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EXPERIMENTAL STUDY OF PANTOHEMATOGEN AS A FUNCTIONAL INGREDIENT FOR DIETARY SUPPLEMENTS: TOXICITY

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ABSTRACT

The research was aimed at examining the safety of pantohematogen, which is produced from velvet antlers of the Altai Wapiti and is used as a functional ingredient in a range of supplements. Wistar rats (males and females) were injected with pantohematogen intragastricly in the maximum dose possible. During a six-month-long study, the influence of pantohematogen on the overall well-being, weight, peripheral blood, bone marrow, liver, kidneys, central nervous system, and cardiovascular function was examined. Throughout the experiment, the rats behaved as usual, their skin, hair, and appetite remained normal, reflexes were preserved, and the digestive and excretory systems were functioning properly. Therefore, no manifestations of the toxic effect of pantohematogen, administered intragastrically, were observed. The findings of the study revealed that doses of 250 and 500 mg/kg of pantohematogen led to increased liver weight, as well as decreased testicles in the male rats (the condition remained 2 weeks after the end of the administration). Examination of other internal organs in all experimental groups did not reveal any pathology when compared with the control and the intact animals. The doses of pantohematogen used in the experiments exceeded the doses for humans (per 1 kg of weight) by 2, 10, and 20 times. The findings of the study revealed no toxicity of pantohematogen. The study was performed at Tomsk National Research Medical Centre of the Russian Academy of Sciences and supervised by Suslov N.I., Doctor of Medical Science.

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Introduction

Antler products are widely used in a variety of dietary supplements for their adaptogenic properties [1-6]. The use of pantohematogen, produced from velvet antlers of the Altai Wapiti, has recently grown in popularity. The present study aims to examine the safety of pantohematogen [1, 7-14].

Materials and Methods

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The study was performed at Tomsk National Research Medical Centre of the Russian Academy of Sciences and supervised by Suslov N.I., Doctor of Medical Science.

A six-month-long series of experiments on Wistar rats (males and females) was performed to examine the effect of Pantohematogen on overall well-being, weight, peripheral blood, bone marrow, liver, kidneys, central nervous system, and cardiovascular function [15, 16]. Pantohematogen was injected intragastricly [17, 18].

Acute Toxicity

For the experiments, both male and female Wistar rats weighing 170-240 g and CBA mice were kept in a standard vivarium. The object of the study was the dosage form of pantohematogen. Before use, pantohematogen was ground and mixed with a 1% starch solution.

Wistar rats (5 males and 5 females) were administered a single dose of 5000 mg/kg dissolved in a 1% starch solution in the ratio of 1 g of pantohematogen to 4 ml of the solution. A two-week observation detected no deviations from the norm. Throughout the experiment, the rats behaved as usual, their skin, hair, and appetite remained normal, reflexes were preserved, and the digestive and excretory systems were functioning properly. Therefore, no manifestations of the toxic effect of pantohematogen, administered intragastrically in the maximum dose possible, were observed.

CBA mice (5 males and 5 females) were administered 5000 mg/kg dissolved in a 1% starch solution (in the ratio of 1:4). To avoid exceeding the maximum dose allowed for intragastric administration, the dose was divided into two parts. A two-week monitoring was performed. On Day 1, there was a reduction in activity and appetite. On Days 2 and 3, the mice started sneezing, their coats were unkempt, and their activity was low. On Day 6, their activity was back to normal. For the rest of the two weeks, the mice behaved normally, demonstrating no manifestations of toxicity.

CBA mice (5 males and 5 females) were administered 10000 mg/kg divided into three parts and injected on the same day. A two-week monitoring was performed. On Day 1, there was a greater decrease in activity and appetite when compared with the mice administered 5000 mg/kg. On Days 2 and 3, the mice started sneezing, their coats were unkempt. Sneezing and unkempt appearance continued throughout Days 4, 5, and 6. For the rest of the two weeks, there were neither manifestations of toxicity nor deaths, therefore, LDSO could not be calculated.

