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REPRODUCTION STUDIES WITH PRANOPROFEN; A NONSTEROIDAL ANTIINFLAMMATORY AGENT IN RATS–PERINATAL AND POSTNATAL TEST.

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ABSTRACT: *prenatal and postanatal study on pranoprofen, as an antiinflammatory agent, was conducted by oral administration in Sprague–Dawley pregnancy rats from day 17 of gestation to day 21 of after delivery. Pranoprofen was intubated doses of 1.0, 2.5 and 5.0 mg/kg/day and dose of 5.0mg/kg/day of Indomethacin was used as positive control. After delivery, several study indexes such as length of gestation, No. of implantations, No. of live pups, No. of perinatal deaths, sex ratio and No. of malformation were checked and then all the newborns were feeded and investigated physical and behavioral changes. At the highest dose level of 5.0mg/kg/day of pranoprofen, a marked high mortality rate of pups as 59% was observed and 51% and 16% respectively at 2.5 and 1.0 mg/kg/day of pranoprofen dose level. At Indomethacin treated group, most pregnancy rats showed delay of delivery and death without delivery and all the 15 delivered pups were dead within 1 day. At non–treated and CMC control groups showed no problem during pregnancy, delivery and feeding. From the above results, administration of 1.0, 2.5 and 5.0mg/kg/day of pranoprofen to pregnancy rats showed toxic effects to implantations so that high perinatal death and delay of initiation were observed.*

INTRODUCTION

Pranoprofen, a propionic acid derivative with three cyclic structure, is used as analgesic, antipyretic and anti–inflammatory agent (Drugs, 1983). It was newly developed in 1981 by Gil Boo Pharmacy Company in Japan. This non–steroidal antiinflammatory agent, like drugs of this kind, is used clinically in the treatment of rheumatoid and other types of arthritis (Gaut Z.N. 1975). Pranoprofen, salicylates and pharmacologically similar antiinflammatory agents such as indometacin blocks the biosynthesis of prostaglandin by inhibiting the enzyme, prostaglandin synthetase (Piper and Vane, 1969; Vane, 1971), and subsequent reduction of peripheral prostaglandin level occurs.

Generally, non–steroidal antiinflammatory analgesic agent is known to have some side–effects on the gastrointestinal systems and the reproduction system (Aiken, 1972; Wanka,

1964; Lövgren, 1964; Hucker, 1966; Rotemich, 1966; Menguy, 1967; Selye, 1969; Menasse and Gdynia 1974).

In case of indomethacin, several defects were reported that the maternal death and decreased body weight, the fetal death and decreased body weight, decreased fetal ossification, deceased uterine contraction and delayed initiation during animal experiments (Chester, 1972).

From 1984, fertility and teratological effects of pranoprofen were examined (Moon H.H., 1984, 1985) in our laboratory. In present study, perinatal and postnatal study were conducted for the evaluation of toxic effect on pregnancy rats. This study was performed by the NIH guideline No. 267 (1985. 4. 1), guidelines for the test method of specific toxicity test on drugs mainly and several behavioral test methods (Masahiro, 1983; Koichi, 1984; Watanabe, 1984, 1985).

MATERIALS AND METHODS

Test Substance

Pranoprofen, 2 (5H–[1]benzopyrano (2,3–6) pyridine–7–yl), propionic acid with slightly bitter taste, white or yellowish white colored crystal compound was used in this test.

Experimental Animal and Feeding Condition

Sprague–Dawley rats were bred in NIH rat breeding room. 5 weeks old male and female rats were purchased from Sung Hun experimental animal farm and bred in NIH animal room for 8 weeks. Animals were maintained at $23\pm2°C$ and $55\pm5\%$ humidity with a 12 hour light–dark cycle. Animals were fed autoclaved Hanil Chow diet and tap water ad libitum. Good conditioned and normal body weighed 12 weeks old female rats without pregnancy history and 13 weeks old male rats were used in the experiment.

Method of Mating

Estrus cycles of female rats were investigated by Long and Avance methods. Primiparous female rats were mated with male rats from 5 P.M. to 9 A.M. next morning and then confirmed sperm from vaginal fluid by microscoping. This is day zero of gestation when sperms were observed. The period of mating was from day zero of gestation to the second estrus.

Doses Finding and Administration Method

The appropriate amount of pranoprofen was suspended in 100 ml of 0.5% carboxy methyl cellulose (CMC) with 2 drops of Tween 80 added The suspensions were prepared freshly each day just prior to use. Pranoprofen was administered daily by oral intubation for the period of 29 days from day 17 of gestation to 21 days of after delivery with a dose volume of 10 mg/kg. The pregnant rats were divided into 6 groups and each group consisted of 10 female rats; non–treated control group, CMC vehicle control group, indomethacin group as positive control, 1.0, 2.5, and 5.0 mg/kg of pranoprofen treat groups. Pranoprofen doses were selected on the base of maximum tolerance level and non–toxic level derived from chronic toxicity test (浜田佑二, 今村, 1976). Indomethacin was used as a comparative compound on the base of reported teratogenicity test (Physicians Desk Reference 1985).

