RESULTS

1. ANTI-INFLAMMATORY TEST

1.1 Effects of PNQ-4482 and phenylbutazone on EPP-induced ear edema in rats

The inhibitory effect produced by the topical administration of PNQ-4482 on EPP-induced rat ear edema was assessed at 15, 30, 60 and 120 min after EPP application. As shown in Table 1, PNQ-4482 at the doses of 1, 2 and 4 mg/ear produced significant and dose-dependent inhibition of the edema formation at all assessment times. The percent inhibition on the ear edema formation gradually increased as the dose was increased. The results showed that a marked effect of PNQ-4482 was obtained with the highest dose used in this test i.e. 4 mg/ear. This dose showed significant inhibitory effect on edema formation of 70%, 65%, 58% and 63% at 15, 30, 60 and 120 min, respectively, after topical application of EPP. Phenylbutazone, the nonsteroidal anti-inflammatory drug, at the dose of 1 mg/ear, exhibited significant inhibitory activity on the edema formation at all determination times. It produced marked antiedema activity of 56%, 60%, 57% and 48% at 15, 30, 60 and 120 min, respectively.

1.2 Effects of PNQ-4482, phenlbutazone and phenidone on arachidonic acid-induced ear edema in rats

Results obtained from the rat ear edema induced by AA are demonstrated in Table 2. Phenylbutazone, a cyclooxygenase inhibitor, at the dose of 1 mg/ear did not show any inhibitory effect on AA-induced edema during the first 30 min. Anyhow, when assessment was done at 60 and 120 min, phenylbutazone exhibited slight edema inhibition. In contrast, phenidone, a dual inhibitor of AA metabolism, exhibited marked inhibitory activity on the edema formation of 61%, 52%, 52% and 56% when assessment was done at 15, 30, 60 and 120 min after AA application. At doses of 1, 2 and 4 mg/ear, PNQ-4482 possessed profound inhibitory effect on AA-induced rat ear edema at all assessment times. The percent inhibition on the edema formation gradually increased

Table 1. Effects of PNQ-4482 and phenylbutazone on EPP-induced ear edema in rats

Group	Dose (mg/ear)	Time after topical application of EPP							
		15 min		30 min		1h		2 h	
		ED (μm)	EDI (%)	ED (μm)	EDI (%)	ED (μm)	EDI (%)	ED (μm)	EDI (%)
Control	-	210.00 ± 11.94	-	373.33 ± 6.43	-	380.00 ± 8.63	-	276.67 ± 12.61	-
Phenylbutazone	1	93.33 ± 6.43*	56	146.67 ± 14.66*	60	163.33 ± 11.59*	57	143.33 ± 12.61*	48
PNQ-4482	1	143.33 ± 18.91*	32	260.00 ± 11.14*	31	263.33 ± 12.61*	31	160.00 ± 17.96*	42
	2	110.00 ± 4.31*	48	213.33 ± 9.54*	43	226.67 ± 9.54*	40	133.33 ± 8.13*	52
	4	56.67 ± 5.93*	70	130.00 ± 9.64*	65	160.00 ± 9.96*	58	103.33 ± 10.47*	63

Test drugs were applied topically to both inner and outer surfaces of the ear. Control = received vehicle (acetone) only.

Values are expressed as mean ± S.E.M. (N = 10). Significantly different from control: * $P < 0.001$.

ED = edema thickness at time, % ED = percent edema inhibition of test compound at time

as the dose was increased. The result showed that a marked effect of PNQ-4482 was obtained with the highest dose used in this test i.e. 4 mg/ear. This dose showed significant inhibitory effect on edema formation of 69%, 74%, 70% and 74% at 15, 30, 60 and 120 min, respectively, after topical application of AA.

1.3 Effects of PNQ-4482 and aspirin on carrageenin-induced hind paw edema in rats

The inhibitory activity on carrageenin-induced rat hind paw edema caused by an oral administration of PNQ-4482 and aspirin at various times after carrageenin injection is shown in Table 3.

Aspirin, a cyclooxygenase inhibitor, at the dose of 300 mg/kg exhibited significant edema inhibitory activity. PNQ-4482 at doses of 37.5, 70, and 150 mg/kg possessed profound inhibitory effect on carrageenin-induced paw edema at all assessment times. The anti-inflammatory effect of PNQ-4482 in this animal model was gradually increased with the increasing doses. The inhibitory activity of PNQ-4482 on the paw edema formation at the dose of 150 mg/kg was greater than that of aspirin at the dose of 300 mg/kg. The percent edema inhibition produced by the dose of 150 mg/kg of PNQ-4482 on carrageenin-induced edema formation of the rat paw were 79, 64 and 66 whereas those produced by aspirin were 53, 57 and 58 at the 1st, 3rd and 5th h, respectively, after carrageenin injection.

1.4 Effects of PNQ-4482, aspirin and prednisolone on carrageenin-induced pleurisy in rats

The effects of test drugs on pleural exudate formation, leukocyte accumulation and PGE_2-like substance were assessed in the rat pleurisy model induced by carrageenin.

1.4.1 Pleural exudate volume

The anti-exudative formation effect of PNQ-4482, aspirin and prednisolone obtained from rat pleurisy model is shown in Table 4. The pleural exudate formed by an intrapleural injection of carrageenin was harvested 3 h after carrageenin injection. The results obtained revealed that the three drugs tested exhibited significantly inhibitory

Table 2. Effects of PNQ-4482, phenylbutazone and phenidone on AA-induced ear edema in rats

Group	Dose (mg/ear)	Time after topical application of AA							
		15 min		30 min		1h		2 h	
		ED (um)	EDI (%)	ED (um)	EDI (%)	ED (um)	EDI (%)	ED (um)	EDI (%)
Control	-	180.00 ± 9.96	-	276.67 ± 7.74	-	290.00 ± 4.31	-	266.67 ± 4.07	-
Phenylbutazone	1	166.67 ± 8.13	7	260.00 ± 7.04	6	263.33 ± 9.21*	9	236.67 ± 7.74*	11
Phenidone	1	70.00 ± 11.94**	61	133.33 ± 4.07**	52	140.00 ± 4.98**	52	116.66 ± 7.74**	56
PNQ-4482	1	116.67 ± 3.21**	35	203.33 ± 9.21**	27	226.67 ± 8.13**	22	210.00 ± 8.26**	21
	2	86.67 ± 6.43**	52	106.67 ± 6.43**	61	113.33 ± 9.54**	61	100.00 ± 11.14**	63
	4	56.67 ± 5.93**	69	73.33 ± 9.54**	74	86.67 ± 9.54**	70	70.00 ± 8.20**	74

Test drugs were applied topically to both inner and outer surfaces of the ear. Control = received vehicle (acetone) only.