According to the data obtained during the experiment, pantohematogen can be identified as a relatively harmless substance (Class 4 - low-hazard substance, GOST (Official State Standard, Russia) 12.1.007-76).

Chronic Toxicity

To assess the chronic toxicity of pantohematogen, 130 Wistar rats (65 females and 65 males) were used. Animals were administered an experimental solution prepared with 10 parts of pantohematogen, 20 parts of glucose, and 1 part of ascorbic acid. The three doses used were as follows: Group 1 (effective) - 155 mg/kg (50 mg/kg of pantohematogen), Group 2 (intermediate) - 775 mg/kg (250 mg/kg of pantohematogen), and Group 3 (maximum) - 1550 mg/kg (500 mg/kg of pantohematogen).

The animals from the control group were administered a 21% solution of 20 parts of glucose and 1 part of ascorbic acid (1050 mg/kg) **(Table 1)**. Both the experimental solution and the control solution were injected 7 days a week for 6 months.

Table 1. Groups and doses

Group	Dose (pantohematogen content)	Males	Females
Group 1	50 mg/kg	15	15
Group 2	250 mg/kg	15	15
Group 3	500 mg/kg	15	15
Control	Control solution	10	10
Intact	Intact animals	10	10

During the experiment, the data on changes in overall well-being, weight, peripheral blood, bone marrow, liver, kidneys, central nervous system, and cardiovascular function were collected and analyzed [19, 20]. The morphological study of the lungs, kidneys, heart, brain, spleen, thymus, lymph nodes, adrenal glands, gonads, as well as the gastrointestinal tract was performed [21, 22]. The examinations of the rats from Groups 1, 2, and 3 were carried out at 3 and 6 months after the start of the experimental study, and 2 weeks after the administration (at 6.5 months). The control group was examined at 3 and 6 months, the intact rats were examined at 3 and 6.5 months. The student's test was applied to evaluate the data.

Results and Discussion

Overall Well-Being and Weight

During the 6 months of the intragastric administration of the experimental solution, no pathological changes in the overall well-being (behavior, appetite, coat, mucous membranes, and pupils) were observed. The rats were weighed once a week for the first three months and once a fortnight for the rest of the experiment. No statistically significant differences were observed in female rats from Groups 1, 2, and 3 when compared with the data on the control group and intact animals. Male rats from Group 3 (500mg/kg of pantohematogen) gained more weight (1.5-2 times) than male rats from Groups 1 and 2, the control

group and intact animals. No animals died during the experiment.

Peripheral Blood

Samples of peripheral blood were taken from the tail vein in the rats. Traditional methods were applied to study hemoglobin, the erythrocyte, reticulocyte, platelet, leukocyte counts, and leukogram. The erythrocyte osmotic resistance test was carried out to assess erythrocyte resistance to hemolysis0. The intensity of hemolysis was measured by studying the hemoglobin content in the supernatant obtained after incubation of erythrocytes (0.04 ml of citrated blood) in a hypertonic solution (0.2 ml of 6.5% NaCl solution) for 60 minutes. After incubation, the cell suspension was centrifuged for 5 min at 1500 rpm and left in the refrigerator for 2 days. Then, we took 0.08 ml of the supernatant, mixed with 3 ml of the transforming solution, and analyzed the samples with a GF-C-104 haemoglobinometer in the wavelength range between 325 and 500 nm. Samples were tested against donor plasma in a 0.9% solution at the dilution ratio of 1:5. Samples of peripheral blood from all the experimental (50, 250, and 500 mg/kg of pantohematogen) and control groups (glucose and ascorbic acid, 20:1), as well as intact animals.

