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Single Oral Toxicity Study of 3-Methoxy-6-Allylthiopyridazine in Rats

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Abstract – The single dose toxicity of 3-methoxy-6-allylthiopyridazine (K-6) was studied with Sprague-Dawley rats. K-6 was administered orally with dosages of 4.0, 3.0, 2.0, 1.5, 1.0, 0.5, 0.25 and 0.1 g/kg to the rats. We observed daily the number of death, clinical signs, body weights and gross findings. All rats (6) were dead within a day after administration when doses of 4.0 and 3.0 g/kg were administered. When dose of 2.0 g/kg was administered, 2 rats among 3 rats were dead. Any significant toxicity below 1.5 g/kg of K6 was not observed when the different doses of 1.5, 1.0, 0.5, 0.25 and 0.1 g/kg were administered to 6 rats respectively.

Keywords □ 3-Methoxy-6-allythiopyridazine, single oral toxicity, rats

INTRODUCTION

Dietary organosulfur compunds of garlic were shown to inhibit the proliferation of tumor cells (Sundaram *et al.*, 1996; Siegers *et al.*, 1999; Thatte *et al.*, 2000). Since hepatocellular carcinoma is one of the most lethal malignancies and there is no effective and chemotherapeutic potential of the synthetic allythiopyridazine derivatives on hepatocarcinoma cells (Fukushima *et al.*, 1997; Reddy *et al.*,;1993; Takahashi *et al.*, 1992; Takada *et al.*, 1994; Jung M.Y. *et al.*, 2001).

One of the most well known ingredient of garlic is allicin, which is chemically unstable and has evil-smelling odor. It is necessary to develop other stable and odorless compounds, that have chemopreventive and chemotherapeutic activities (Dausch *et al.*, 1990; Dorant *et al.*, 1990; Hayes *et al.*, 1987; Shin H.S. *et al.*, 2002; Kwon S.K. *et al.*, 1998; Sparnins *et al.*, 1998).

MATERIALS AND METHODS

Animals husbandry and maintenance

The male or female SD rats (110-160 g) were supplied from Seoul Clinical Laboratories. All SD rats were used after one week of acclimating period.

All animals were house in wire cages at 23±3°C and 55±15% humidity and provided diet and water

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Treatments

In the preliminary test five different doses of K-6 (4.0, 3.0, 2.0, 1.5 and 1.0 g/kg) were administered to 3 rats respectively and in the main test five different doses of K-6 (1.5, 1.0, 0.5, 0.25 and 0.1 g/kg) were administered to 6 rats respectively.

Effects on general behavior

The methods used were based on the procedures described by Irwin(Irwin, 1968).

Rats were administered orally with K-6(1.0 and 1.5g/kg), and the general behavior were observed at 0.5, 1, 2 and 4 hrs after the drug administration.

Body weight

Individual body weights of rats were measured shortly before the test article administration and on day 1, 3, 7 and 14 after the treatment thereafter.

Necropsy findings

On day 14 after the treatment, all rats were necropsied with special attention to all vital organs and tissues.

Statistical analysis

Body weight value were presented by mean±S.D.

The statistical significances between groups were assessed by ANOVA F-test.

Differences is statistically significant($p<0.001$).

RESULTS AND DISCUSSION

Preliminary test

After administering 1.0, 1.5, 2.0, 3.0 and 4.0g/kg as a preliminary test, all rats(6) were dead within a day after administration when doses of 4.0 and 3.0g/kg were administered. When dose of 2.0g/kg was administered, 2 rats among 3 rats were dead. We tested for dose level of below 1.5g/kg at which no rats were dead(Table I, II).

Effects on general behavior

Oral administration of K-6 at does of 1.0 and 1.5g/kg showed no observable changes in behavioral, neurological and autonomic profiles in rats during 4 hrs periods(Table III).

Clinical observation

Clinical signs were checked for 14 days after dosing and then no rats in both sexes showed changes(Table IV).

Body weight

There were no notable changes which could be attributed to the treatment of test article(Table V).

Gross finding

Neither female nor male rats were found dead below 1.5g/kg of K6 during the testing period (Table III, IV). In this experiment, we observed decreased locomotor activity, prone position, crouching position or ataxic agit. In 0.1 g/kg treated group, both female and made rats showed those behaviors 2 hour after administration. In 0.5 g/kg treated group, 2 rats showed decreased locomotor activity, crouching position and ataxic agit 2 hours after administration, but didn't show those abnormal behaviors one day after administration. In 0.1 and 0.25g/kg treated groups, we couldn't observe abnormal behaviors. Any significant toxicity below 1.5g/kg of K-6 was not observed when the different doses of 1.5, 1.0, 0.5, 0.25 and 1.0g/kg were

Table III. General behavior of rats administered orally with K-6

Items	0.5	1	1.5	2	4(hr)
	ABC	ABC	ABC	ABC	ABC
Locomotor activity	000	000	000	000	000
Writhing response	000	000	000	000	000
Fighting	000	000	000	000	000
Stereotyped behavior	000	000	000	000	000
Convulsion	000	000	000	000	000
Tremor	000	000	000	000	000
Exophthalmos	000	000	000	000	000
Ptosis	000	000	000	000	000
Piloerection	000	000	000	000	000
Tail elevation	000	000	000	000	000
Traction	000	000	000	000	000
Motor incoordination	000	000	000	000	000
Muscle tone	000	000	000	000	000
Catalepsy	000	000	000	000	000
Righting reflex	000	000	000	000	000
Pain response	000	000	000	000	000
Pinna reflex	000	000	000	000	000
Pupil reflex	000	000	000	000	000
Skin color	000	000	000	000	000
Respiration	000	000	000	000	000
Lecrimation	000	000	000	000	000
Salivation	000	000	000	000	000
Urination	000	000	000	000	000
Diarrhea	000	000	000	000	000
Vocalization	000	000	000	000	000
Death	000	000	000	000	000