Values are expressed as mean ± S.E.M. (N = 10). Significantly different from control: * $P < 0.05$, ** $P < 0.001$.

ED = edema thickness at time, % ED = percent edema inhibition of test compound at time

Table 3. Effects of PNQ-4482 and aspirin on carrageenin-induced paw edema in rats

Group	Dose (mg/kg)	Time after carrageenin injection					
		1 h		3 h		5 h	
		EV (ml)	EI (%)	EV (ml)	EI (%)	EV (ml)	EI (%)
Control	-	0.34 ± 0.02	-	0.96 ± 0.01	-	0.79 ± 0.01	-
Aspirin	300	0.16 ± 0.02**	53	0.41 ± 0.03**	57	0.33 ± 0.02**	58
PNQ-4482	37.5	0.19 ± 0.03*	44	0.59 ± 0.02**	39	0.50 ± 0.02**	37
	75	0.13 ± 0.04**	62	0.48 ± 0.06**	50	0.36 ± 0.06**	54
	150	0.07 ± 0.03**	79	0.35 ± 0.06**	64	0.27 ± 0.06**	66

Test drugs were orally administered 1 h before carrageenin injection. Control = received 5% Tween 80 only.

Values are expressed as mean ± S.E.M. ($N = 6$). Significantly different from control: *$P < 0.01$, ** $P < 0.001$.

EV = edema volume (ml) at time, % EI = percent edema inhibition of test compound at time

activity on the formation of the pleural exudate. PNQ-4482 at a dose of 150 mg/kg showed stronger anti-exudative activity (72%) than aspirin at the dose of 300 mg/kg (66%) and prednisolone (the steroidal anti-inflammatory drug) at a dose of 5 mg/kg (64%).

1.4.2 Total leukocytes count in the pleural exudate

Table 5 demonstrates inhibitory effects of PNQ-4482, aspirin and prednisolone on the number of the total leukocytes in the pleural exudate which was determined 3 h after carrageenin injection. The results obtained revealed that PNQ-4482 (150 mg/kg), aspirin (300 mg/kg) and prednisolone (5 mg/kg) exhibited significant inhibitory effect on the accumulation of the total leukocytes. PNQ-4482 and aspirin were found to possess larger inhibitory effects on leukocyte accumulation than prednisolone. The percent inhibition on the leukocytic mobilization of PNQ-4482, aspirin and prednisolone was found to be 82, 78 and 41, respectively.

1.4.3 PGE_2-like substance activity

Contractile responses of rat fundus strip preparation induced by exudative PGE_2-like substances and standard PGE_2 are shown in Figure 14. Inhibitory effect of test drugs on PGE_2-like substances presented in pleural exudates is shown in Table 6. The amounts of PGE_2-like substances at the 3rd hour obtained from the exudates of aspirin (300 mg/kg)-, prednisolone (5 mg/kg)- and PNQ-4482 (150 mg/kg)-treated groups were 7.04 $\pm$ 0.19, 9.68 $\pm$ 0.19 and 5.97 $\pm$ 0.31 ng/ml respectively whereas that from control group was 14.05 $\pm$ 0.42 ng/ml. The results obtained showed that all test drugs i.e. aspirin, prednisolone and PNQ-4482 exhibited significantly inhibitory effect on the production of PGE_2-like substances with the percent of inhibition of 50, 31 and 58, respectively.

1.5 Effects of PNQ-4482, aspirin and prednisolone on the cotton pellet-induced granuloma formation in rats

The inhibitory effect of PNQ-4482 and the two reference drugs, i.e. aspirin and prednisolone, on the cotton pellet-induced granuloma formation in rats was examined on the eighth day after the daily oral administration of test drugs for 7 days.

Table 4. Effects of PNQ-4482, aspirin and prednisolone on pleural exudate volume of carrageenin-induced rat pleurisy

Group	Dose (mg/kg)	3 hr after carrageenin injection	
		exudate volume (ml)	inhibition (%)
Control	-	1.77 ± 0.08	-
Aspirin	300	0.60 ± 0.04*	66
Prednisolone	5	0.63 ± 0.04*	64
PNQ-4482	150	0.50 ± 0.04*	72

Test drugs were orally administered 1 h before induced pleurisy.

Values are expressed as mean ± S.E.M. ($N = 6$).

Significantly different from control: * $P < 0.001$.

Control = received 5% Tween 80 only.

Table 5. Effects of PNQ-4482, aspirin and prednisolone on total leukocyte count of carrageenin-induced rat pleurisy

Group	Dose (mg/kg)	3 hr after carrageenin injection.	
		Total leukocyte no. (x 10^7/ mm^3)	inhibition (%)
Control	-	106.45 ± 16.44	-
Aspirin	300	23.37 ± 7.80*	78
Prednisolone	5	62.78 ± 10.56*	41
PNQ-4482	150	19.20 ± 6.80*	82

Test drugs were orally administered 1 h before induced pleurisy.

Values are expressed as mean ± S.E.M. (N = 6).

Significantly different from control: * $P < 0.001$.

Control = received 5% Tween 80 only.