Three-Month Administration

A higher leukocyte count with an increased number of lymphocytes was recorded in the male rats of the control group, compared with the intact animals. At the same time, the female rats of the control group had a lower hemoglobin count. The male rats in Group 1 had lower hemoglobin and erythrocyte counts than the intact animals. The male rats in Group 2 had lower reticulocyte and leukocyte counts, compared with the control group. The female rats in Group 1 had a lower hemoglobin count, compared with the control group, while monocyte and lymphocyte counts were higher than in the control group and the intact animals. The noted changes were not dose-related and were within the reference ranges.

Six-Month Administration

The male rats in Group 1 had higher hemoglobin and erythrocyte counts than the control group and the intact animals. A lower reticulocyte count was not dose-related in the male rats in Groups 1 and 2, since the control group and the intact animals had higher indicators at the beginning. The increased value of the osmotic resistance in rats of both sexes in the experimental groups was not dose-related and was transient, since two weeks after the end of the administration (6.5 months), these values returned to normal.

Group 3 had a higher segmented neutrophil count, compared with the intact animals, while the male rats in Group 2 had eosinopenia and monocytosis. At 6.5 months the values were within the reference range. The changes recorded in the female rats were not dose-related either and were within the reference range at 6.5 months. The reason for the moderate leukocytosis in the male rats in Group 3 at 6.5 months due to neutrophilia, as well as in the female rats in Group 3 at 6 months due to lymphocytosis, remains unclear. The lab results showed changes in peripheral blood parameters that occur during daily six-month administration of pantohematogen (50, 250, and 500 mg/kg), however, these changes are transient and not dose-related. Therefore, the administration of pantohematogen in the applied doses does not have a toxic effect on peripheral blood.

Bone Marrow

The total number of karyocytes per femur (10 million/femur) was determined in the bone marrow. A homogenate of the bone marrow obtained from a segment of the sternum with autologous serum (1: 1) was used for Nocht and Maksimov stained smears. The testing was performed at 3 and 6 months of the experimental solution administration (Groups 1, 2, and 3) and the control solution administration (the control group), as well as at 6.5 months.

At 3 and 6 months, there were no statistically significant differences in most bone marrow parameters between the intact animals and the control. It should be noted, that at 3 months, the female rats in the control group had a higher count of bone marrow cells of the erythroid lineage, compared with the intact animals. However, at 6 months, there was a decrease, but within the reference range.

At 6 months, the female rats in the control group had a higher immature granulocyte count, compared with the intact animals. At the same time, the male rats in the control group had higher lymphocyte and monocyte counts than those of the intact animals. The female rats in Group 3 had a lower count of bone marrow cells of the erythroid lineage, compared with the control and the intact animals, and a higher mature (stab and segmented) neutrophil count. The male rats had a lower eosinophil count than the intact animals. At 6 months, the female rats in Group 3 had a higher immature granulocyte count, compared with the control and the intact animals. The female rats in all groups had a lower count of bone marrow cells of the erythroid lineage, compared with the intact animals.

At two weeks after the end of the administration (6.5 months), the nucleated erythrocyte count was within the normal values. At 6 months, the male rats in Group 3 had a higher nucleated erythrocyte count than the control and the intact animals. The male rats in all groups had a higher lymphocyte count. Other changes were either minor or irregular. All changes in the bone marrow parameters in the rats in the experimental groups were transient. Therefore, a six-month-long administration of pantohematogen in different doses does not have a toxic effect on bone marrow.

The Central Nervous System

At 3, 6, and 6.5 months, the Open Field test was performed to assess anxiety and exploratory behavior in the rats (male and female were in separate groups). The results obtained were compared with the results from the control group and the intact

animals. A square open field maze with white walls measuring 100x100x60 cm was used. The floor of the chamber was divided into 16 squares, each with a round hole in diameter of 6 cm. A 100-watt electric incandescent lamp was located 1 m above the floor of the chamber. The rats were placed in a corner of the maze. For two minutes, line crossing (number of crossed horizontal squares), hole-poking, rearing, grooming, and defecation were recorded.