A. I Group:Non treated group
B. II Group:CMC vehicle control group
C. III Group: Indomethacin treated group
D. IV Group:1.0 mg/kg pranoprofen treated group
E. V Group:2.5 mg/kg pranoprofen treated group
F. VI Group:5.0 mg/kg pranoprofen treated group

Test Method

Using 10 pregnancy female rats in each group, general symptom and death were observed every day. Body weight and consumption of food and water were checked from day 0 of gestation to day 21 of lactation every day. All pregnancy female rat were done normal delivery and made day 0 of delivery when completed delivery. Just after the finishing delivery, duration of gestation, No. of pups deliveried, No. of death, sex, weight, and external abnormality were recorded. During lactation, behavioral changes and physical development were checked and on day 21, all the F0 rats were killed and autopsied and then weight of several organs and No. of implantations were recorded.

On the other hand, young animals were checked weight on the 1st day and 1,2 and 3 weeks after birth, date of eye opening, date of ear opening, date of fur formation, date of normal walking and several physical and behavioral change were checked by below method:

1) Vision (light–dark vision): direct pupillary reflex after incidence of light from a torch in a darkened room.
2) Hearing: the hearing was tested at the end of lactation period using Prey's reflex (pinna twitch reflex) of both ears to stimuli from a Galton's whistle. The duration of stimuli was 0.14 sec. the intervals between the stimuli were 2 sec. and the frequency was 6.7 KHx. In order to maintain the duration and frequency of the stimuli constant, the whistle was actuated with compressed air using an electromagnetic valve. The pulses were initiated by a synchronous motor. The experimental animals sat about 25 cm away from the Galton's whistle on a platform which was attached to a stand such that a direct effect on the animals due to the flow of air was avoided. The design of this experiment was derived from the method of the bioassay of Kanamycin auditory toxicity. (Vernier and Alleva, 1968).
3) Test of rotorod and the learning ability: at the end of the rearing period, the animals were subjected to a balancing test on a rotating cylinder (3 cm diameter) on 3 consecutive days. The rotating cyliner was brought from test immediatedly to 10 rpm by means of a synchronous motor. The animals were required to remain for a maximum of 1 min. on the rotating cylinder in each test. The learning ability was measured on the basis of the improvement in the running performance in 3 days.
4) Multiple water Y–maze test: using Y–maze swimming system sized 80 cm length, 60 cm width, and 30 height and complicated 2 Y–mazes. From day 35 to day 42 of lactation, 8 young female and male rats were tested more than 5 times of Y–maze swimming performace test and numbers. of error were recorded.
5) Active avoidance test: using shuttle box, in case of conditioned stimulation set 80 dB and unconditioned stimulation set 3–4 mA each 5 sec. and total session was 40 sec. For 6 female and 6 male young rats in one group, were tested and for one rat 10 sessions were tested at once and from 6 weeks after delivery tests were started and repeated 5 times till 8 weeks old. During test no. of avoidance responses were recorded.
6) Fertility test on the F1 generation: 7 male and female animals from each group were reared for about 8 weeks up to sexual maturity. They were kept in type III Makrolon cages containing a maximum of 3 animals, separated according to sex and dose. For mating within the groups, the male and female animals were placed individually and they were mated overnight in the ratio 1:1 in the cages of the males. Inseminated females (sperm in a vaginal smear) were kept individually and were permitted to give birth. Care was taken that brother–sister mating did not take place. The criteria for assessment employed were: 1. mating and impregnation rates 2. number of corpora lutea and implantation. Statistics: for the statistical analysis of the results obtained was performed

according to the method of t–test and Chi–squared test. Unless otherwise indicated, a difference is regarded as being significant if, in the statistical computatio, the probability of error that it is wrongly accepted is less than 5% ($P<0.05$).

RESULTS

Effect on FO dams

As shown in Fig. 1, Table 1. and Table 2. maternal body weight gains, and food and