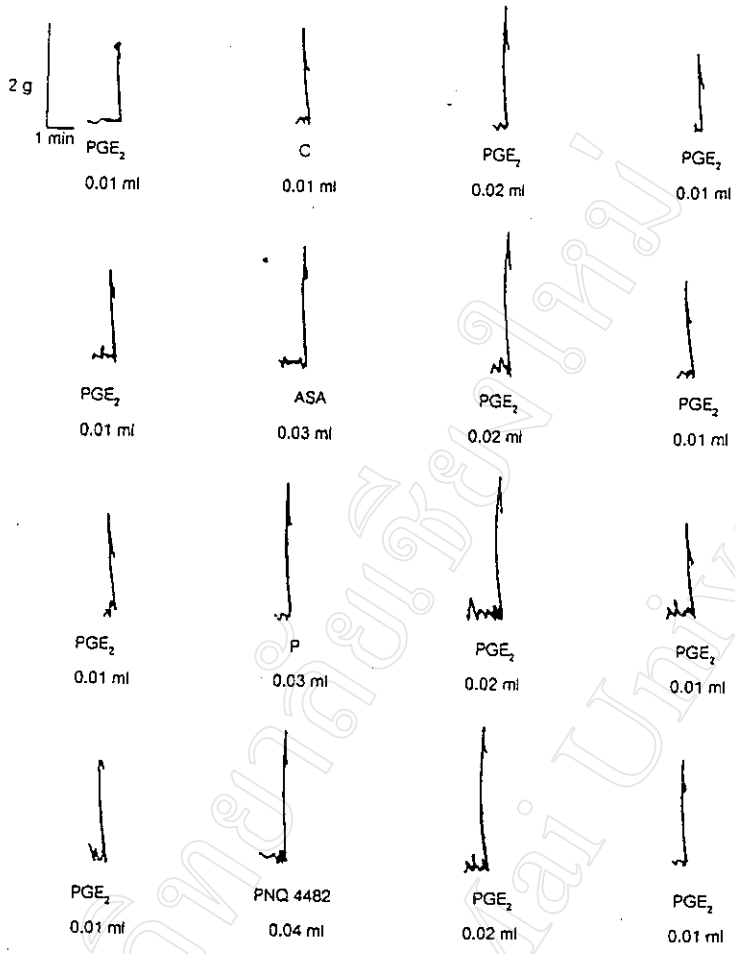

Figure 14. Response of rat fundus strip to standard PGE_2 (100 ng/ml) to the pleural exudate of drug-treated groups obtained 3 h after intrapleural injection of carrageenin :

C = control group

ASA = aspirin-treated group (300 mg/kg)

P = prednisolone-treated group (5 mg/kg)

PNQ-4482 = PNQ-4482-treated group (150 mg/kg)

Table 6. Effects of PNQ-4482, aspirin and prednisolone on PGE_2-like activity of carrageenin-induced rat pleurisy

Group	Dose (mg/kg)	3 h after carrageenin injection.	
		exudate (ng/ml)	inhibition (%)
Control	-	14.05 ± 0.42	-
Aspirin	300	7.04 ± 0.19*	50
Prednisolone	5	9.68 ± 0.19*	31
PNQ-4482	150	5.97 ± 0.31*	58

Test drugs were orally administered 1 h before induced pleurisy.

Values are expressed as mean ± S.E.M. (N = 6).

Significantly different from control: * $P < 0.001$.

Control = received 5% Tween 80 only.

1.5.1 Granuloma formation and transudation

Table 7 depicts the inhibitory effcect of PNQ-4482 and reference drugs on the granuloma formation and transudative weight induced by cotton pellet implantation. It was found that aspirin, and PNQ-4482 exhibited slight inhibitory effect on the granuloma formation of 16% and 10 %, respectively. Prednisolone, on the contrary, elicited marked antigranuloma formation of 41 %. The transudative weight of the control group was found to be 407.63 mg. PNQ-4482, prednisolone and aspirin, significantly reduced the weight of the transudate to 360.35, 226.73 and 321.02 mg, respectively. It indicated that all test drugs inhibited the formation of transudate.

1.5.2 Body weight gain and thymus weight

Table 8 shows the body weight gain during the first and the last day of the experimental period as well as the dry weight of thymuses of the rats implanted with cotton pellets. In the control group the body weight gain was 46.67 $\pm$ 3.33 g. The gain of the weight of the PNQ-4482-treated group was 42.50 $\pm$ 4.03 g, which was not significantly different from that of the control group. On the contrary, prednisolone, at the dose of 5 mg/kg markedly reduced the gain of the body weight to 15.00 $\pm$ 1.83 g. Aspirin at the dose of 300 mg/kg slightly but significantly decreased the weight gain of the animals to 36.67 $\pm$ 3.33 g.

Dry thymus weight of rats in the control group was 51.05 $\pm$ 1.18 mg/100 g body weight. Both aspirin and PNQ-4482 did not show any suppressive effect on the thymus weight of the rats (49.74 $\pm$ 1.58 and 50.91 $\pm$ 1.42 mg/100 g body weight, respectively) when compared with the control group, whereas prednisolone significantly reduced the thymus weight of the animals to 22.62 $\pm$ 2.02 mg/100 g body weight.

1.5.3 Alkaline phosphatase activity

The effects of test drugs on alkaline phosphatase activity in rats implanted with cotton pellets are shown in Table 9. Significant elevated alkaline phosphatase level in the serum of rats in control group was observed (55.47 x 10^{-4} U of enz./mg of serum protein) when compared with that of normal or non-implanted rats (43.74 x 10^{-4} U of enz./mg of serum protein). The increase in serum alkaline phosphatase caused by cotton pellet implantation was reduced to normal level by PNQ-4482 at the dose of 150

Table 7. Effects of PNQ-4482, aspirin and prednisolone on granuloma formation and transudation of cotton pellet-induced granuloma in rats

Group	Dose (mg/kg)	Granuloma wet weight (mg)	Granuloma dry weight (mg)	Transudative weight (mg)	Granuloma weight (mg/mg cotton)	GI (%)
Control	-	483.48 ± 5.01	75.85 ± 1.32	407.63 ± 3.84	2.80 ± 0.01	-
Aspirin	300	385.55 ± 4.68**	64.53 ± 1.08**	321.02 ± 4.81**	2.35 ± 0.01**	16
Prednisolone	5	279.90 ± 6.05**	53.17 ± 0.85**	226.73 ± 5.60**	1.66 ± 0.00**	41
PNQ-4482	150	430.50 ± 7.45**	70.15 ± 0.68**	360.35 ± 7.10**	2.51 ± 0.00*	10

Values are expressed as mean ± S.E.M. ($N = 6$).