A semi-quantitative method was applied to assess responses to being caught in a home cage; being caught on a flat surface; being approached with forceps. While assessing responses, defecation, urination, squeaking, and muscle tension were also registered. All responses were given points (from 1 to 4); the total sum was then used to assess emotional reactions. The null hypothesis was applied to evaluate the differences.

The findings of the study revealed certain behavioral changes in the rats administered pantohematogen. These changes are slightly different for male and female rats and are more evident over time. At 3 months, the male rats in Group 3 demonstrated increased emotional reactions, locomotor activity was higher in Group 2. However, the same changes were more obvious in female rats and only in Group 2.

At 6 months, the male rats in Group 3 showed slightly higher locomotor activity, while the emotional reaction was similar to the control group. The female rats in Group 3 showed a lower emotional reaction.

At 6.5 months, the male rats in Group 2 had reduced emotional reactions, while the level of locomotor activity did not change. The female rats did not differ from the control.

Thus, the first three months of administration of pantohematogen resulted in a significant increase in emotional reaction and locomotor behavior. The six-month-long administration of pantohematogen decreased locomotor activity to comparable with that of the control group; while the emotional reaction of the male rats was lower than in the control.

Liver Functioning

The liver function tests including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), total protein were taken. The blood glucose was measured to assess carbohydrate metabolism in the liver. Urea and creatinine were measured to study nitrogen metabolism in the liver.

The non-inhalation anesthetics (like Hexobarbital and Thiopental) that are degraded in the liver are known to help assess the antitoxic function of the liver, as well as microsomal oxidation. For the Hexobarbital Sleep Test, a 1% solution of Hexobarbital (80 mg/kg) was used.

The duration of hexobarbital sleeping time in the male rats in Groups 1 and 2 was reduced both at 3 and 6 months. However, the female rats from Group 2 had a longer hexobarbital sleeping time at 6 months. At two weeks after the end of the administration, the duration of hexobarbital sleeping time in all experimental groups was similar to the control group and the intact animals. At 3 months, there were no significant changes in blood biochemical parameters **(Table 2)**.

Table 2. Blood biochemical parameters.

Groups and doses	Males	Females
At 3 months		
Intact animals	31.25±7.33	116.20±8.82
The control group	31.80±2.89	106.60±11.99
Group 1 (50 mg/kg)	28.80±3.67	122.20±5.13
Group 2 (250 mg/kg)	15.00±6.16	129.20±1.53
Group 3 (500 mg/kg)	26.75±5.56	119.80±2.40
At 6 months		
Intact animals	39.00±2.26	89.60±6.10
The control group	31.80±2.89	93.80±5.28
Group 1 (50 mg/kg)	26.20±3.37	99.00±8.33
Group 2 (250 mg/kg)	40.20±3.23	113.00±6.17
Group 3 (500 mg/kg)	35.50±0.50	85.60±3.92
Two weeks after the end of the administration		
Intact animals	39.00±2.26	89.60±6.10
The control group	31.80±2.89	93.80±5.28
Group 1 (50 mg/kg)	32.40±1.12	98.00±21.97
Group 2 (250 mg/kg)	38.00±3.00	96.48±13.94
Group 3 (500 mg/kg)	39.80±3.35	95.00±12.33

At 6 months, the male rats in Group 3 had a higher AST level, compared with the control group. The statistically significant difference in ALT levels when compared with the intact animals was registered in Groups 1 and 2. In some groups, the blood glucose level was higher, while urea and creatinine were reduced. It should be noted, that the female rats were less affected by

pantohematogen than the male rats. Two weeks after the end of the administration, blood biochemical parameters in the experimental groups were similar to the control group and the intact animals. Little changes throughout the entire observation period were transient and not dose-related. Therefore, a six-month-long administration of pantohematogen in different doses (50, 250, and 500 mg/kg of pantohematogen) does not damage the liver.

Kidney Functioning

To assess kidney function, we analyzed urine production, pH, protein, glucose, creatinine, and urea. For urine collection, the rats were kept in individual cages for a day (water intake accounted for 2% of body weight). The protein in the urine (up to 0.3 g/l) and pH remained within the reference range **(Table 3)**.

