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Pantohematogen Safety Study: Embryotoxicity or Teratogenicity

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ABSTRACT

The research was aimed at examining the embryotoxic or teratogenic effects of pantohematogen, which is produced from velvet antlers of the Altai Wapiti and is used as a functional ingredient in a range of confectionery products. A total of 180 female Wistar rats were studied. During the study, the rats were administered different doses of the experimental solution, although, no significant differences in either appearance or behaviour were observed. The findings of the study indicate that pantohematogen neither affects fertility nor increases embryonic death. However, the female pups from the dams injected with the subtoxic dose of the experimental solution demonstrated delayed puberty. With a lower dose of the experimental solution, no delays in the onset of puberty were observed. The findings of the research suggest that pantohematogen does not possess any undesirable properties and is safe to use as a functional ingredient. The research was performed at Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk National Research Medical Centre of the Russian Academy of Sciences, and supervised by Tatyana G. Borovskaya, D.Sc., Professor, Head of the Laboratory of Pharmacology of the Reproductive System.

Keywords: Dietary supplement, Pantohematogen, Deer antler, Effectiveness, Safety.

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INTRODUCTION

Supplements with adaptogenic substances have been used in herbal medicine for centuries, and now antler products that contain pantohematogen are experiencing growing popularity [1-9], therefore, it is very important to examine the safety of pantohematogen. In Russia, pantohematogen is produced from velvet antlers of the Altai Wapiti. In previous studies, velvet antler was found to be of value for its anti-inflammatory effect, immune-boosting potential, as well as its stress-relieving effect [10-13].

MATERIALS AND METHODS

The present study was performed at Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk National Research Medical Centre of the Russian Academy of Sciences and supervised by Tatyana G. Borovskaya, D.Sc., Professor, Head of the Laboratory of Pharmacology of the Reproductive System.

To study the effect of pantohematogen on antenatal development, 180 female Wistar rats were selected to form groups of 14-17 rats. The

female rats were housed with intact males in the 2:1 ratio. The day of detection of spermatozoa in a vaginal smear was considered to be the first day of pregnancy. For the experimental solution, 10 parts of pantohematogen were mixed with 20 parts of glucose and 1 part of ascorbic acid to be injected into the stomach once daily. The solution for the control groups consisted of 20 parts of glucose and 1 part of ascorbic acid.

The following doses of the experimental solution were applied: a subtoxic dose of 1550 mg/kg (500 mg/kg of pantohematogen), an intermediate dose of 775 mg/kg (250 mg/kg of pantohematogen), and a therapeutic dose of 155 mg/kg (50 mg/kg of pantohematogen). The solution for the control groups was dosed likewise (1050, 525, and 105 mg/kg). The rats were weighed on Days 1, 7, and 14; their appearance and behavior were continuously observed.

On Day 20, manual cervical dislocation was carried out and the reproductive organs and the foetuses were studied. We examined corpora lutea, implantation sites, and live and dead fetuses, and calculated pre- and post-implantation death rates. The foetuses were weighed, their macroscopic examination was performed, the cranio-caudal size was measured, and the sex was determined. Half of the foetuses were then placed in Bouin solution for fixing and further examination of the visceral organs with the Wilson technique, while the rest were fixed and stained by the Dawson method for skeletal examination. For statistical analysis, a litter was taken as a unit of observation, i.e. data obtained during the autopsy of one female rat.

To study the effect of pantohematogen on postnatal development, pregnant rats from the experimental group were injected with 1550 mg/kg of experimental solution from Day 6 until delivery; while pregnant rats from the control group received the solution of glucose and ascorbic acid (1050 mg/kg). During pregnancy, rats were weighed, and their appearance and behavior were observed. On Day 18 rats were separated and put into individual cages to prepare for the delivery. Upon delivery, the delivery date, litter size, and sex were recorded. Twenty-four hours after the delivery only four male pups and four female pups were selected from each litter. Dams were removed between Days 25 and 30.