Significantly different from control: * $P < 0.01$, ** $P < 0.001$.

GI = granuloma inhibition. Control = received 5% Tween 80 only.

Table 8. Effects of PNQ-4482, aspirin and prednisolone on body weight and thymus weight of cotton pellet-induced granuloma in rats

Group	Dose (mg/kg)	Body weight (g)			Dry thymus weight (mg/100 g)
		Initial	Final	Gain	
Control	-	193.33 ± 4.22	240.00 ± 2.58	46.67 ± 3.33	51.05 ± 1.18
Aspirin	300	193.33 ± 4.22	228.33 ± 3.07*	36.67 ± 3.33*	49.74 ± 1.58
Prednisolone	5	190.00 ± 4.72	203.33 ± 4.01**	15.00 ± 1.83**	22.62 ± 2.02**
PNQ-4482	150	186.67 ± 4.22	229.17 ± 0.83*	42.50 ± 4.03	50.91 ± 1.42

Values are expressed as mean ± S.E.M. ($N = 6$).

Significantly different from control: * $P < 0.05$, ** $P < 0.001$.

Control = received 5% Tween 80 only.

mg/kg (39.35 x 10^{-4} U of enz./mg of serum protein) as well as by both aspirin at the dose of 300 mg/kg (39.63 x 10^{-4} U of enz./mg of serum protein) and prednisolone at the dose of 5 mg/kg (40.32 x 10^{-4} U of enz./mg of serum protein).

2. ANALGESIC TEST

2.1 Effects of PNQ-4482, morphine and aspirin on acetic acid-induced writhing response in mice

The analgesic effect of PNQ-4482 was assessed in comparison with morphine and aspirin. As shown in Table 10, morphine at a dose of 10 mg/kg completely abolished the writhing response induced in mice by acetic acid. Aspirin at a dose of 150 mg/kg also exerted significant inhibition of the number of writhes (90 % inhibition). PNQ-4482 was found to possess profound inhibitory activity on the writhing response. At doses of 37.5, 75 and 150 mg/kg, PNQ-4482 showed the reduction of the number of writhes, with the percentage of 45, 78 and 89, respectively.

2.2 Effects of PNQ-4482, morphine and aspirin on the formalin test in mice

The inhibitory effect of PNQ-4482 on algesia induced by formalin at the hind paw of mice was investigated both in early phase and late phase, using licking of the paw as a criterion for algesia.

2.2.1 Early phase

The results on licking response in the early phase of the formalin test are shown in Table 11. Morphine at a dose of 10 mg/kg completely inhibited the licking response. Aspirin at a dose of 150 mg/kg slightly inhibited the licking response with the percentage of 17. PNQ-4482, at doses of 150, 300 and 600 mg/kg, similarly possessing analgesic activity, showed inhibitory effect on licking response with the percentages of 12, 33, and 39, respectively.

2.2.2 Late phase

Inhibition of licking response of the test drugs in the late phase of the formalin test is shown in Table 12. Morphine, at a dose of 10 mg/kg, exerted a pronounced effect with complete inhibition of licking response. Aspirin and PNQ4482 at a dose of

Table 9. Effects of PNQ-4482, aspirin and prednisolone on alkaline phosphatase activity in the serum of cotton pellet-induced granuloma in rats

Group	Dose (mg/kg)	Alkaline phosphatase (units/l)	Total protein (g/dl)	Serum alkaline phoshatase activity (U of enz./mg of serum protein x 10^{-4})
Normal	-	235.83 ± 4.17	5.50 ± 0.01	43.74 ± 0.42
Control	-	317.00 ± 6.52	5.73 ± 0.01	55.47 ± 1.35[a*]
Aspirin	300	209.33 ± 4.98	5.28 ± 0.13	39.63 ± 0.18[b*]
Prednisolone	5	249.17 ± 3.39	5.95 ± 0.21	40.32 ± 0.86[b*]
PNQ-4482	150	202.67 ± 6.87	5.15 ± 0.17	39.35 ± 0.33[b*]

Values are expressed as mean ± S.E.M. ($N = 6$).

a = significant from normal: * $P < 0.001$.. b = significant from control: * $P < 0.001$.

Normal = non-implanted group; Control = implanted group, received 5% Tween 80 only.

Table 10. Effects of PNQ-4482, morphine and aspirin on acetic acid induced writhing response in mice

Group	Dose (mg/kg)	No. of writhes	Inhibition of Writhing Response (%)
Control	-	19.67 ± 0.76	-
Morphine	10	0.0*	100
Aspirin	150	2.00 ± 0.45*	90
PNQ-4482	37.5	10.83 ± 0.60*	45
	75	4.33 ± 0.49*	78
	150	2.17 ± 0.31*	89

Test drugs were intraperitoneally administered 30 min before acetic acid injection.

Values are expressed as mean ± S.E.M. ($N = 6$).

Significantly different from control: * $P < 0.001$.

Control = received 5% Tween 80 only.

Table 11. Effects of PNQ-4482, morphine and aspirin in the early phase of the formalin test in mice

Group	Dose (mg/kg)	Licking time (s)	Inhibition of Licking response (%)
Control	-	91.67 ± 3.09	-
Morphine	10	0.0***	100
Aspirin	150	76.50 ± 4.90**	17
PNQ-4482	150	81.00 ± 4.53*	12
	300	61.17 ± 2.20***	33
	600	55.50 ± 0.85***	39

Test drugs were intraperitoneally administered 30 min before 1% formalin injection.

Values are expressed as mean ± S.E.M. ($N = 6$).

Significantly different from control: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Control = received 5% Tween 80 only.

150 mg/kg elicited analgesic effect in the late phase with the percentage of inhibition of 58. At doses of 300 and 600 mg/kg, PNQ-4482 exhibited an intensive analgesic activity with percentage of inhibition of 81 and 88, respectively. The results show that PNQ-4482 and aspirin possess similar analgesic activity in the late phase of the formalin test.