Table 3. The kidney function at 3 months, (X±m).

Parameters	**Doses (mg/kg)**				
	50	250	500	Control	Intact
			Males		
pH	6-7	6-7	6-7	6-8	5-7
Daily urine production, ml	13.80±0.78	15.05±0.60	11.12±9.66	10.18±1.17	13.50±1.44
Creatinine, μmol/ml	2.00±0.11	3.30±0.31	5.15±0.63	3.39±1.18	4.76±0.34
Creatinine, μmol/day	27.63±2.20	47.28±7.30	56.49±5.97	30.52±7.32	63.20±4.27
Urea, mol/l	102.1±11.1	145.2±18.5	151.0±29.9	141.7±30.2	91.9±6.7
Urea, mol/day	1.41±0.17	2.20±0.32	1.81±0.31	1.36±0.22	1.23±0.11
			Females		
pH	6-7	6-7	6-7	6-8	5-7
Daily urine production, ml	12.75±1.36	9.35±0.81	10.18±0.77	10.30±0.10	11.70±1.84
Creatinine, μmol/ml	0.92±0.13	1.93±0.14	4.03±0.53	1.37±0.14	4.89±0.48
Creatinine, μmol/day	11.51±1.96	18.12±1.97	40.37±4.53	14.21±1.42	48.15±4.00
Urea, mol/l	85.0±13.8	117.1±9.6	118.5±8.8	109.1±20.1	106.6±31.4
Urea, mol/day	1.07±0.22	1.11±0.19	1.20±0.09	1.12±0.20	1.17±0.27

There were changes in the concentration and excretion of creatinine and urea.

At 6 months, some groups demonstrated increased urine production and higher levels of creatinine and urea. The most changes were in the male rats.

Two weeks after the end of the administration, some parameters of the kidney function were different from the control. Having analyzed the data obtained, it was concluded that the changes were random, not dose-related, and remained within the reference range. Therefore, a six-month-long administration of pantohematogen in different doses (50, 250, and 500 mg/kg of pantohematogen) does not damage the kidneys.

Heart Functioning

To assess the effect of pantohematogen on the heart, electrocardiography (ECG) data was analyzed. The rats were anesthetized and placed in the supine position, the electrical activity was recorded in 3 standard leads, with an amplification of I mV = 20 mm and a speed of 100 mm/s.

ECG was performed in Wistar rats (males and females) of Groups 1, 2, and 3, the control group, as well as the intact animals at 3 months, 6 months, and 6.5 months (two weeks after the end of administration). No differences in ECG data were found, thus, the sex was not taken into account when performing the statistical analyses. The amplitude of the P, R, and T waves (mV) and the duration of the P-Q, Q-T, and R-R intervals (mm) were measured **(Table 4)**.

Table 4. The electrical activity of the heart, (X±m)

Parameters	**Doses (mg/kg)**				
ECG	**Intact**	**Control**	**50**	**250**	**500**
			At 3 months		
P-Q, s	0.02±0.00	0.03±0.00	0.02±0.00	0.03±0.00	0.03±0.00
Q-T, s	0.12±0.00	0.14±0.01	0.11±0.01	0.11±0.01	0.12±0.01
R-R, s	0.14±0.01	0.16±0.01	0.14±0.01	0.14±0.01	0.15±0.01
P, mV	0.09±0.02	0.06±0.01	0.05±0.01	0.07±0.02	0.04±0.01
R, mV	0.59±0.07	0.48±0.04	0.52±0.08	0.40±0.07	0.57±0.09
T, m	0.23±0.03	0.22±0.03	0.14±0.03	0.15±0.01	0.12±0.03

