Environmental Mutagens & Carcinogens 21-2 : 77-81 (2001)

Prenatal Treatment Effects of Oriental Herbal Medicine Kamijadowhan on Developmental and Reproductive Toxicity in Rats

Young-Jin Park, Jung-Ran Kim, Jae-Chun Ryu, Bum-Sang Shim[1], Seung-Hoon Choi[1], and Oh-Seung Kwon*

Toxicology Lab., Bioanalysis and Biotransformation Research Center, Korea Institute of Science and Technology, Seoul, Korea 136-791, and [1]Department of Pathology, College of Oriental Medicine, Kyung Hee University, Seoul, Korea.

(Received October 20, 2001 / Accepted November 15, 2001)

ABSTRACT : **Kamijadowhan (KMD), an oriental herbal medicine used for anti-angiogenic effect, was extracted with 80% ethanol from mixture of source materials and lyophilized. KMD was orally administered to plug-positive pregnant rats from gestational days 12 to 20, dividing into three groups including vehicle-treated control, 0.5 g/kg or 3 g/kg KMD-treated groups. Dam weight during gestation and post-gestation, weight of pre- and post-weaning offsprings in male and female, and reproductive and developmental endpoints including incisor eruption, eye opening and testes descent were measured. No significant alterations in development of physical landmarks in offspring, maternal weight gain during gestation and post-gestation, and offspring weight were observed in KMD-treated group. The measurement of organ weight at post-gestational days 21 was not changed in dams. In 0.5 g/kg KMD-treated rats, kidney weights in male and female offsprings were significantly increased, and the body weight in male offspring was also increased. Liver and brain weights were not changed. Taken together, these data suggest that KMD may not significantly cross the placenta and produce no reproductive and developmental toxicity at maternally non-toxic dosages.**

Keywords : Kamijadowhan, Oriental herbal medicine, Developmental toxicity, Reproductive toxicity, Pregnant rats.

Introduction

A plethora of attempt has been made to discover a new compound with potent activity in treating various kinds of cancers or tumors from plants for a long time. Until now, however, the goal was not achieved and still we are striking to accomplish the same goal. Especially, oriental herbal medicine can give good clues and initiatives for this kind of approach. Herbal medicine was used to treat various human disease for a long time in the oriental society and many results from basic scientific study for herbal medicine were accumulated.

Kamijadowhan (KMD), a traditional oriental herbal medicine, has been used to inhibit recurrence and metastasis of cancer in clinical practice such as breast-cancers and brain tumors. Recently, Choi group reported that KMD has anti-angiogenic effects and it may be a potential agent for clinical chemoprevention since it inhibits angiogenesis in ECV304 cell line and blocks angiogenesis measured by the aortic ring assay (Kang *et al.*, 2000; Wang, 2000; Wang *et al.*, 2001).

With the overall purpose of developing a new agent for potent anti-angiogenesis effect and a new preparation form of oriental medicine as well as finding effective lead compounds, toxicities of KMD as one of series of collaboration work were studied. The reproductive and developmental toxicities of KMD remain unclear and no report has been made yet. In oriental herbal medicine, systematic study for toxicity evaluation was not defined and established although it is necessary to make assessment of toxicity in that herbal medicine consisting of mixture of various components was chronically treated to patients.

In this experiment, reproductive and developmental toxicity studies covering the segment II period were performed in pregnant rats treated with two different doses of KMD and toxicity endpoints in maternal and offspring were measured.

Materials and Methods

Kamijadowhan

An oriental herbal medicine, Kamijadowhan, was prepared by Pathology Laboratory of College of Oriental Medicine, Kyung-Hee University (Seoul, Korea) after herbal source materials for KMD were purchased from Kyung-Hee Medical Center (Seoul, Korea). The method of preparation was described below as described in previous reports (Kang *et al.*, 2000; Wang, 2000); *Cremastrae Appendiculatae* (25 g), *Persicae Semen* (25 g), *Coicis Semen* (10 g), *Hippocampus* (3 g), *Trionycis Carapax* (3 g) and *Curcumae Radix* (3 g)

*To whom correspondence should be addressed

were added to 3000 ml of a round flask containing 2000 ml of 80% ethanol. Source materials were extracted for 4 hr under circulating water for cooling and repeated this procedure. The extract was filtered and the filtrate was concentrated on a vacuum evaporator (Eyela, Japan). The concentrates were lyophilized by a freeze-drier (Eyela, Japan) overnight. Finally, about twelve gram of the powder was obtained.