We monitored the physical development of the pups, the dynamics of their weight gain, and assessed their motor activities, their exploratory behavior and general activity with the Open Field Test, their learning ability (CRPI), as well as their adaptive behaviour (the stress response).

To study the effect of pantohematogen on fertility, two experimental studies involving 60 male rats and 190 female rats were performed.

In the first experimental study, for 15 days the experimental group of female rats received 1550, 775, and 155 mg/kg of the experimental solution, while the control group received the solution of glucose and ascorbic acid (1050 mg/kg). Then, for ten days the female rats were kept with intact male rats in a ratio of 2:1. Vaginal smears were used to detect pregnancy. Between Days 17-20, some pregnant rats underwent manual cervical dislocation. Corpora lutea, implantation sites, and live foetuses were studied; pre- and post-implantation death rates were calculated. The rest of the rats delivered the pups, which were then observed to evaluate their physical development. The survival rate was also determined.

In the second experimental study, for 60 days the experimental group of male Wistar rats received 1550 mg/kg of the experimental solution, while the control group received the solution of glucose and ascorbic acid (1050 mg/kg). On Day 61 male rats were separated into individual cages with two intact female Wilstar rats each and kept for 10 days. Vaginal smears were used to detect pregnancy. Between Days 17-20, some pregnant rats underwent manual cervical dislocation. Corpora lutea, implantation sites, and live foetuses were studied; the fertility indices and pre-and post-implantation death rates were calculated. The rest of the pregnant rats delivered the pups, which were then observed to evaluate their physical development. The survival rate was also determined.

The male Wistar rats, which received the experimental solution for 60 days, were killed; their testicles were removed and fixed in Carnoy's solution. Slices of tissue (5-7 μm thick) underwent paraffin sectioning and were stained with hematoxylin and eosin for a morphological analysis.

The obtained data were processed with the Student's test, the Mann-Whitney U test, and the Fisher's angular transformation. The Data from

experimental parameters was carried out using control values, as well as historical control data (HCD).

RESULTS AND DISCUSSION

The findings did not reveal any changes in the appearance or behaviour of the rats from the experimental groups. The dynamics of body weight change demonstrated by the rats from the experimental groups injected with the experimental solution from Day 1 to Day 6 and from Day 6 to Day 16 (the subtoxic dose), as well as from Day 6 to Day 16 (the intermediate dose) were similar to the dynamics of body weight change of the rats from the control groups. However, when comparing the dynamics of body weight change of the control groups to HCD, we observed lower weight gain in the first two weeks of pregnancy in those rats that received the mixture of glucose and ascorbic acid from Day 1 to Day 6 **(Table 1)**.

The experimental group that received the therapeutic dose of the experimental solution demonstrated a reduction in weight gain between Day 7 and Day 14 when compared to both the control group and HCD. Although, this reduction made no significant impact on the overall pregnancy weight gain.

The injections of the subtoxic dose of the experimental solution between Day 16 and Day 20 resulted in a reduction in weight gain when compared to both the control group and HCD. It should also be noted that for the same period weight gain in rats from the control group was lower when compared to HCD.