At 6 months				
P-Q, s	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00
Q-T, s	0.12±0.00	0.10±0.00	0.12±0.01	0.11±0.01
R-R, s	0.14±0.01	0.12±0.00	0.13±0.01	0.13±0.01
P, mV	0.06±0.01	0.05±0.01	0.04±0.01	0.08±0.01
R, mV	0.53±0.04	0.56±0.08	0.49±0.06	0.59±0.08
T, m	0.21±0.04	0.17±0.04	0.14±0.02	0.17±0.02
At 6,5 months				
P-Q, s	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00
Q-T, s	0.11±0.00	0.12±0.00	0.11±0.00	0.12±0.01
R-R, s	0.13±0.00	0.14±0.00	0.13±0.01	0.14±0.01
P, mV	0.07±0.01	0.06±0.01	0.05±0.01	0.05±0.01
R, mV	0.46±0.05	0.66±0.02	0.53±0.09	0.53±0.09
T, m	0.15±0.02	0.21±0.04	0.19±0.03	0.18±0.03

No specific rhythm and conduction abnormalities were found. The rats in the experimental groups had the amplitude and time parameters of the ECG comparable to the control and the intact animals. Although, at 3 months, some parameters in the rats in Group 2 differed from the control and the intact animals, by the end of the six months, there was no difference.

Two weeks after the end of the administration, a statistically significant increase in the R wave was registered in the rats in Group 1 (50 mg/kg of pantohematogen), compared with the control, however, the parameter was similar to the intact animals. Therefore, pantohematogen in different doses (50, 250, and 500 mg/kg of pantohematogen) does not affect the electrical activity of the heart.

Chronic Toxicity Study

To assess chronic toxicity of pantohematogen, Wistar rats weighing 250-300g were intragastricly injected 50, 250, and 500 mg/kg of pantohematogen 7 days a week for 6 months. Each experimental group consisted of thirty animals (15 male rats and 15 female rats). Twenty animals (10 male rats and 10 female rats) comprised the control group and were intragastricly injected with the control solution. Twenty animals (10 male rats and 10 female rats) remained intact.

At 3 months, decapitation was carried out for six rats from each group (3 females and 3 males); at 6 months, for ten rats from each group (5 males and 5 females), at 6.5 months (2 weeks after the end of the administration) for the rest of the rats.

The rats in the control group and the intact animals had similar body weights. However, the body weight in the male rats in Group 3 administered the experimental solution for 3 and 6 months was significantly higher than in the control.

Macroscopic examination of internal organs at autopsy did not reveal any abnormalities in the experimental animals. After dissection, parenchymal organs were isolated and weighed. The weight of the internal organs of the rats in the control and the intact animals did not differ during all periods of the study. In Group 3 (500mg/kg of pantohematogen, at 6 months), there was lower testis weight, while some internal organs were significantly heavier, compared with the control. However, the weight coefficients of the organs did not differ from those in the control, except the thymus (250 and 500 mg/kg of pantohematogen, at 3 months), liver (500 mg/kg of pantohematogen, at 6 months), and testicles (all doses, at 6 months, and 250 and 500 mg/kg of pantohematogen, at 6.5 months).

At 6 months and 6.5 months, microscopic examinations of the brain, pituitary gland, heart, lung, liver, kidney, stomach, small and large intestines, pancreas and thyroid glands, spleen, thymus, adrenal gland, ovary, uterus, and testicles were conducted. Tissues were fixed in formalin and embedded in paraffin. Deparaffinized sections were stained with hematoxylin and eosin.

Microscopic examination of the internal organs of the experimental rats showed that their structure was normal and did not differ from the control and the intact animals. The pia mater of the brain was neither hyperemic nor infiltrated. No symptoms of edema, hyperemia, or infiltration were found in the brain. The cortex and basal ganglia were normal, without any symptoms of dystrophy; the number of glial cells was within the reference range. No symptoms of edema, hyperemia, or infiltration were found in the myocardium of the left and right ventricles of the heart; cardiomyocytes were of normal size; transverse striations were visible in the structure of cells and nuclei. The lumens of the airways and alveoli of the lungs were free, the interalveolar septa were thin, and the vessels of the lungs were not dilated. The thymus was divided into two main lobes with normal cellularity and small Hassall's corpuscles. There were no symptoms of hyperemia in the liver, the structure of the lobules was normal, and hepatocytes showed no signs of abnormalities. Lymphocyte and macrophage accumulation was registered around the vessels and portal tracts in all the rats, in several cases, there was focal lymph macrophage infiltration.