Animals and treatment

The method with a modification was followed as described in Ferguson *et al.* (1993). Plug-positive Sprague-Dawley rats (Dae-Han BioLink Inc., Eumsung, Chungbuk, Korea)) were used and housed individually in polycarbonate cages with woodchip bedding (plug date = gestational day 0). The rats were divided randomly into 3 groups (n = 6-7 rats/group). The experiment was completed by two replicates with the interval of one week. Food and water were provided *ad lib.* The housing room was maintained on a 12:12 hour light-dark cycle, and the temperature and humidity were maintained at 23±3°C and 50±20%, respectively.

Administration of Kamijadowhan to rats by a gavage

Each rat was gavaged on gestational days 12-20 with an aqueous solution of control (n = 6), 0.5 g KMD/kg (n = 7), or 3 g KMD/kg (n=7). The day of birth was designated postnatal day (PND) 1. Litters were weighed on PND 1 and culled to 10 pups, maintaining equal numbers of each sex where possible. All litters remained with their biological dam; no cross-fostering was done. Each pup was tattooed on the dorsal surface of the paw for identification purposes. Weaning was made on PND 22.

Maternal measurement

A body weight was measured for each dam on gestation days 4, 7, 10, 12 to 21 and approximately weekly through post-parturition day 21. Gestation length, number of non-pregnant dams, and number of dams giving birth were measured. At post-parturition days 21, dams were sacrificed and the weights of principle organs were measured.

Offspring measurement

Body weight: Pre- and post-weaning body weight of offspring were measured on PND 1, 7, 14, 21, 30 and 60 in designated male and female of litters.

Physical landmark: Endpoints used for physical development of offspring were included measurement of the day of incisor eruption, eye opening, and testes descending. The criteria of recording these endpoints were clear appearance of bilateral incisor, full opening of bilateral eyes, and full testes descent using methods previously described (Mohammad and St. Omer, 1986).

Statistical treatment

Data are presented as mean±SE. The general linear model procedure of SAS (SAS Institute Inc., Cary, NC) was used. Analysis for repeated measures over day or session were evaluated with multivariate techniques. Duncan's multiple range test and probability difference were used for one-way analysis when F-values showed significance. Values were considered statistically significant when $p < 0.05$.

Results

Development of physical landmarks in offspring

Results of physical landmarks are shown in Table 1. KMD treatment had no effects on incisor eruption and eye opening both in male and female. Time taking to the patent development of testes descent was not changed. No statistical difference in development of physical landmarks in offsprings was observed between KMD-treated and vehicle-treated control groups.

Developmental and reproductive endpoints in offspring

As shown in Table 2, the offspring weight at PND 1 did not show dose-dependency and statistical significance. Percentages of pregnancy were 83.3, 85.7, and 57.1 in control, 0.5 g/kg and 3 g/kg KMD-treated groups, respectively. There was no significant difference in numbers of male or female offsprings, gestation length, and gender ratios.

Maternal body weight gain during gestation

Body weight gain of dams during gestation and post-parturition showed that KMD treatment has no effects on

Table 1. Development of physical landmarks in offspring

Endpoints	Dose of Kamijadowhan		
	Control (n=5)	0.5 g/kg (n=6)	3.0 g/kg (n=4)
Male			
Incisor eruption (days)	11.6±0.9	11.2±0.3	11.5±0.3
Eye opening (days)	15.8±0.5	14.7±0.4	15.5±0.5
Testes descent (days)	20.6±0.4	20.7±0.2	21.0±0.0
Female			
Incisor eruption (days)	10.2±0.5	11.3±0.2	11.3±0.6
Eye opening (days)	14.8±0.2	14.7±0.2	15.5±0.6

Each value presents mean±SE of rats (n=4-6 rats per group).