Table 1. The dynamics of body weight change during pregnancy

Doses and Periods	1-7	7-14	14-20	1-20
HCD	34.41±3.32	26.47±2.60	35.88±1.56	96.18±4.90
Control group, 1050 mg/kg, Days 1-6	25.36±2.13*	15.71±3.05*	45.36±2.06*	87.50±3.13*
Experimental group, 1550 mg/kg, Days 1-6	20.67±2.57	20.33±2.51	40.00±2.63	87.00±4.19
Control group, 1050 mg/kg, Days 6-16	25.14±1.87	27.14±2.44	39.64±4.01	88.21±6.54
Experimental group, 1550 mg/kg, Days 6-16	22.00±4.01	25.31±2.21	41.56±2.40	81.25±6.25
Control group, 525 mg/kg, Days 6-16	31.67±1.18	30.00±3.23	30.00±4.25	91.67±6.07
Experimental group, 775 mg/kg, Days 6-16	15.71±3.09	29.64±3.25	33.93±3.42	79.29±4.86
Control group, 105 mg/kg, Days 6-16	30.56±2.27	32.22±2.90	38.89±2.98	100.56±3.48
Experimental group, 155 mg/kg, Days 6-16	34.64±2.15	15.00±1.62***	42.00±5.12	91.67±5.81
Control group, 1050 mg/kg, Days 16-20	32.31±3.61	30.77±1.86	30.38±2.00*	93.46±3.86
Experimental group, 1550 mg/kg, Days 16-20	37.5±2.15	34.6±3.72	21.43±3.16***	88.93±5.50
Control group, 525 mg/kg, Days 16-20	22.22±2.65	16.11±1.62	49.00±3.00	68.33±5.27
Experimental group, 775 mg/kg, Days 16-20	21.00±2.77	32.00±1.86	33.00±3.89	86.00±5.57

Note: the differences are significant when comparing:
* - The experimental group with the control group and the control group with HCD;
** - the experimental group with HCD;
*** - the experimental group with the control group and HCD.

No influence of doses or injection periods on the sex ratio of pups was registered. The data received indicates that the experimental group of rats treated with the subtoxic dose from Day 1 to Day 6 demonstrated a similar number of corpora letua and implantation sites, and a similar number of live foetuses (per a dam) when compared with the control group. The average weight and the cranio-caudal size were also similar to those of the control group. However, the post-implantation survival rate, on the one hand, was higher when compared with the control group, but, on the other hand, it was similar to HCD. When comparing the post-implantation survival rate in the control group to HCD, we observed a significant reduction **(Table 2)**.

Table 2. The impact of the experimental solution

Groups and Doses	Corpora letua	Implantation sites	Live foetuses	Pre-implantation survival rate, %	Post-implantation survival rate, %	Foetus weight, g	Foetus size, mm
Days 1-6							
HCD, intact	11.85±0.37	10.38±0.53	9.71±0.61	12.62±4.10	7.52±2.19	2.47±0.06	29.57±0.24
Control group, 1050 mg/kg	12.43±0.31	11.15±0.71	11.07±0.62	10.36±4.45	1.07±0.73*	2.37±0.06	27.42±1.89
Experimental group, 1550 mg/kg	11.47±0.46	10.87±0.65	9.93±0.73	6.63±3.18	9.60±2.42*	2.49±0.46	30.06±0.29
Days 6-16							
HCD, intact	11.85±0.37	10.38±0.53	9.71±0.61	12.62±4.10	7.52±2.19	2.47±0.06	29.57±0.24
Control group, 1050 mg/kg	11.47±0.62	10.14±0.86	9.50±0.82	13.36±5.70	5.43±2.93	2.56±0.11	30.02±0.19
Experimental group, 1550 mg/kg	12.39±0.35	11.00±0.82	10.00±0.71	12.12±5.49	7.62±2.10	2.52±0.07	29.79±3.29
Control group, 525 mg/kg	11.11±0.61	10.67±0.58	9.22±0.76	3.78±1.52	14.38±5.76	2.48±0.08	29.70±0.42
Experimental group, 775 mg/kg	11.79±0.53	10.93±0.62	10.07±0.65	7.86±2.95	10.00±3.42	2.37±0.10	29.21±0.47
Control group, 105 mg/kg	11.87±0.57	11.44±0.67	11.11±0.61	4.25±2.35	3.62±1.46	2.54±0.04	29.92±0.19
Experimental group, 155 mg/kg	11.07±0.45	10.00±0.62	9.13±0.77	9.33±3.62	10.47±3.49	2.49±0.05	29.69±0.24
Days 16-20							
HCD, intact	11.85±0.37	10.38±0.58	9.71±0.61	12.62±4.10	7.52±2.19	2.47±0.06	29.57±0.24
Control group, 1050 mg/kg	10.85±0.74	9.23±0.99	8.59±0.95	13.08±7.17	6.92±2.24	2.49±0.05	29.73±0.19
Experimental group, 1550 mg/kg	11.86±0.54	10.93±0.63	10.00±0.48	8.36±2.34	7.36±2.34	2.37±0.04	28.97±0.66
Control group, 525 mg/kg	11.89±0.54	11.11±0.70	10.00±0.67	6.33±4.59	11.11±0.97	2.49±0.04	29.96±0.22
Experimental group, 775 mg/kg	11.00±2.57	10.70±0.65	9.40±0.78	5.40±3.13	9.30±3.23	2.51±0.06	30.73±0.79

