did not have significant differences, compared to the indicators in control, and also did not reveal changes in the weight of the placenta, fetuses and their cranio-caudal size.

2. The drug "BTF plus", under conditions of 5-day subcutaneous administration to white mice in doses (based on the absolute weight of the drug) of 200.0 and 2000.0 mg/kg of body weight, does not show a carcinogenic effect (during microscopic studies, the proportion of polychromatophilic erythrocytes had no probable deviations among themselves and amounted to 0.117–0.133 %, which is within the normal range of up to 0.2 %).

Prospects for further research. Further studies will be the next stage of pre-registration tests to study the ecotoxicity of "BTF plus", which is mandatory material of the "Safety and residue studies" section of the dossier for this medicinal product.

Conflict of interest

The authors declare that there is no conflict of interest.

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