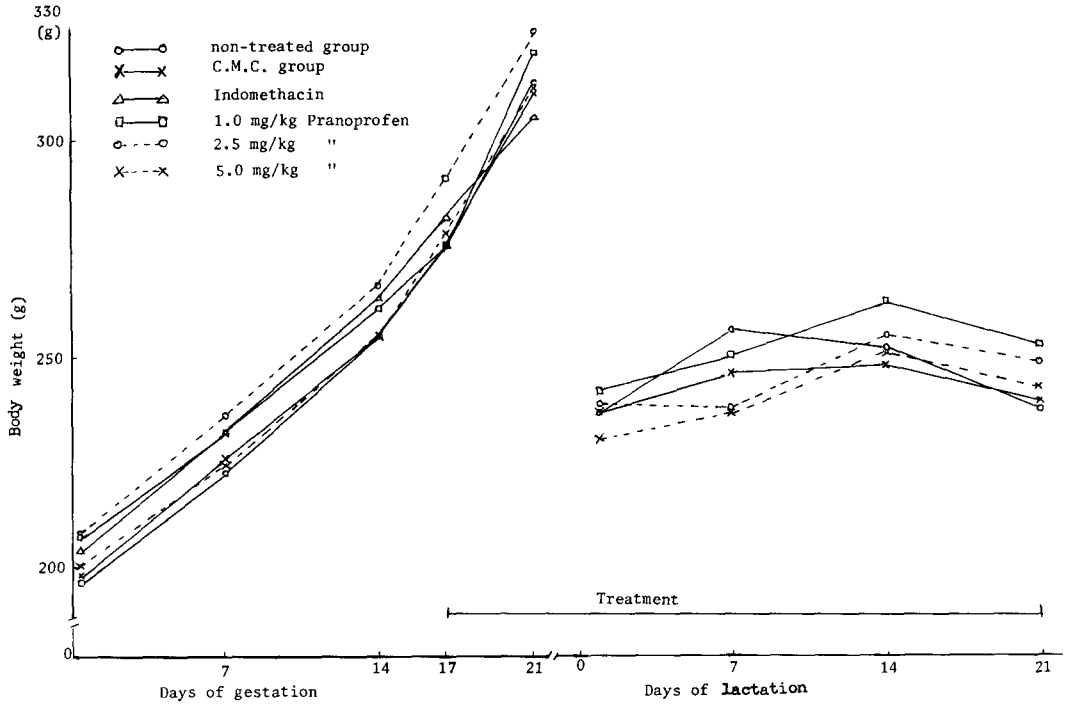

Fig. 1. Body weights of FP dams during gestation and lactation

Table 1. Mean food and water consumptions by dams during gestation period.

Compound	Control			Pranoprofen Treated		
Dose (mg/kg)	Non–treated	C.M.C.	Indomethacin	1.0	2.5	5.0
No. of dams	10	10	10	11	11	11
Food (g/day)	(AV.±S.E.)					
Day 13–16	38.7±2.8	29.5±2.2	28.8± 5.68	25.4±11.45	28.4± 8.98	26.4±2.94**
Day 16–19	37.7±3.3	26.0±0.0	28.7±14.42	35.3±10.76	36.8±23.17	25.68±6.83
Day 19–21	39.5±2.5	17 ±7.0	27.8± 5.21	25.0± 8.21	14.5± 9.95*	18.3±8.88**
Water (ml/day)	(AV.±S.E)					
' 31.5±3.52	30.4±2.35	29.3± 4.35	44.8±17.2	30.0±3.69	34.4±4.76	
32.7±2.31	29.7±0.47	28.6±12.03	33.6± 8.0	32.1±4.98	34.2±2.90	
30.0±2.0	24.0±0.0	27.8±14.0	30.7± 4.19	24.2±9.72	28.2±5.98	

*$0.05<P<0.1$, **$P<0.05$ significant difference from control (non–treated): Student's t–test

Table 2. Mean food and water consumptions by dams during lactation period.

Compound	Control		Pranoprofen Treated		
Dose (mg/kg)	Non-treated	C.M.C.	1.0	2.5	5.0
No. of dams	9	10	9	7	5
Water (ml/day)	(Av. ±S.E)				
Day 0–3	30.3± 1.67	21.3± 4.67	31.2± 1.56	21.0± 4.01	22.8± 3.71
Day 3–6	37.0± 2.89	38.0± 1.56	32.2± 2.45	39.0± 2.00	28.3± 4.91
Day 6–9	40.0± 1.00	44.0± 1.53	38.2± 5.60	47.0± 3.22	33.5± 4.81
Day 9–12	38.3± 6.34	40.3± 2.85	48.5± 2.76	47.7± 2.67	43.7±11.12
Day 12–15	48.7± 1.20	50.0± 4.36	47.8± 8.75	69.0± 3.79	42.8± 6.80
Day 15–18	61.7± 1.66	71.0± 7.01	59.3± 6.11	70.3± 2.19	51.3±10.78
Day 18–21	69.0±10.23	99.3± 7.54	85.2±10.19	84.3± 6.97	54.3±13.40
Food (g/day)	(Av. ±S.E)				
Day 0–3	23.3± 3.18	20.0± 6.68	24.7± 1.50	28.3± 3.67	16.3± 3.51
Day 3–6	35.3± 3.28	27.8± 0.34	28.8± 2.83	32.0± 3.01	27.3± 2.51
Day 6–9	27.0±12.03	35.0± 2.65	33.8± 3.95	42.7± 0.88	29.2± 3.38
Day 9–12	33.7± 0.33	31.0± 5.69	39.5± 1.93	43.7± 0.66	33.2± 4.35
Day 12–15	39.0± 1.53	36.7± 0.31	42.8± 4.82	40.7± 3.53	32.7± 4.51
Day 15–18	35.3±14.68	48.0± 2.00	45.0± 2.05	48.0± 1.00	35.3± 5.04
Day 18–21	69.0±10.23	76.0±13.88	51.7± 6.06	53.7± 3.18	34.0± 6.88

water consumptions in 1.0, 2.5 and 5.0 mg/kg pranoprofen treated groups were similar to those of control groups and no marked changes in general appearance or behavior were observed till day 21 of gestation. From day 22 of gestation, in 2.5 and 5.0 mg/kg pranoprofen treated groups as well as indomethanic group showed no food intake and no activity and syncopic state. In 2.5 and 5.0 mg/kg pranoprofen treated groups and indomethacin group showed delay of delivery. From Table 4, in length of gestation period, 2.5 and 5.0 mg/kg pranoprofen treated group and indomethacin group showed long gestation period as 23.0±0.36, 23.0±0.30 and 23.0±0.40 respectively compared those of 22.0±0.33 and 21.7±0.21 gestation period of non-treated and C.M.C. control groups. It showed 1 or 1.3 day delay of delivery than control groups.