Table 2. Summary of reproductive and developmental endpoints in offspring

Endpoints	Dose of Kamijadowhan		
	Control (n=5)	0.5 g/kg (n=6)	3.0 g/kg (n=4)
Pregnant	5	6	4
Non-pregnat	1	1	3
Total pup no./litter	12.0±2.4	11.8±1.82	13.0 ±0.4
Male pup no. (M)	6.0±1.4	5.5±1.3	4.8 ±1.3
Female pup no (F)	6.0±1.5	6.3±1.2	8.3 ±1.1
Ratios (M/F)	1.56±0.65	1.38±0.57	0.67±0.25
Body weight (g)			
Male	7.24±0.56	7.39±0.001	6.80±0.001
Female	6.90±0.57	7.00±0.35	6.50±0.35
Total	7.04±0.54	7.16±0.33	6.61±0.33
Gestation (days)	22.4±0.51	22.0±0.26	22.5±0.29

Each value presents mean±SE of rats (n=4-6 rats /group).

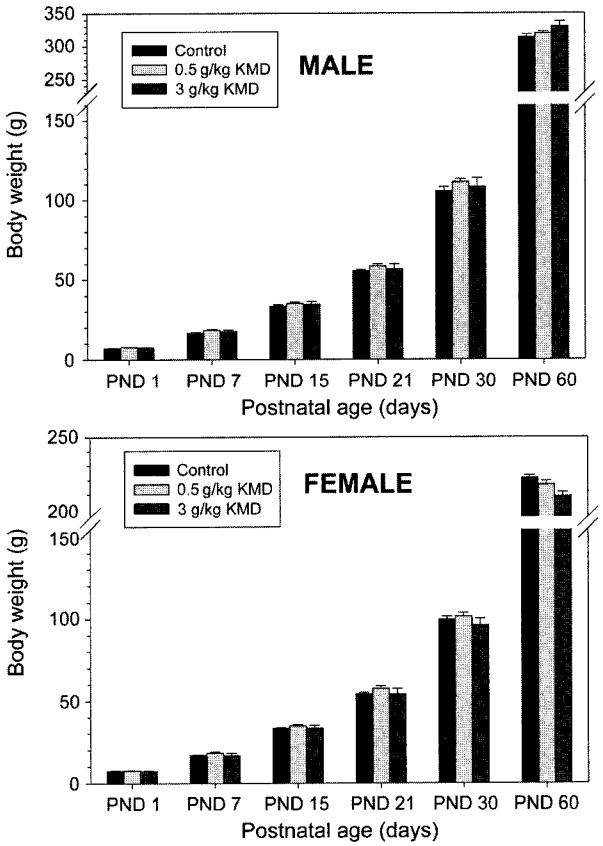

Fig. 1. Maternal body weight gains during gestation and post-gestation. Kamijadowhan (KMD) were administered to rats by a gavage on gestational days 12 (G12) to 20 (G20). Body weight gains were calculated based on the weight of gestational days 2 of each group. Each point represents mean±SE of rats. No statistical difference was found in body weight gains.
Abbreviation: PND, postnatal days.

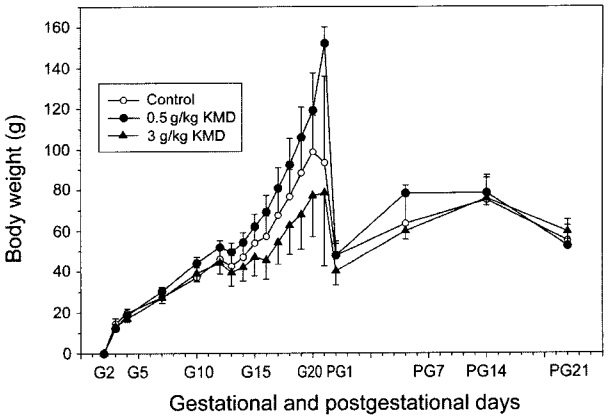

Fig. 2. Offspring body weight gains after prenatal treatment of Kamijadowhan (KMD) from postnatal days (PND) 1 to PND 60 in female and male offspring. Each bar represents mean SE of rats. No treatment effect of Kamijadowhan was observed.
Abbreviations: G, gestational days; PG, postgestational days.

changes of body weight gain (Fig. 1).

Offspring weights

Body weight of pre- and post-weaning offspring in both sex are not significantly different in KMD-treated group, compared to the control, as shown in Fig. 2, suggesting that prenatal exposure of these doses on gestational days 12-20 may not be potentially toxic.