The subtoxic and intermediate doses of the experimental solution administered during organogenesis and growth (between Days 6 and 16 and Days 16 and 20), as well as the therapeutic dose from Day 6 to Day 16, did not cause the reduction of the number of corpora letua, implantation sites, or live foetuse. Macroscopic examinations of 1610 foetuses from the experimental and control groups did not reveal any external malformations. Although, the rats treated with the subtoxic doses of the experimental solution between Days 1 to 6 and Days 6 to 16 had 1,5 or 2 times as many foetuses with subcutaneous hemorrhage as observed in the control group and HCD ($p < 0.05$). No significant difference in the number of foetuses with subcutaneous hemorrhage was registered in the rats treated with the subtoxic and intermediate doses between Days 16 and 20 ($p > 0.05$).

We applied the Wilson technique to assess foetal abnormalities of 805 foetuses. When comparing foetuses with malformations of the visceral organs, we did not observe a statistically significant difference between the control group, HCD, the experimental group treated with the subtoxic dose from Day 1 to Day 6, and from Day 16 to 20, and the experimental groups treated with the intermediate and therapeutic doses from Day 6 to Day 16. In some cases, there was a statistically significant decrease in the number of foetuses with hydrocephalus and hydronephrosis compared with the control group and HCD. The examination of foetuses from the experimental group treated with the subtoxic dose during organogenesis revealed hydronephrosis in 5.2% of cases, while no hydronephrosis was registered in the foetuses from the control group. The injections of the intermediate dose from Day 16 to Day 20 resulted in a statistically significant increase in the number of foetuses with hydrocephalus when compared with HCD; however, when compared with the control group, the value was not statistically significant **(Table 3)**.

It should be noted that the injections of glucose and ascorbic acid caused abnormalities. The increased number of foetuses with hydrocephalus was recorded in the control group treated with 1050 mg/kg from Day 1 to Day 6 (when compared with HCD).

Table 3. The data on foetal abnormalities.

Group	Dose	Period	Number of foetuses, %			
			Subcutaneous haemorrhage	Cholestasis	Hydrocephalus	Hydronephrosis
HCD	-	-	39.1	43.4	7.2	10.4
Control group	1050	1-6	19.1	30.1	20.5	2.7
Experimental group	1550	1-6	20.3	29.7	7.8	1.6
Control group	1050	6-16	17.3	39.1	13.0	0.0
Experimental group	1550	6-16	21.0	54.7	14.7	5.2
Control group	525	6-16	43.9	34.1	12.1	2.4
Experimental group	775	6-16	38.8	27.7	0.0	0.0
Control group	105	6-16	43.0	37.2	13.1	1.9
Experimental group	155	6-16	28.0	42.0	15.8	2.8
Control group	1050	16-20	27.5	20.0	18.7	8.7
Experimental group	1550	16-20	43.4	28.0	2.1	8.7
Control group	525	16-20	40.0	27.0	9.8	13.7
Experimental group	775	16-20	39.0	21.8	18.7	1.5

The skeletal examination of 788 foetuses was performed. The statistically significant difference in bone ossification was observed. The foetuses of the dams treated with the subtoxic dose for the whole gestation period demonstrated faster ossification of the sternum, wrist, and sacrum ($p < 0.05$). When compared with HCD, more intense bone formation was also observed if the experimental solution was injected between Days 16 and 20. Even with the reduced dose, the effect continued.