3. ANTIPYRETIC TEST

Effects of PNQ-4482 and aspirin on yeast-iduced hyperthermia in rats

As shown in Table 13, in the group which received aspirin at the dose of 300 mg/kg, significant reduction of the rectal temperature was observed. Aspirin could reduce the rectal temperature of the rats from 39.28 ± 0.00 °C to 38.83 ± 0.13, 38.15 ± 0.22, 37.83 ± 0.22 and 37.65 ± 0.23°C when measurement was made 30, 60, 90 and 120 min, respectively, after drug administration. All doses of PNQ-4482, (75, 150 and 300 mg/kg) significantly reduced the rectal temperature of hyperthermic rats at all assessment times. PNQ-4482, at a dose of 300 mg/kg elicited pronounced effects in reduction of rectal temperature after yeast-induced body temperature from 39.20 ± 0.10 °C to 38.02 ± 0.21, 37.17 ± 0.15, 37.07 ± 0.01 and 36.85 ± 0.01 °C at 30, 60, 90 and 120 min, respectively.

4. ANTI-ULCEROGENIC TEST

4.1 Ethanol/hydrochloric acid-induced gastric lesions in rats

PNQ-4482 was assessed against EtOH/hydrochloric acid-induced gastric lesions in rats. The results obtained are shown in Table 14. It was found that PNQ-4482 significantly elicited anti-ulcerogenic activity in this animal model. The gastric lesion in the group receiving vehicle was 144.25 ± 8.65. PNQ-4482 at doses of 150, 300 and 600 mg/kg reduced the formation of gastric lesions to 140.00 ± 11.67, 6.52 ± 2.88 and 0.00 mm, with the percentage of inhibition of 3, 96 and 100, respectively. Cimetidine, a reference anti-ulcer agent at the dose of 100 mg/kg, exhibited moderate anti-ulcer activity; the formation of gastric lesion in cimetidine-treated group was 89.27 ± 6.95 mm, with percent inhibition of 38.

Table 12. Effects of PNQ-4482, morphine and aspirin in the late phase of the formalin test in mice

Group	Dose (mg/kg)	Licking time (s)	Inhibition of Licking response (%)
Control	-	103.50 ± 1.75	-
Morphine	10	0.0*	100
Aspirin	150	43.50 ± 3.31*	58
PNQ-4482	150	43.33 ± 2.46*	58
	300	20.00 ± 1.15*	81
	600	12.50 ± 0.76*	88

Test drugs were intraperitoneally administered 10 min before 1% formalin injection.

Values are expressed as mean ± S.E.M. (N = 6).

Significantly different from control: * $P < 0.001$.

Control = received 5% Tween 80 only.

Table 13. Effects of PNQ-4482 and aspirin on yeast- induced hyperthermia in rats

Group	Dose (mg/kg)	Rectal temperature (°C)					
		Before Yeast injection	18 h after yeast injection	Time after medication (min)			
			0	30	60	90	120
Control	-	38.07 ± 0.26	39.45 ± 0.01	39.63 ± 0.12	39.58 ± 0.12	39.53 ± 0.01	39.45 ± 0.01
Aspirin	300	37.60 ± 0.24	39.28 ± 0.00	38.83 ± 0.13***	38.15 ± 0.22***	37.83 ± 0.22***	37.65 ± 0.23***
PNQ-4482	75	37.93 ± 0.23	39.32 ± 0.01	39.18 ± 0.01*	39.02 ± 0.15*	38.95 ± 0.18*	38.80 ± 0.22*
	150	38.05 ± 0.12	39.67 ± 0.15	39.03 ± 0.17**	38.92 ± 0.16**	38.67 ± 0.15***	38.47 ± 0.17***
	300	37.73 ± 0.30	39.20 ± 0.10	38.02 ± 0.21***	37.17 ± 0.15***	37.07 ± 0.01***	36.85 ± 0.01***

Test drugs were orally administered 18 h after yeast injection.

Values are expressed as mean ± S.E.M. ($N = 8$).

Significantly different from the rectal temperature after yeast injection 18 h: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Control = received 5% Tween 80 only.

The results indicated that the gastric lesions were markedly reduced and completely abolished by PNQ-4482 at the doses of 300 and 600 mg/kg, respectively. Whereas the dose of 150 mg/kg elicited only mild and nonsignificant reduction of gastric lesions.

4.2 Indomethacin-induced gastric lesions in rats

Table 15 demonstrates the data obtained with PNQ-4482 and cimetidine (the reference drug) on indomethacin-induced gastric lesion in rats. PNQ-4482 was also found to exhibit marked anti-ulcerogenic activity in this model. The gastric lesions observed with PNQ-4482 administered at the doses of 150, 300and 600 mg/kg were 3.25 $\pm$ 1.37, 0.53 $\pm$ 0.32 and 0.12 $\pm$ 0.12, with percent inhibition 20, 87 and 97, respectively. It was found that the inhibition of ulcer formation increased with the increasing dose of PNQ-4482. The results obtained indicated that while the doses of PNQ-4482 at 300 and 600 mg/kg possessed pronounced anti-ulcerogenic activity, the dose of 150 mg/kg exerted a nonsignificant inhibition on gastric lesions induced by indomethacin. Cimetidine showed anti-ulcerogenic activity with the ulcer index of 0.49 $\pm$ 0.30 and with percent inhibition of 88.

4.3 Restraint water immersion stress-induced gastric lesions in rats

The inhibitory effects of PNQ-4482 and cimetidine on restraint water immersion stress-induced gastric lesions in rats are shown in Table 16. Cimetidine, a reference anti-ulcer agent and PNQ-4482 significantly inhibited the ulcer formation. Ulcers induced by restraint water immersion stress as observed in the control group resulted in gastric lesions of 22.60 $\pm$ 1.67 mm. Cimetidine at the dose of 100 mg/kg and PNQ-4482 at the dose of 150 mg/kg significantly reduced the formation of gastric lesions to 10.92 $\pm$ 1.55 and 15.83 $\pm$ 1.38, respectively. The percent inhibition of gastric lesions produced by cimetidine and PNQ-4482 were 52 and 30, respectively.