In indomethacin group, 8 maternals among 10 showed death without delivery. And in 2.5 and 5.0 mg/kg pranoprofen treated group, they showed 110 and 100 number of pups deliveried but 51 and 59 pups were dead respectively. it means 46% and 59% of high perinatal death rate compared that of 2.9% of control group. Indomethacin group showed 100% perinatal death rate as 15 death among 15 pups as suspected. Among live birth, the sex ratio, viability index and weaning index showed no difference between control groups and treated groups.

After delivery, all the maternals include control and treated groups showed normal take care of neonates, lactation etc.

On day 21 of laction, all the FO dams were autopsied and eye observation and organ weight were measured. All the dams were showed no special pathological changes but in organ weight especially concerned with lung and spleen of 5.0 mg/kg pranoprofen treated group were significatly lighter than control as 1.28±0.15 g and 0.71±0.4 g compared with those of 1.65±0.15 g and 0.92±0.11 g of control as shown in Table 3.

Effect on Young Animals

1) General observation: among live born pups only one case in 2.5 mg/kg pranoprofen treated group showed malformation and rest of them were normal. During lactation

Table 3. Organ weights of FO dams autopsied on day 21 of lactation.

Dose (mg/kg)	Control		Pranoprofen Treated		
	non-treated	C.M.C.	1.0	2.5	5.0
No. of dams examined	9	10	9	7	5
Body weight (g) (Av. ± SE)	238.2 ± 8.82	236.9 ± 9.98	253.3 ± 11.87	249.1 ± 8.34	243.2 ± 12.83
Organ weight (Av. ± SE)					
Heart (g)	0.81 ± 0.05	0.85 ± 0.04	0.88 ± 0.02	0.87 ± 0.02	0.83 ± 0.06
Liver (g)	1.65 ± 0.15	1.71 ± 0.13	1.50 ± 0.29	1.59 ± 0.40	1.28 ± 0.15
Lungs (g)	11.58 ± 0.86	12.08 ± 0.57	13.59 ± 0.78	12.23 ± 0.23	12.72 ± 0.91
Kidneys (g)	1.97 ± 0.08	1.95 ± 0.07	1.90 ± 0.15	2.04 ± 0.06	1.88 ± 0.08
Spleen (g)	0.92 ± 0.11	0.81 ± 0.06	0.80 ± 0.08	0.75 ± 0.06	0.71 ± 0.01
Thyroids (mg)	12.4 ± 2.20	12.3 ± 1.50	13.6 ± 4.80	16.0 ± 2.00	15.50 ± 0.01
Thymus (g)	0.17 ± 0.04	0.17 ± 0.01	0.15 ± 0.02	0.16 ± 0.02	0.17 ± 0.01
Adrenals (mg)	68.5 ± 4.50	67.60 ± 3.60	70.6 ± 4.80	80.7 ± 10.80	64.00 ± 0.01
Ovaries (mg)	44.75 ± 9.70	27.20 ± 6.20	23.1 ± 11.00	28.0 ± 12.00	38.00 ± 0.03
Uterus (g)	1.23 ± 0.01	0.24 ± 0.02	0.26 ± 0.38	0.26 ± 0.03	0.24 ± 0.03
Cecum (g)	10.16 ± 0.01	9.98 ± 1.34	8.36 ± 1.04	9.07 ± 0.82	10.19 ± 3.68

Table 5. Physical development of F1 rats.

Dose (mg/kg)	Control		Pranoprofen Treated		
	non-treated	C.M.C.	1.0	2.5	5.0
No. of animals positive/examined(%)					
Pinna unfolding on day 6	89.2	90.2	85.7	84.3	87.6
Tooth eruption on day 14	92.3	93.5	90.3	91.5	90.2
Ear opening on day 14	93.5	94.1	92.4	92.7	91.5
Eye opening on day 15	96.2	93.0	94.3	92.5	90.7
Testis descent on day 21	99.6	98.7	100.0	98.5	98.7
Vaginal opening on day 37	85.2	80.6	81.4	82.3	83.3

period, no special symptoms and deaths were observed.

2) Physical development: as shown in Table 5., pinna unfolding tooth eruption, ear opening, eye opening, testis descent and vaginal opening were tested. Pinna unfolding was examined on day 6 after birth and there were no significant difference among control and treated groups. On day 14 after birth, tooth eruption and eye opening were examined and there were no significant difference among control and treated groups. Basis on day 15 after birth, eye opening positive rate was high in non-treated control group as 96.2% and lowest in 5 mg/kg pranoprofen treated group as 90.7%. In testis descent and vaginal opening, there were no significant differences among control and treated groups.
3) Rotorod coordination: as shown in Table 6. using rotorod mill, behavioral ability was examined for 25 female and 25 male rats in each group. During 3 min. test, number of falls was recorded. There were no significant difference between control and treated groups and no significant difference between male and female rats.
4) Y-maze performance test: Using Y-maze, young animals were tested swimming activity 5 times each and number of error were checked. In all group, young animals showed good learning ability so that the number of error was fewer than previous test. There were no

Table 4. Observation of FO with respect to the birth of F1.