No symptoms of hyperemia, edema, or infiltration were registered in the gastric mucosa. The epithelium had a normal structure without any dystrophic changes or desquamation. Gastric glands were not dilated, and the secretory cells were of the usual shape and color. The mucosa of the small and large intestines showed no symptoms of hyperemia, edema, or infiltration. The epithelium in the crypts and villi of the small intestine had the usual structure with 3-4 mitotic figures in the crypts.

No symptoms of edema, hyperemia, and infiltration were observed in the pancreas. The interlobular connective tissue septa were thin, the structure of the acini was usual, the zymogenic zone was clearly defined, and the cell nuclei were large, clearly stained, and located near the basement membrane. No desquamated epithelial cells in the excretory ducts were found. The

islets of Langerhans were rather large, the capillaries were moderately dilated, and the secretory cells were of the usual shape and color.

There was an indefinite venous plethora in the kidney tissue. The glomerulus was of usual structure and cellularity, the capillaries were moderately plethoric. The epithelium of the tubules in the male rats was normal.

Most of the female rats of all groups had focal deposition of calcium salts in the epithelium of the tubules, without a perifocal reaction. The zones of the adrenal cortex were normal. The pulp was moderately plethoric and contained macrophages, megakaryocytes, lymphocytes, and a small amount of siderophages.

No edema, hyperemia, or infiltration was observed in the testicles. All layers of the spermatogenic epithelium were present in the convoluted seminiferous tubules, however, in the rats from the experimental groups, a non-dose-related reduction of the number of cells was observed. Desquamated epithelial cells were rare.

The number of tubules with 12 stages of meiosis was within the reference range (2-3 per 100 convoluted seminiferous tubules). The number and arrangement of Leydig cells were normal.

No hemodynamic disturbances were observed in the ovaries. There were follicles and corpus luteum at all stages of development, single atretic follicles, and atretic bodies. The thickness of the endometrium was normal, the endometrial glands were moderately tortuous, lined with cuboidal epithelium with light cytoplasm, the lumen of the glands was enlarged, and no desquamated epithelial cells were noted. The connective tissue between the glands was abundantly infiltrated with leukocytes, predominantly eosinophilic. The myometrium was moderately plethoric.

In all rats (the experimental and control groups), the thyroid gland was slightly enlarged, the thyroid tissue was plethoric, and the layers of connective tissue between the lobules were thickened. Moderate lymphoid infiltration of the gland tissue (similar in structure to diffuse parenchymal goiter) was registered. In individual rats, moderate hyperemia of the anterior lobe of the pituitary gland was observed.

Conclusion

Therefore, doses of 155, 775, and 1550 mg/kg of the experimental solution (50,250, and 500mg /kg of pantohematogen) did not affect the overall well-being, peripheral blood parameters, bone marrow, liver, kidneys, or heart.

However, a dose of 500 mg/kg resulted in (a) greater weight gain in the male rats; (b) increased emotional reaction at 3 months (both sexes); and (c) increased locomotor activity in the female rats.

The findings of the morphological study revealed that doses of 250 and 500 mg/kg of pantohematogen led to increased liver weight, as well as decreased testicles in the male rats (the condition remained 2 weeks after the end of the administration).

Histological examination of other internal organs in all experimental groups did not reveal any pathologies when compared with the control and the intact animals.

The doses of pantohematogen used in the experiments exceeded the doses for humans (per 1 kg of weight) by 2, 10, and 20 times. The findings of the study revealed no toxicity of pantohematogen.

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