Meanwhile, the foetuses of the dams, treated with the intermediate and therapeutic doses of the experimental solution during organogenesis, showed slower ossification of the sternum, metatarsus, wrist, and sacrum ($p<0.05$). The injections of glucose and ascorbic acid also influenced the ossification speed. The foetuses of the dams injected with 1050 mg/kg of the control solution demonstrated a statistically significant reduction in the ossification speed of the sternum and sacrum when compared with HCD. With the reduction of the dose of glucose and ascorbic acid, the ossification accelerated. The foetuses from the dams injected with 525 and 105 mg/kg of the control solution during organogenesis had a faster ossification of the sternum, metatarsus, sacrum, and metacarpus when compared with HCD ($p < 0.05$).

Observation revealed no significant difference in appearance, weight, or behaviour of the rats treated with 1550 mg/kg of the experimental solution between Days 6 and 22 and the rats from the control group **(Table 4)**. Both the experimental and the control groups demonstrated a lower body weight gain when compared with HCD.

The pups were born at term. For 60 days, we recorded their body weight change. The female pups of the experimental group gained more weight than the female pups from the control group, although their weight gain was comparable with HCD. It should also be noted that the fifteen-day-old and thirty-day-old pups from both the experimental and the control groups weighed less when compared with HCD **(Table 4)**. The assessment of the physical development, hair growth, incisor growth, eye-opening, and testicular descent showed no statistically significant differences between the pups from all the studied groups. The puberty (vaginal opening) in the female pup rats from the control group occurred with an average delay of 5 days.

The horizontal rope walking test performed on the five-day-old pups demonstrated no statistically significant differences between the pups of the experimental and control groups. The righting reflex and the cliff avoidance tests were also performed on the five-day-old pups to evaluate motor skills **(Table 4)**.

To measure the muscular strength of fifteen-day-old pups, grip strength tests were applied. The ability to grasp with forelimbs and hind limbs and the time spent on the bar were measured. We also conducted pull-up tests **(Table 4)**.

Further testing included the open field tests performed on one-month-old pups, with crossing, rearing, and grooming analyzed **(Table 4)**.

Table 4. The dynamics of body weight change during pregnancy

Group	1-7	7-14	14-22	1-22
HCD	31.00±2.45	30.00±2.47	49.00±6.00	110.00±7.58
Control group	23.00±3.73	19.17±0.83*	42.50±4.23	85.83±6.76*
Experimental group	26.00±1.87	15.00±1.58**	49.00±2.92	90.00±3.54**

The dynamics of body weight change of the pups (g)

Group	Sex	Pups' age, days			
		5	15	30	60
HCD	Female pups	9.48±0.20	28.12±1.11	64.15±2.18	155.82±4.91
	Male pups	9.71±0.23	29.04±2.53	66.66±5.38	157.88±7.09
Control group	Female pups	8.68±0.56	22.70±1.30*	55.05±2.90*	136.48±5.44*
	Male pups	8.79±0.46	22.55±1.02*	56.46±2.37*	140.15±5.74
Experimental group	Female pups	8.96±0.53	22.11±1.47±**	55.76±1.15**	164.00±4.97*
	Male pups	9.26±0.61	19.37±1.21**	54.51±1.71**	152.566±4.83

The integration of the sensory and motor systems of the five-day-old pups (x±m)

Group	Sex	Horizontal rope walking test, % of pups	Horizontal rope walking test length, sec	Developed cliff avoidance response, %	The righting reflex, sec
HCD	Female pups	100.00	8.25±1.44	65.00	12.85±2.82
	Male pups	100.00	7.80±1.51	61.60	8.12±1.44
Control group	Female pups	90.33	10.08±1.64	69.50	9.46±3.90
	Male pups	95.83	10.07±1.74	55.50	5.67±2.04
Experimental group	Female pups	100.00	9.68±1.05	53.40	5.78±1.74
	Male pups	100.00	9.50±0.36	35.00	4.75±0.86