Compound	Control			Pranoprofen Treated		
Dose (mg/kg)	Non-treated	C.M.C	Indomethacin	1.0	2.5	5.0
No. of litters (FO)	10	10	10	11	11	11
No. of implantations						
Total	133	129	100	118	120	115
Av. ± S.E.	13.3 ± 0.7	12.9 ± 0.6	10.0 ± 0.8**	10.7 ± 0.7**	10.9 ± 0.5**	10.4 ± 0.8**
Mean length of gestation Period (Day) Mean ± S.D.	22.0 ± 0.33	21.7 ± 0.21	23.0 ± 0.40*	22.1 ± 0.37	23.0 ± 0.36**	23.0 ± 0.36**
No. of pups deliveried	101	96	15	105	110	100
(Av. ± S.E.)	11.1 ± 0.55	9.6 ± 0.86	7.5 ± 2.29	9.5 ± 0.84	10 ± 0.65	9.1 ± 0.87
No. of Perinatal deaths	3	5	15	16	51	59
perinatal deaths/pups	3/101	5/96	15/15	16/105	51/110	59/100
delivered (%)	(2.97)	(5.21)	(100)	(15.2)	(46.36)	(59.0)
No. of live born pups	98	91	-	83	59	41
(Av. ± S.E)	(9.1 ± 0.58)	(9.1 ± 1.08)		(8.5 ± 1.23)	(5.4 ± 1.53)**	(3.7 ± 1.51)**
Sex ratio of newborns	0.90	0.87	—	0.85	0.90	0.95
(Male/Female)	(43/48)	(41/47)		(33/39)	(28/31)	(20/21).
Live birth index (A) (%)	73.7	70.5		70.3	49.2***	51.3***
Viability index (B) (%)	100	100		100	98.3	100
Weaning index (C) (%)	100	100		100	100	100
No of F1 with malformation		0	0	0	1	0

(A) Live birth index (%) = No. of alive F1 at day 0/No. of implantations × 100
(B) Viability index (%) = No. of alive F1 at day 4/No. of alive F1 at day 0 × 100
(C) Weaning index (%) = No. of weaning F1 at day of 21 after delivery
*0.05 < P < 0.1, **P < 0.05 significant from *control (non-treated): Student's t-test

Table 6. Rotorod Coordination of F1 rats

Dose (mg/kg)	Control		Pranoprofen Treated		
	non-treated	C.M.C.	1.0	2.5	5.0
(Male)					
No. of animals examined	25	23	25	25	24
No. of falls for 3 min. (Av±SE)	8.7±2.12	8.5±1.87	7.5±2.00	6.5±1.55	6.2±1.75
(Female)					
No. of animals examined	25	24	25	23	25
No. of falls for 3 min. (Av±SE)	8.9±1.82	8.3±1.56	7.2±1.78	6.8±1.92	5.9±1.35

Table 7. Water-Filled double Y-Maze performance of F1 rats.

Dose(mg/kg)	Control		Pranoprofen Treated		
	Non-treated	C.M.C.	1.0	2.5	5.0
Male					
No. of errors (AV±SE)					
Session 1	3.00±1.35	3.29±0.75	2.00±1.15	2.57±0.62	2.75±0.25
2	1.13±0.23	2.17±0.79	1.38±0.50	1.00±0.44	1.00±0.40
3	1.00±0.27	3.00±1.00	0.83±0.31	0.67±0.55	0.00±0.00
4	1.38±0.32	0.75±0.25	0.83±0.31	1.20±0.73	1.00±0.41
5	1.00±0.26	0.00±0.00	0.75±0.25	0.60±0.40	0.00±0.00
Female					
No. of errors (AV.±SE)					
Session 1	2.50±0.50	3.89±0.59	1.25±0.48	2.57±0.14	3.75±0.56
2	2.75±1.01	1.44±0.82	1.125±0.40	1.57±0.43	1.75±0.75
3	1.38±0.46	2.57±0.65	0.43±0.20	0.83±0.48	1.25±0.25
4	1.17±0.40	0.86±0.46	0.57±0.30	0.80±0.80	1.50±0.50
5	0.50±0.22	0.29±0.38	0.40±0.24	0.80±0.63	0.25±0.25

significant difference between control and treated groups. (Table 7)

5) Test of avoidance responses: Using Animax Shuttle Box, avoidance responses were tested and number of avoidance responses were checked and compared. As shown in Table 8, there were no significant difference between female and male. And also no significant difference was found between control and treated groups. All the tested animals showed normal responses and there was no effect concerned with pranoprofen administration.

6) Open-field test: Using 9 sectioned, 30 cm square box, open field test were examined. Test were repeated every day from age 6 days to 21 days. The number of passing section was recorded every test. Generally, the number of passing section was increased day by day in all test groups. There were no singificant difference between control and

Table 8. Conditioned avoidance responses of F1 rats.