Table 14. Effects of PNQ-4482 and cimetidine on ethanol/hydrochloric acid-induced gastric lesions in rats

Group	Dose (mg/kg)	Gastric lesion (mm.)	Inhibition (%)
Control	-	144.25 ± 8.65	
Cimetidine	100	89.27 ± 6.95*	38
PNQ-4482	150	140.00 ± 11.67	3
	300	6.52 ± 2.88*	96
	600	0.0*	100

Test drugs were orally administered 1 h before induced gastric lesion.

Values are expressed as mean ± S.E.M. ($N = 6$).

Significantly different from control: * $P < 0.001$.

Control = received 5% Tween 80 only.

Table 15. Effects of PNQ-4482 and cimetidine on indomethacin-induced gastric lesions in rats

Group	Dose (mg/kg)	Gastric lesion (mm.)	Inhibition (%)
Control	-	4.05 ± 1.25	
Cimetidine	100	0.49 ± 0.30*	88
PNQ-4482	150	3.25 ± 1.37	20
	300	0.53 ± 0.32*	87
	600	0.12 ± 0.12*	97

Test drugs were orally administered 1 h before induced gastric lesion.

Values are expressed as mean ± S.E.M. ($N = 6$).

Significantly different from control: * $P < 0.001$.

Control = received 5% Tween 80 only.

Table 16. Effects of PNQ-4482 and cimetidine on restraint water immersion stress-induced gastric lesions in rats

Group	Dose (mg/kg)	Gastric lesion (mm.)	Inhibition (%)
Control	-	22.60 ± 1.67	
Cimetidine	100	10.92 ± 1.55*	52
PNQ-4482	150	15.83 ± 1.38*	30

Test drugs were orally administered 1 h before induced gastric lesion.

Values are expressed as mean ± S.E.M. ($N = 6$).

Significantly different from control: * $P < 0.001$.

Control = received 5% Tween 80 only.

4.4 Pylorus ligation-induced gastric lesions in rats

The effects of PNQ-4482 and cimetidine on the volume of gastric secretion and the total acidity were investigated in the model of pylorus-ligation-induced gastric lesions in rats. The results obtained are depicted in Table 17. A gastric volume of 9.05 ± 0.42 ml and a total acidity of 192.39 mEq were observed in the control group. Both cimetidine(100 mg/kg) and PNQ-4482 (150, 300 and 600 mg/kg) showed an anti-secretory effect, causing a significant decrease of the gastric volume and the total acidity. The group pretreated with cimetidine at a dose of 100 mg/kg reduced the gastric volume and the total acidity to 5.08 ± 0.49 ml and 65.67 ± 7.50 mEq, respectively. The inhibition of PNQ-4482 on the gastric secretion gradually increased as the dose increased. PNQ-4482 at doses of 150, 300 and 600s mg/kg reduced the gastric volume to 8.48 ± 0.42, 5.08 ± 0.24 and 2.75 ± 0.21 ml, and total acidity to 150.27 ±10.37, 85.92 ± 8.14 and 40.17 ± 3.03 mEq, respectively.

5. HIPPOCRATIC SCREEN

Changes in general behavior of animals caused by PNQ-4482 were studied in conscious rats. As PNQ-4482 at the dose of 5,000 mg/kg given orally did not produce any signs or symptoms in rats, the test substance was then given by an intraperitoneal route. Any signs and symptoms observed following drug administration were recorded in the standard working sheet. Two doses levels, an ineffective and a lethal dose, were first determined. The doses of 2,000 and 5,000 mg/kg were selected. The results showed that no change in general behavior of rats was observed from the dose of 2,000 mg/kg whereas the rats receiving 5,000 mg/kg of PNQ-4482 died within 1 h. Symptoms and toxicity observed from PNQ-4482 at the dose of 5,000 mg/kg were as follows: decrease of motor activity, loss of righting reflex, loss of screen grip, decrease of respiratory rate, and death.

The three effective doses were calculated according to the equation in Hippocratic screen using the dose 2,000 mg/kg as an ineffective dose and 5,000 mg/kg as a lethal dose. Five dose levels, i.e. 2,000, 2,500, 3,100, 3,900 and 5,000 mg/kg were obtained. Accordingly, the results revealed that an intraperitoneal dose of 3,900 mg/kg

Table 17. Effects of PNQ-4482 and cimetidine on the volume of gastric secretion and acidity in pylorus ligated (Shay) rats

Groups	Dose (mg/kg)	Volume of gastric secretion (ml)	Titratable acidity (mEq/ml)	Total acid output (mEq)
Control	-	9.05 ± 0.42	21.17 ± 0.67	192.39 ± 13.09
Cimetidine	100	5.08 ± 0.49**	12.92 ± 0.69**	65.67 ± 7.50**
PNQ-4482	150	8.48 ± 0.42*	17.67 ± 0.67**	150.27 ± 10.37**
	300	5.08 ± 0.24**	16.83 ± 1.14**	85.92 ± 8.14**
	600	2.75 ± 0.21**	14.67 ± 0.49**	40.17 ± 3.03**

Test drugs were orally administered 1 h before pylorus ligated rats.

Values are expressed as mean ± S.E.M. ($N = 6$).

Significantly different from control: * $P < 0.01$, ** $P < 0.001$.

Control = received 5% Tween 80 only.

caused death of the rats. Therefore this dose was used as a lethal dose, and the five doses were recalculated. The doses obtained were 2,000, 2,400, 2,900, 3,500 and 3,900 mg/kg. Table 18, 19 and 20 summarize the results of Hippocratic screen. The results obtained show that symptoms and toxicity could be observed in groups of rats treated with 2,400, 2,900, 3,500, and 3,900 mg/kg of PNQ-4482. The intensity of responses increased with the increasing doses. Signs and symptoms which occurred in response to PNQ-4482 were a decrease in motor activity, loss of screen grip, loss of righting reflex and decrease of respiratory rate. Sign of respiratory failure was observed before death in rats received the dose of 3,900 mg/kg. All surviving animals were sacrificed and internal organs were examined. No unusual signs of abnormalities of the internal organs could be detected and they were found to be normal in both size and color.

6. ACUTE TOXICITY STUDY

The results obtained from various doses of PNQ-4482 administered orally showed that no death was found during the 14 day-observation either in the control or treated groups, even at the highest dose of 5,000 mg/kg of PNQ-4482. The rats did not show any signs of toxicity or changes in general behavior or other physiological activities.