The integration of the sensory and motor systems of 15-day-old pups (x±m)

Group	Sex	Number of pups grasping with forelimbs, %	Hang time with forelimbs, sec	Number of pups grasping with hind limbs, %	Number of pups able to perform pull-ups, %
HCD	Female pups	100.00	30.63±10.06	100.00	100.0
	Male pups	100.00	30.83±12.29	100.00	100.00
Control group	Female pups	100.00	9.86±1.49*	91.67	86.17
	Male pups	100.00	10.54±2.25*	73.67	83.33
Experimental group	Female pups	100.00	18.54±3.11*	100.00	100.00
	Male pups	100.00	21.25±2.54*	100.00	100.00

The open field test of one-month-old pups (x±m)

Parameter	HCD		Control group		Experimental group	
	Male pups	Female pups	Male pups	Female pups	Male pups	Female pups
Line crossing	40.95±3.30	49.47±6.86	39.60±3.02	38.59±3.02	48.02±3.76	44.88±6.51
Hole board	5.60±0.90	6.10±1.67	6.54±0.81	7.30±1.01	7.62±0.94	6.47±1.10
Rearing	4.20±1.14	7.10±1.20	5.72±0.98	7.49±4.09	8.53±1.20	3.55±0.65
Grooming	0.45±0.12	0.65±0.23	0.96±0.04	0.96±0.19	0.67±0.15	0.78±0.15
Defecation	1.85±0.38	2.10±0.36	3.07±0.30	2.79±0.61	2.38±0.15	2.56±0.21

The passive avoidance test was applied to assess the learning ability and memory. When preparing to assess the passive avoidance response, we allowed the pups to enter both lit and dark compartments on Day 1 and recorded the time spent in each of the compartments. Comparing the data, we can see that the pups from the experimental group had lower learning ability **(Table 5)**. Moreover, the pups from the control group also demonstrated lower learning ability.

To assess the stress response, we analyzed active behaviors: swimming, diving, and climbing. The

data obtained indicates a statistically significant difference in the stress response when compared to HCD. However, the defecation levels of the pups from both the control and the experimental groups were lower than those of HCD **(Table 5)**.

Table 5. The passive avoidance response

Parameter	HCD		Control group		Experimental group	
	Female pups	**Male pups**	**Female pups**	**Male pups**	**Female pups**	**Male pups**
1 Day 1						
Burrowing, %	100.00	100.00	100.00	100.00	100.00	100.00
Time spent in a lit compartment, sec	22.00±4.15	64.60±10.64	22.08±3.98	49.33±13.48	27.33±5.04	39.00±9.41
Time spent in a dark compartment, sec	158.00±4.15	115.40±10.64	157.92±3.98	130.67±13.48	153.67±5.04	141.00±9.41
Day 2						
Number of pups with developed passive avoidance response, %	90.00	100.00±0.00	66.70	75.00*	33.33**	44.44**
Time spent in a lit compartment, sec	163.50±16.50	180.00±0.00	127.00±22.75	135.08±23.46	99.44±22.55**	118.78±24.50**
Time spent in a dark compartment, sec	16.50±16.50	0.0	53.00±22.75	44.92±23.46*	80.56±22.55**	61.22±24.50**
The active stress response						
Parameter	HCD		Control group		Experimental group	
	Male pups	Female pups	Male pups	Female pups	Male pups	Female pups
Number of diving pups, %	80.0	90.0	70.0	28.57*	72.8	60.0
Time spent in an inner cylinder, sec	81.25±20.85	63.00±13.22	51.14±23.58	63.75±23.57	35.00±14.08	44.88±6.51
Time spent in an outer cylinder, sec	17.38±5.76	13.00±7.08	8.86±3.59	16.75±3.57	10.50±2.67	11.50±2.58
Climbing (mesh), sec	2.50±0.33	11.00±6.14	3.43±1.19	2.50±0.50	2.12±0.23	3.00±0.63
Defecation	4.25±0.75	2.56±0.75	1.71±0.57*	1.75±0.63	1.38±0.42*	0.83±0.17

To assess fertility and pre- and post-implantation mortality, corpora lutea, implantation sites, and live and dead foetuses were compared. No statistically significant influence of the subtoxic dose of the experimental solution injected for 15 days prior to mating with intact males was found **(Table 6)**.