Dose (mg/kg)	Control		Pranoprofen Treated	
	Non-treated	C.M.C.	1.0	2.5
Male				
No. of animals examined	6	6	6	6
No. of avoidance responses (Av. ± SE)				
Session 1	7.0±3.06	7.1±2.26	6.7±0.47	7.1±0.80
2	7.3±0.69	8.2±2.59	7.6±0.58	6.7±3.20
3	8.9±4.00	8.6±2.60	8.9±0.71	8.0±2.29
4	9.8±3.60	8.7±0.47	9.1±0.98	8.9±3.55
5	10.2±2.53	9.8±0.88	10.0±2.25	9.7±1.78
Total	43.2±0.65	42.4±0.44	42.3±0.58	40.4±0.45
Female				
No. of animal examined	6	6	6	6
No. of avoidance responses (AV. ± SE)				
Session 1	7.5±2.54	5.9±2.60	6.9±1.82	7.2±0.96
2	7.8±3.40	6.8±3.02	7.5±2.45	7.4±0.09
3	9.3±1.13	7.6±2.00	9.3±0.65	9.1±0.45
4	8.9±3.08	8.9±0.55	9.7±0.75	9.5±0.55
5	10.4±3.21	10.4±1.44	10.5±0.25	10.3±1.20
Total	43.9±0.52	39.6±0.79	43.9±0.68	43.5±0.60

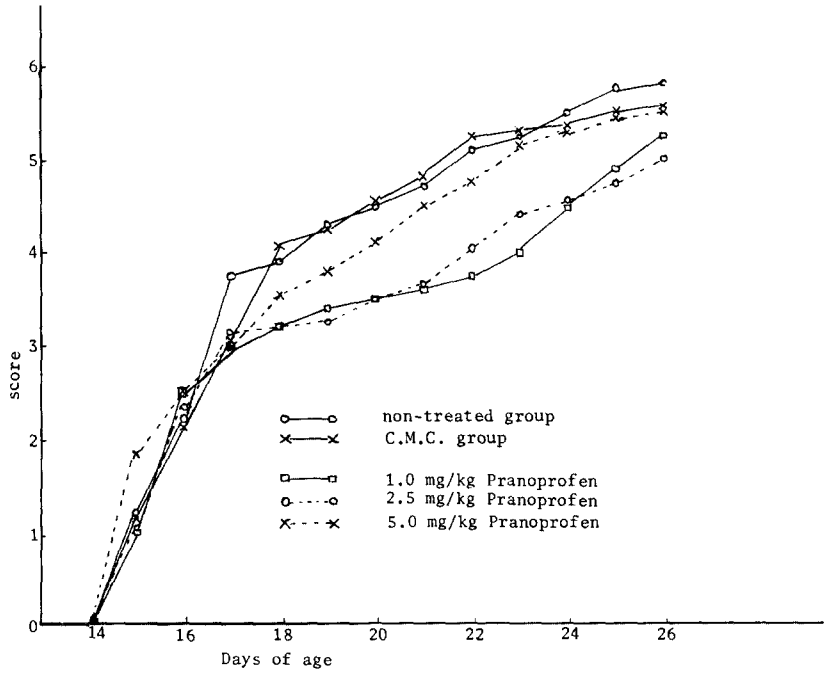

Fig. 2. Climbing down a rope in F1 female Rats

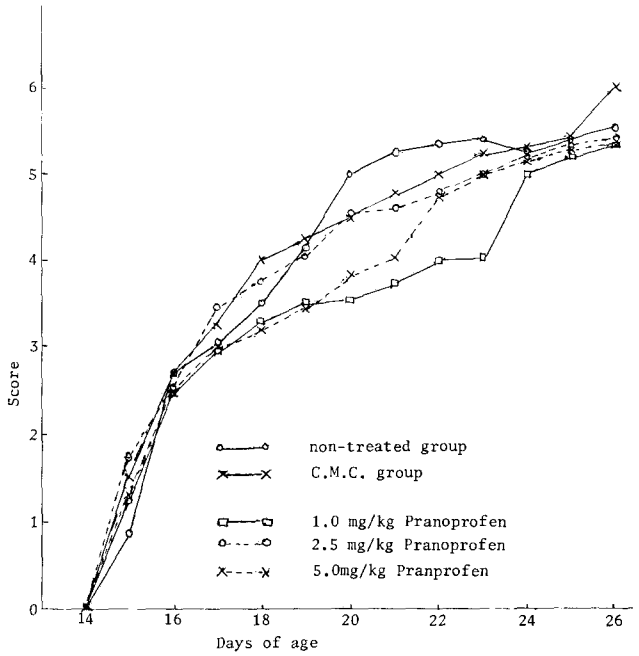

Fig. 3. Climbing Down a Rope in F1 Male Rats

treated groups. (Fig. 2 and Fig. 3)

7) Rope test: Using 50 cm rope, diameter 30mm, climbing down test were performed. The test were started age 14 days and continued to age 26 day once everyday. In the test, when rat climb down well, scored 6 and when rat drop down, scored 0, and when rat descent down, scored 3. At first test all the test animals dropped down but two or three times repeating most of them learned how to climb down the rope. There were no significant difference between control and treated groups. (Fig. 4 and Fig. 5)