The body and organ weights of male and female rats from the control and PNQ-4482-treated groups (5,000 mg/kg) are presented in Table 21 and 22. It was noted that there were no significant differences of the body and organ weights between the control and PNQ-4482-treated groups.

The pathological examination of the internal organs at a gross level revealed that there were no detectable abnormalities and no differences between the control and PNQ-4482-treated groups.

Table 18. Effects of PNQ-4482 on motor activity and respiratory rate in conscious rats (Hippocratic screen)

	Minutes after drug administration									
PNQ-4482	Decrease of motor activity					Decrease of respiratory rate				
(mg/kg)	5	15	30	60	120	5	15	30	60	120
2,000	0	0	0	0	0	0	0	0	0	0
2,400	0	+1	+1	+1	0	0	0	+1	+1	0
2,900	0	+1	+2	+2	+1	0	0	+1	+2	0
3,500	0	+1	+2	+3	+2	0	0	+1	+2	+2
3,900*	0	+1	+3	+4	+4	0	+2	+3	+4	+4

* died within 6 hr., n = 8 (male = 4, female = 4)

PNQ-4482 were intraperitoneal administered.

Remarks

Decrease of motor activity

+1 = does not move spontaneously, but when handled will move rapidly

+2 = when handled will move slowly

+3 = when handled will move sluggishly

+4 = when handled will not move at all

Decrease in respiratory rate

+1 = 10% decrease in respiratory rate

+2 = 20% decrease in respiratory rate

+3 = 40% decrease in respiratory rate

+4 = 80% decrease in respiratory rate

Table 19. Effects of PNQ-4482 on righting reflex and screen grip in conscious rats (Hippocratic screen)

	Minutes after drug administration									
PNQ-4482	Loss of righting reflex					Loss of screen grip				
(mg/kg)	5	15	30	60	120	5	15	30	60	120
2,000	0	0	0	0	0	0	0	0	0	0
2,400	0	0	+1	+1	0	0	0	+1	+1	0
2,900	0	0	+1	+2	0	0	0	+1	+1	+1
3,500	0	0	+1	+2	+2	0	0	+1	+3	+2
3,900*	0	0	+1	+3	+4	0	0	+1	+3	+4

* died within 6 hr., n = 8 (male = 4, female = 4)

PNQ-4482 were intraperitoneal administered.

Remarks

Loss of righting reflex

+1 = can be placed only on one side

+2 = can be placed on either side equally well

+3 = can be placed on back as well as either side

+4 = can not be aroused from back position by the hind leg toe pinch

Loss of screen grip

+1 = falls at first shake of screen

+2 = falls off when screen has been inverted

+3 = falls off when the screen is at a 90° angle

+4 = the rat falls off as the screen is tilted to a 45° angle

Table 20. Effect of PNQ-4482 in conscious rats recorded 1 h after drug administration.

Signs and symptoms	PNQ-4482 (mg/kg)				
	2,000	2,400	2,900	3,500	3,900*
Decrease of motor activity	0	+1	+2	+3	+4
Decrease in respiratory rate	0	+1	+2	+2	+3
Loss of righting reflex	0	+1	+2	+2	+3
Loss of screen grip	0	+1	+2	+3	+3
Time of death (h)	-	-	-	-	6

* died within 6 h, n = 8 (male = 4, female = 4)

PNQ-4482 were intraperitoneal administered

Remarks

Decrease of motor activity

+1 = does not move spontaneously, but when handled will move rapidly

+2 = when handled will move slowly

+3 = when handled will move sluggishly

+4 = when handled will not move at all

Decrease in respiratory rate

+1 = 10% decrease in respiratory rate

+2 = 20% decrease in respiratory rate

+3 = 40% decrease in respiratory rate

+4 = 80% decrease in respiratory rate

Loss of righting reflex

+1 = can be placed only on one side

+2 = can be placed on either side equally well

+3 = can be placed on back as well as either side

+4 = can not be aroused from back position by the hind leg toe pinch

Loss of screen grip

+1 = falls at first shake of screen

+2 = falls off when screen has been inverted

+3 = falls off when the screen is at a 90° angle

+4 = the rat falls off as the screen is tilted to a 45° angle

Table 21. Body weights of rats in the acute toxicity study of PNQ-4482

	Body weight (g)	
Group	Control (5%Tween80)	PNQ-4482 (5,000 mg/kg)
Female		
Day 0	178.00 ± 1.97	181.80 ±1.88
Day 7	205.40 ± 3.36	200.30 ± 3.72
Day 14	235.70 ± 2.72	235.70 ± 1.36
Increased	57.70 ± 2.91	53.90 ± 2.73
Male		
Day 0	176.90 ± 1.47	177.40 ± 1.71
Day 7	227.70 ± 1.81	227.20 ± 3.31
Day 14	256.50 ± 1.49	257.90 ± 1.31
Increased	79.60 ± 1.32	80.50 ± 2.13

Values are expressed as mean ± S.E.M. (N = 10)

Table 22. Organ weights of rats in the acute toxicity study of PNQ-4482

Group		Organ weight (g)	
		Control (5% Tween 80)	PNQ-4482 (5,000 mg/kg)
Female			
	Lung	1.04 ± 0.10	0.91 ± 0.02
	Heart	0.89 ± 0.02	0.89 ± 0.03
	Liver	7.90 ± 0.20	8.31 ± 0.31
	Spleen	0.72 ± 0.09	0.70 ± 0.07
	Adrenal	0.03 ± 0.00	0.03 ± 0.00
	Kidney	0.84 ± 0.02	0.83 ± 0.04
	Ovary	0.06 ± 0.00	0.06 ± 0.00
Male			
	Lung	1.35 ± 0.05	1.34 ± 0.07
	Heart	1.35 ± 0.06	1.32 ± 0.06
	Liver	12.14 ± 0.35	12.71 ± 0.62
	Spleen	0.82 ± 0.01	0.96 ± 0.07
	Adrenal	0.03 ± 0.00	0.03 ± 0.00
	Kidney	1.28 ± 0.04	1.31 ± 0.03
	Testis	1.68 ± 0.03	1.63 ± 0.02

Values are expressed as mean ± S.E.M. (N = 10)

7. SUBACUTE TOXICITY STUDY

As PNQ-4482 given orally at a dose of 5,000 mg/kg did not produce any toxicity (acute toxicity test), in the subacute toxicity test the dose of 1,000 mg/kg given daily for 14 days was chosen (according to the OECD guidelines for testing of chemicals).