Organ weight of dams

At postnatal days 21, organ weights including liver, kidney and brain were not significantly changed compared to vehicle-treated control. Body weight in pregnant rats treated with 3 g/kg KMD showed insignificant decreasing tendency compared to the body weight of the control (Table 3).

Organ weights of offspring

At postnatal days 21, designated female and male offsprings

Table 3. Organ weights of dams

	Control (n=5)	0.5 g/kg (n=6)	3 g/kg (n=4)
Body weight (g)	296.40±10.47	281.33±4.51	276.65±6.68
Liver (g)	13.17± 0.69	12.42±0.27	12.48±0.40
Kidney (g)	1.95± 0.08	1.86±0.03	1.82±0.05
Brain (g)	1.73± 0.007	1.69±0.03	1.65±0.02
Ratios to body weight			
Liver	0.044±0.001	0.044±0.001	0.045±0.001
Kidney	0.007±0.0001	0.007±0.0001	0.007±0.0001
Brain	0.006±0.0001	0.006±0.0001	0.006±0.0001

Each value presents mean±SE of rats (n=4-6 rats/ group).

Table 4. Organ weights of male offspring at PND 21

	Control (n=5)	0.5 g/kg (n=6)	3 g/kg (n=4)
Body weight (g)	57.36±1.60	59.25±2.08	57.23±3.88
Liver (g)	2.01±0.69	2.26±0.12	2.06±0.11
Kidney (g)	0.53±0.02	0.61±0.02*	0.54±0.04
Brain (g)	1.39±0.02	1.41±0.02	1.35±0.02
Ratios to body weight			
Liver	0.037 ±0.001	0.038 ±0.001	0.036 ±0.001
Kidney	0.0098±0.0001	0.0104±0.0001*	0.0094±0.0001
Brain	0.026 ±0.001	0.024 ±0.001	0.024 ±0.001

Each value presents mean±SE of rats (n=46 rats/group).
*Significantly dfferent from control, $p<0.05$.

Table 5. Organ weights of male offspring at PND 21

	Control (n=5)	0.5 g/kg (n=6)	3 g/kg (n=4)
Body weight (g)	49.92±2.07	57.85±1.73*	52.98±3.58
Liver (g)	1.74±0.08	2.08±0.10	1.89±0.15
Kidney (g)	0.47±0.02	0.60±0.02*	0.51±0.03
Brain (g)	1.32±0.02	1.37±0.02	1.34±0.02
Ratios to body weight			
Liver	0.035 ±0.001	0.036 ±0.001	0.036 ±0.001
Kidney	0.0095±0.0001	0.0103±0.0001*	0.0095±0.0001
Brain	0.027 ±0.001	0.024 ±0.001	0.026 ±0.001

Each value presents mean±SE of rats (n=4-6 rats/group).
*Significantly dfferent from control, $p<0.05$.

were sacrificed and principle organ weights were measured. In 0.5 g/kg KMD, body weights in female offspring were increased (Table 5), and kidney weights and their ratios to the body weight in male and female offsprings were significantly increased (Table 4 and Table 5). No other organ weights were changed by KMD treatment.

Discussion

The method used to measure the prenatal effect of a compound in this study has been found to have a high reproducibility in maternal and offspring body weights, appearance of certain physical landmark and behavioral measurement by international collaboration study across 6 laboratories with no statistical significant interaction between dose and laboratory (dose x laboratory) found (Buelke-Sam *et al.*, 1985; Vorhees. 1985).

The inhibitory effects of KMD on angiogenesis were reported by Choi group. In one study conducted with ECV304 cell line, angiogenic tube formation was shown to be inhibited at 200-400 μg/ml of KMD. The inhibition of cell proliferation at 100 μg/ml KMD was also observed (Kang *et al.*, 2000; Wang, 2000). In a study using hexane and ethylacetate fractions of KMD (Wang, 2000a, Wang *et al.*, 2000b), capillary-like tube formation assay and rat aortic ring assay in the same cell line were reported to be 5-10 times more potent in exerting inhibitory action.