DISCUSSION

This study was conducted by the guidelines for the method of specific toxicity test on drugs (NIH Guidelines No. 267). As an anti–inflammatory and analgesic agent, pranoprofen, was administered in pregnancy rats from day of 17 gestation till day of lactation period for the investigation of peri and post natal toxic effect on maternal and young animals. Pranoprofen, pharmacologically similar to non–steroidal anti–inflammatory, analgesic, and antipyretic agent such as indomethacin is reported to be more effect than indomethacin. Indomethacin and other non–steroidal anti–inflammatory agents were reported as an inhibitor of prostaglandin synthesis, resulting an inhibition of ovulation, decreased uterine contraction, prolongation of parturition and delayed initiation (Gaut. *et al.* 1975., McClain. *et al.* 1980., Tanaka. *et al.* 1973., Tsafriri. *et al.* 1972., and 1973, Vane *et al.* 1968., Ferreira. *et al.* 1971 and 1974, Aiken 1972). Doses were selected on the base of the maximum tolerance dose and no effect dose from the chronic toxicity tests of pranoprofen. (Edanaga. *et al.* 1976). In this study, the 1.0, 2.5 and 5.0 mg/kg/day pranoprofen were administered to pregnancy rats. From indomethacin treated group, as a positive control, showed 24 ± 1 hours delay of initiation compared with control group and high maternal