Each value represents the number of abnormalities on general behavior (n=6).
A; Control(corn oil), B; K-6 1.0 g/kg, C; K-6 1.5 g/kg

Table I. Gross observation of necropsy in rats administered orally with K-6 Preliminary test

Sex	Dose (mg/kg)	Frequency	
		Decedents	Surivors
Male	1.0	0/0	3/3
	1.5	0/0	3/3
	2.0	2/3	1/3
	3.0	3/3	0/0
	4.0	3/3	0/0

Table II. Body weights of rats administered orally with K-6

Sex	Dose(g/kg)	Body weights after treatment (days)				
		0	1	3	7	14
Male	1.0	156.33±1.53(3)	182.0±11.36(3)	229.93±27.59(3)	268.67±26.86(3)	260.67±17.24(3)
	1.5	155.67±2.08(3)	173.67±7.01(3)	212.67±25.01(3)	244.0±50.48(3)	269.33±23.86(3)
	2.0	175.27±1.15(3)	149.33±1.15(3)	153.5±0.71(2)	162(1)	172(1)
	3.0	157.20±3.03(5)	(0)	(0)	(0)	(0)
	4.0	157.20±3.03(5)	(0)	(0)	(0)	(0)

Table IV. Clinical signs of rats administered orally with K6

Sex	Dose(g/kg)	observations	Range(Day)
Male	Control	Appears normal	0 ~ 14
	0.1	Appears normal	0 ~ 14
	0.25	Appears normal	0 ~ 14
	0.5	Appears normal	0 ~ 14
	1.0	Appears normal	0 ~ 14
	1.5	Appears normal	0 ~ 14
Female	Control	Appears normal	0 ~ 14
	0.1	Appears normal	0 ~ 14
	0.25	Appears normal	0 ~ 14
	0.5	Appears normal	0 ~ 14
	1.0	Appears normal	0 ~ 14
	1.5	Appears normal	0 ~ 14

administered to 6 rats respectively(Table VI). The single dose toxicity of 3-methoxy-6-allylthiopyridazine (K-6) was studied with Sprague-Dawley rats. K-6 was administered orally with dosages of 4.0, 3.0, 2.0, 1.5, 1.0, 0.5, 0.25 and 0.1g/kg to the rats. We observed daily the number of death, clinical signs, body weights and gross findings. Based on the results, it was concluded that a single oral dose of K-6 did not produce any toxic effect in Sprague-Dawley rats at dose levels of 1.5g/kg or below. According to above results, the minimal lethal dose was considered to be over 1.5g/kg body weight for rats.

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Table VI. Gross observation of necropsy in rats administered orally with K-6 main test

Sex	Dos (mg/kg)	Observation	Frequency	
			Decedents	Survivors
Male	0.1	N.G.F.a)	0/0b)	6/6
	0.25	N.G.F.	0/0	6/6
	0.5	N.G.F.	0/0	6/6
	1.0	N.G.F.	0/0	6/6
	1.5	N.G.F.	0/0	6/6
Female	0.1	N.G.F.	0/0	6/6
	0.25	N.G.F.	0/0	6/6
	0.5	N.G.F.	0/0	6/6
	1.0	N.G.F.	0/0	6/6
	1.5	N.G.F.	0/0	6/6

a) No gross finding
b) Values are expressed as animal numbers

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Table V. Body weights of rats administered orally with K-6 main test

Sex	Dose(g/kg)	Body weights after treatment (days)				
		0	1	3	7	14
Male	Control	153.00±1.58(6)[a)]	166.20±1.10(6)	206.40±3.85(6)	245.20±7.29(6)	300.00±8.72(6)
	0.1	153.00±0.71(6)	154.40±1.67(6)	182.40±11.87(6)	203.60±0.89(6)	276.00±4.69(6)
	0.25	151.80±2.17(6)	156.00±2.45(6)	178.00±4.00(6)	182.40±4.34(6)	268.40±9.53(6)
	0.5	152.80±0.84(6)	162.80±5.21(6)	189.60±8.05(6)	187.60±8.29(6)	270.00±10.58(6)
	1.0	158.50±1.72(6)	173.17±4.54(6)	187.50±6.22(6)	189.33±5.64(6)	278.17±5.85(6)
	1.5	155.67±1.63(6)	176.00±5.25(6)	178.33±3.72(6)	184.50±3.67(6)	277.17±5.53(6)
	Control	118.40±1.14(6)	128.80±3.35(6)	155.60±3.86(6)	158.80±3.63(6)	212.40±14.79(6)
	0.1	121.80±1.14(6)	136.00±5.10(6)	156.00±4.47(6)	168.80±8.56(6)	199.60±14.31(6)
	0.25	124.00±7.31(6)	133.20±6.42(6)	155.20±11.10(6)	167.20±12.05(6)	202.40±14.38(6)
	0.5	120.60±1.52(6)	122.80±2.28(6)	135.20±13.31(6)	144.40±7.13(6)	198.80±15.59(6)
	1.0	120.83±2.32(6)	126.00±3.58(6)	158.67±1.21(6)	156.50±0.84(6)	216.67±4.87(6)
	1.5	120.83±1.72(6)	131.83±4.54(6)	158.00±1.67(6)	159.33±1.37(6)	215.67±6.35(6)

a) (n) = number of animals
Defferences is statistically significant ($p<0.001$).
Each value represents the mean ± S.D.

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