In our results, oral administration of 0.5 and 3 g/kg KMD for gestational day 12-20 did not show significant effects in maternal and offspring endpoints except the minimal significant changes of kidney weight in male and female offsprings. Increases of kidney weight and its ratios to body weight in male and female offsprings showed a inconsistent dose-dependency. It is not clear that why the kidney weight of offsprings was affected by treatment of 0.5 g/kg KMD and not by treatment of 3 g/kg KMD.

KMD fraction used in this work was extracted with 80% ethanol as mentioned in the Experimental section. Even though there is not enough analytical information relating to compound or content of components of KMD, it is considered that 80% ethanol extract may contain more polar components, compared to the fraction obtained with hexane and ethyl acetate, based on reported results from the tube formation and rat aortic assays. Practically, in a view of patient's taking a mass amount of an aqueous extract of herbal medicine for a long period, 80% ethanol fraction did not correctly reflect clinical situation. This may be one of limitation in studying mixture of various component of herbal medicine. Absorption of KMD might be very low because water solubility of the 80% ethanol extract is poor and suspended aqueous solution (yellow, turbid) of KMD was administered to rats by a gavage. Apparent behavioral changes including diarrhea and increased or decreased movement were not observed through experimental period. Growth pattern in male and female offsprings may be normal because no significant treatment effects were observed in body weight of offsprings. Treatment-unrelated effects of gender and time were statistically significant ($p<0.05$).

Various interactions between the mixtures of compound in exerting effects will be occurred. Synergistic or antagonistic effects of compound mixture are also important in considering treatment effects of herbal medicine. Therefore, in designing desirable study of herbal medicine of complex mixture extracts, the type and content of mixture compounds according to the solvent used for extraction and the interaction between main components should be known, remaining further works.

In conclusion, this study suggests that KMD may not significantly cross the placenta and produce no direct maternal toxic effect.

Acknowledgement

This research was supported by grants to Dr. S.H. Choi from the Ministry of Health and Welfare, Republic of Korea (HMP-99-O-01-0004) and authors thank to Mr. J.

H. Park very much for his assistance of Kamijadowhan preparation.

References

Buelke-Sam, J., Kimmel, C.A., Adams, J., Nelson, C.J., Vorhees, C.V., Wright, D.C., Omer, V. St., Korol, B.A., Butcher, R.E., Geyer, M.A., Holson, J.F., Kutscher, C.L., and Wayner, M.J. (1985): Collaborative behavioral teratology study: Results. *Neurobehabioral Toxicology and Teratology*, **7**, 591-624.

Ferguson, S.A., Racey, F.D., Paule, M.G., and Holson, R.R. (1993): Behavioral effects of methylazoxymethanol-induced microencephalsy. *Behav. Neurosci.*, **107**, 1067-1076.

Kang. H.S., Kim, S.H., Shim, B.S., Choi, S.H., and Ahn, K.S. Study on the effect of Kamijadowhan on angiogenic inhibition mechanism. *Korean Journal of Oriental Medical Pathology*, **14**, 168-181 (2000).

Mohammad, F.K. and St. Omer., V.E.V. (1986). Behavioral and developmental effects in rats following in utero exposure to 2,4-D/2,4,5-T mixture. *Neurobehav. Toxcol. Teratol.*, **8**, 551-560.

Vorhees. C.V. (1985): Comparison of the collaborative behavioral teratology study and Cincinnati behavioral teratology test batteries. *Neurobehabioral Toxicology and Teratology*, **7**, 625-633.

Wang, Teh-Chung. Study of the effect of Jiaweicitaowan (Kamijadowhan) ingredients on angiogenic inhibition. *Ph.D. Dissertation Thesis* (38 pages), Kyung Hee University (2000a).

Wang, T.C., Park, J.H., Yu, Y.B., Shim, B.S., Ahn, K.S., and Choi, S.H. (2000b): Study on the effects of Jiaweicitaowan ingredients on angiogenic inhibition (I). *Korean Journal of Oriental Medical Pathology.* **14**(2), 155-167.

Wang, T.C., Park, J.H., Yu, Y.B., Shim, B.S., Ahn, K.S., and Choi, S.H. (2001) Study on the effects of Jiaweicitaowan ingredients on angiogenic inhibition (II). *Korean Journal of Oriental Medical Physiology & Pathology.* **15**(2), 235-240.