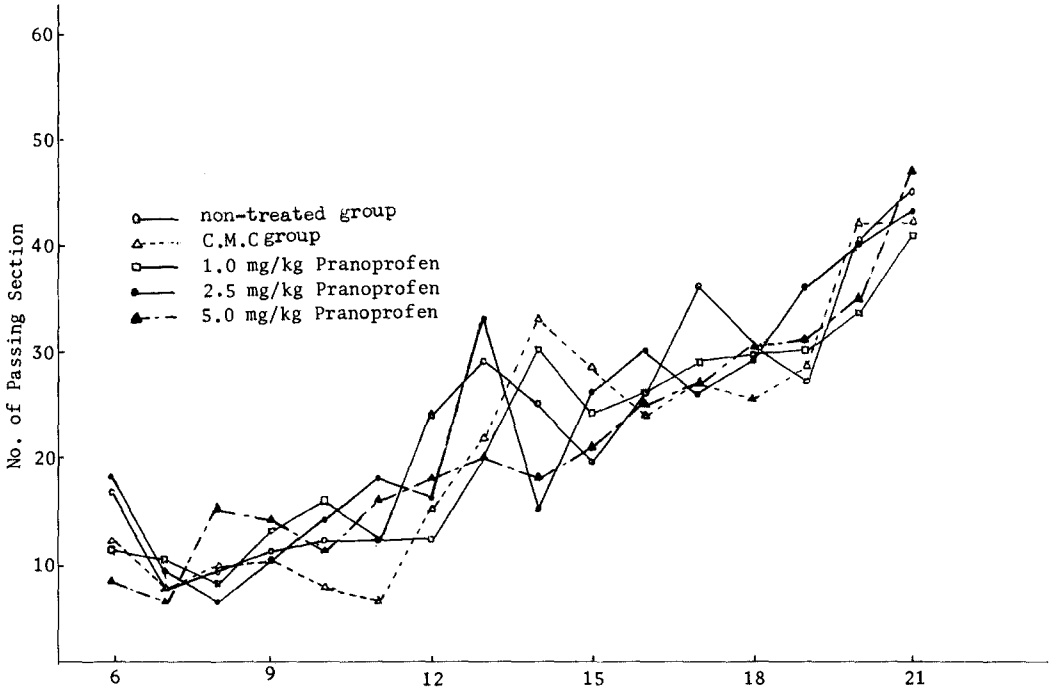

Fig. 4. Results of Open-Field test of F1 female rats

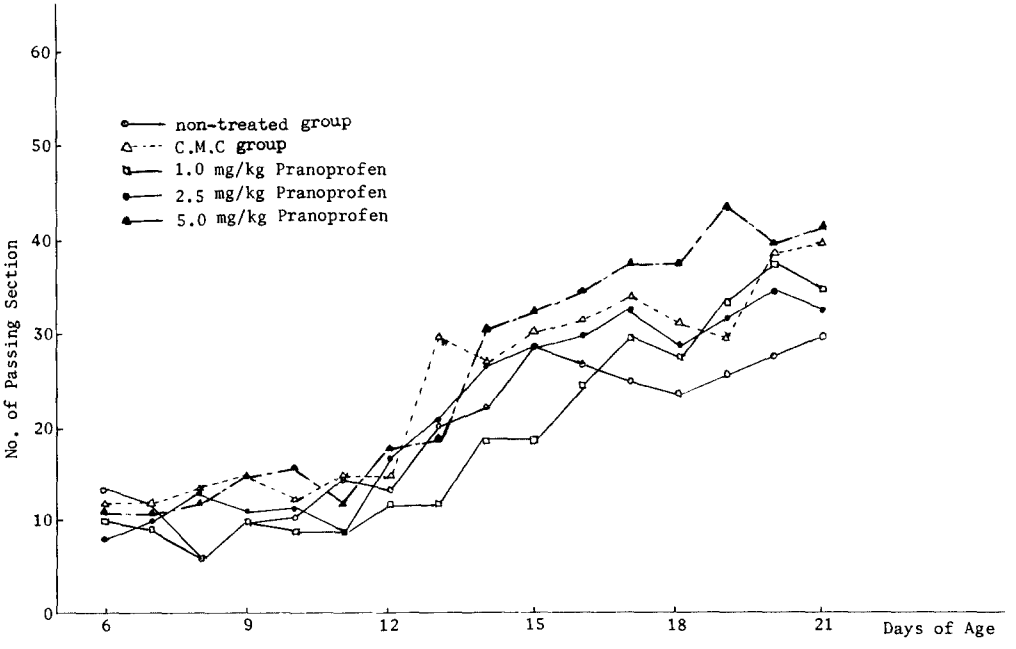

Fig. 5. Results of Open-Field test of F1 male rats

Table 9. Mating and fertility data and cesarean section data of F1 rats.

Dose (mg/kg)	Control		Pranoprofen Treated		
	Non-treated	C.M.C.	1.0	2.5	5.0
Mating and fertility data Male					
No. mated/No. paired	5/7	7/7	6/7	6/7	5/7
No. impregnating/ No. paired	5/7	6/7	6/7	6/7	6/7
Female					
No. mated/No. paired	5/7	7/7	6/7	6/7	5/7
No. pregnant/No paired	5/7	6/7	6/7	6/7	5/7
Cesarean section data					
No. of dams examined	5	6	6	6	5
No. of corpora lutea	72	92	94	90	76
(AV.±SE)	14.4±0.87	15.3±0.80	15.7±0.47	15.0±0.86	15.2±0.87
No. of implantation	62	77	80	78	64
(AV.±SE)	12.4±0.83	12.8±0.76	13.3±0.82	13.0±0.56	12.8±0.39
Implantation/ Corpora lutea (%)	86.1	83.6	84.7	86.7	84.2

death rate without delivery. Eight out of ten tested pregnancy rats in indomethancin treated group showed maternal death and rest of two cases showed 15 stillbirths. It means that indomethacin was a strong toxic substance especially concerned with delivery. And in 2.5 and 5.0 mg/kg/day pranoprofen treated groups, delay of initiation were observed as 24± 0.09 and 24±0.03 hours respectively and high stillbirth rate as 46.4% and 59% compared that of control groups. Although there was no report on pranoprofen that delay of initiation and high stillbirth were occurred (Hamada. *et al.* 1976) before, 2.5 and 5.0 mg/kg/day pranoprofen treated group showed delay of initiation and high stillbirth rate. On the other hand, for the investigation of physical and behavioral changes, several tests and observations were performed. From the early 1940, the behavioral teratology test were performed (Harned. 1944; Hamilton, 1944) with sodium bromide at first. At 1963, the term of "behavioral teratology" was used (Werboff and Gottlib, 1963) first when they studied about the effect of "drugs in pregnancy". During the middle of 1970, several reports were presented that without any malformation occurred dose level, certain behavioral changes were occurred. (Rodier. 1978; Coyle, *et al.*, 1980; Clark *et al.*, 1970; Butcher *et al.*, 1976; Spyker *et al.*, 1975; Vorhees 1974). After that period, every country considered that the importance of behavioral teratology and prepared a special guidelines on that subject upon the basis of several studies (Buelke-Sam and Keinmel 1979; Vorhees *et al.*, 1979; Bucher *et al.*, 1979; and Vorhees *et al.*, 1981). In this study, several physical developments such as pinna unfolding, tooth eruption, ear opening, eye opening, testis descent and vaginal opening were observated for young animals. There were no significatn difference between control and pranoprofen treated goups. And for the investigation of learning ability, rotorod test, Y-maze performance, avoidance responses, open-field test and rope test were done for young animals. There were no significant difference between control and pranoprofen treated groups also.

For the observation of fertility ability, each 7 Fl female rats were mated in each group with male rats of same group and checked mating rate, pregnancy rate and then did cesarean section on day 20 of gestation and investigated number of corpora lutea and implantations. There were no significant difference between control and treated groups in

many checking points.

CONCLUSION

Following the NIH special toxicity guidelines of peri and postnatal test, 1, 2.5 and 5 mg/kg/day pranoprofen were given to pregnant female rats on day 17 of gestation to end of lactation and observated the toxic effect during gestation, delivery and lactation period on maternal and investigated physical developments and learning ability on young animals. From this studies, the following results were obtained;

1. In 2.5 and 5.0 mg/kg/day pranoprofen treated groups, pregnant females showed delay of initiation about 24 ± 1 hour longer than control group.
2. In 2.5 and 5.0 mg/kg/day pranoprofen treated groups, pregnant females showed high stillbirth rate as 46.4 and 59 per cent respectively compare than that of 2.9 per cent at non-treated control group.
3. Indomethacin (positive control) group showed delay of initiation and death without delivery.
4. After delivery, all the pups showed normal physical development and behavioral changes in control and in pranoprofen treated groups.
5. All the F7 female tested showed good ability in mating, fertility and had no significant difference in the number of corpora lutea and implantations between control and treated groups.

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