The subtoxic dose of the experimental solution injected for 15 days prior to mating with intact males did not significantly affect the survival rate and weight gain. While no puberty delays were observed in groups with the intermediate and therapeutic doses.

The subtoxic dose of the experimental solution days prior to mating with intact females did not affect litter. The survival rate, physical development, and weight gain were comparable. The analysis of the testicular morphometry did not reveal significant differences between HCD and the control group **(Table 6)**.

Table 6. The subtoxic dose of the experimental solution and the fertility: females (x±m)

Parameter	Group		
	HCD	**Control group**	**Experimental group**
Corpora lutea per female	12.55±0.58	12.42±0.57	13.36±0.59
Implantation sites per female	12.00±0.70	11.58±0.60	12.18±1.19

Live foetuses per female	7.20±3.12	11.25±0.60	11.64±1.19
Pre-implantation mortality, %	10.09±0.91	6.66±2.55	11.04±6.33
Post-implantation mortality, %	13.82±4.86	3.33±0.15	5.05±1.84
Fertility index, %	76.2	100.0	89.5
Pregnancy index, %	100.0	100.0	85.0
The subtoxic dose of the experimental solution and the fertility: males (x±m)			
Parameter	Group		
	HCD	Control group	Experimental group
Corpora lutea per female	12.55±0.58	13.33±0.37	13.22±0.80
Implantation sites per female	12.00±0.70	11.89±1.30	12.67±0.83
Live foetuses per female	7.20±3.12	11.33±1.29	11.56±0.69
Pre-implantation mortality, %	10.09±0.91	11.11±9.30	4.00±3.04
Post-implantation mortality, %	13.82±4.86	4.33±2.61	8.00±3.37
Fertility index, %	76.2	83.3	63.7
Pregnancy index, %	100.0	93.0	100.00

The findings of the study indicate that the subtoxic dose of the experimental solution injected between Days 16 and 20 and the therapeutic dose of the experimental solution injected between Days 6 and 16 of the pregnancy result in slower weight gain in certain pregnancy periods. A higher post-implantation mortality was recorded in the rats from the experimental group injected with the subtoxic dose during the implantation period. Moreover, there was an increase in foetuses with subcutaneous hemorrhage, when the subtoxic dose was injected during the implantation period and organogenesis. Bone ossification was recorded to be influenced by pantohematogen, as the subtoxic dose of the experimental solution accelerated the process of bone formation. When injected during fetogenesis, the subtoxic dose of the experimental solution improved skeletal development. However, the foetuses of the rats injected with the intermediate and therapeutic doses during organogenesis demonstrated slower ossification.

Examining the impact of pantohematogen on the postnatal development of the pups from the dams injected with the subtoxic dose of the experimental solution between Days 6 and 22, we did not detect any developmental delays. Although, two-month-old pups from the experimental group weighed more than the pups from the control group. The results obtained from the open field tests were comparable for all the groups.

The pups from the experimental group demonstrated similar learning abilities to the pups from the control group; however, it was lower when compared with HCD.

Assessment of the stress response revealed adequate reaction to stressful situations from all the pups, while the pups from the experimental group exhibited lower anxiety than the intact ones.

CONCLUSION

The findings of the study indicate that pantohematogen neither affects fertility nor increases embryonic death. With a lower dose, no delays in the onset of puberty were observed.

In conclusion, the findings of the study suggested no negative embryotoxic or teratogenic effects of pantohematogen.

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