The animals were weighed and then anesthetized with pentobarbital sodium. The external appearance including the hair and the skin texture as examined before opening the abdominal wall and the thoracic cage. The venous blood was taken for chemical study. All the internal organs were dissected one by one and weighed as wet weight. The solid organs, including hearts, lungs, liver, pancreas, spleen, kidneys, adrenal glands, muscle and sex organs, were serially cut for gross examination whereas the hollow viscera, including digestive tracts, were opened for examination. The calvaria were opened and the brains including the pituitary glands were dissected. The organs of interest were livers, kidneys, brains and sex organs which were involved in chemical metabolism, and reproduction. The livers and kidneys were serially cut into several pieces whereas the others were taken as bisected or in toto. All representative sections were fixed in 10% neutral-buffered formalin before being routinely processed. They were embedded in paraffin as tissue blocks and cut at 4 microns. The tissue sections were stained routinely with hematoxylin and eosin for microscopic examination.

Table 23 demonstrates data of body weights of control and PNQ-4482-treated groups of both male and female rats. It was found that treatment with PNQ-4482 for 14 days did not cause any change in general behavior or other physiological activity of both male and female rats. The body weight gain of the female treated group in 14 days was slightly less than that of the control group, whereas that of a satellite group, which received PNQ-4482 for 14 days followed by no treatment for a further 14 days, was significantly higher than those of both groups. However, the body weight of the three groups of male rats, i.e. control, PNQ-4482-treated group and the satellite group was not different from each other. In fact, the body weight gain of the satellite group should be more than those of the control group and the treated groups.

The organ weights of the control and treated groups of male as well as female rats are given in Table 24. Administration of PNQ-4482 at 1,000 mg/kg daily for 14 days did not cause significant change in organ weight in either male or female rats. The gain of the internal organ weight in the female satellite group was significantly different from the control group as the rats were kept for 14 additional days. However, the weights of liver and spleen of the male satellite group were significantly less than those of the control group.

Data in Table 25 describe the hematological values of control and PNQ4482-treated group. All hematological values of the female group given PNQ-4482 for 14 days were found to be non-significantly different from those of control group. HGB, HCT and MCV values of the female satellite group were slightly less than those of the control group. Both HGB and HCT values of the male group treated with PNQ-4482 for 14 days were also found to be less than those of the control group. Incontrast, the male satellite group showed significant increase of MCH and MCHC values.

Figures for the differentiation of white blood cells in male and female rats are provided in Table 26. The data show no significant difference between the control and treated groups.

Results obtained from the blood chemistry values of female rat are given in Table 27. PNQ-4482 was not found to produce any change in blood chemistry values of animals. Only the albumin value of the satellite group was significantly less than that of control group. The blood chemistry values of male rats are shown in Table 28. The PNQ-4482-treated group did not produce any abnormality of blood chemistry in the rats. In contrast, a significant rise of creatinine and a significant decrease of alkaline phosphatase as observed in the satellite group as compared with the control group. The albumin value of the male satellite group was slightly less than that of the control group.

The pathological observation based on gross and histopathology of male and female rats was examined in all control and treated animals. All the sampling tissue section of the internal organs, both from the control and the treated groups, were within

normal limits. Neither degenerative nor infiltrative lesions were observed. No significant changes referring to cell injury were noted among the animals.

Table 23. Body weights of rats in the subacute toxicity study of PNQ-4482

	Body weight (g)		
Group	Control (5% Acacia)	PNQ-4482 (1,000 mg/kg)[a]	PNQ-4482 (1,000 mg/kg)[b]
Female			
Initial weight	147.88 ± 0.58	148.86 ± 0.51	148.88 ± 0.40
Final weight	212.25 ± 3.65	197.14 ± 4.57*	229.50 ± 5.23*
Increased	64.38 ± 3.41	48.29 ± 4.30*	80.63 ± 5.24*
n	8	7	8
Male			
Initial weight	148.00 ± 0.60	149.13 ± 0.40	148.57 ± 0.57
Final weight	313.50 ± 4.22	302.25 ± 6.50	308.00 ± 7.86
Increased	165.50 ± 4.50	153.13 ± 6.70	160.29 ± 8.52
n	8	8	7

a: A group was given 1,000 mg/kg daily for 14 days

b: A satellite group was given 1,000 mg/kg daily for 14 days followed by no treatment for 14 days

Values are expressed as mean ± S.E.M.

Significantly different from control: * $P<0.05$

Table 24. Organ weights of rats in the subacute toxicity study of PNQ-4482

Group	Organ weight (g)		
	Control (5% Acacia)	PNQ-4482 (1,000 mg/kg)[a]	PNQ-4482 (1,000 mg/kg)[b]
Female			
Lung	0.99 ± 0.03	0.94 ± 0.03	1.35 ± 0.04***
Heart	0.89 ± 0.03	0.81 ± 0.03	1.05 ± 0.04**
Liver	7.94 ± 0.25	8.33 ± 0.41	9.32 ± 0.37**
Spleen	0.74 ± 0.12	0.64 ± 0.02	0.69 ± 0.03
Adrenal	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00*
Kidney	0.84 ± 0.02	0.87 ± 0.04	0.98 ± 0.04**
Ovary	0.06 ± 0.00	0.06 ± 0.01	0.06 ± 0.01
n	8	7	8
Male			
Lung	1.34 ± 0.05	1.30 ± 0.07	1.35 ± 0.13
Heart	1.37 ± 0.07	1.28 ± 0.07	1.20 ± 0.04
Liver	12.46 ± 0.33	12.83 ± 0.74	9.71 ± 0.54**
Spleen	0.83 ± 0.01	0.90 ± 0.05	0.63 ± 0.02***
Adrenal	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Kidney	1.30 ± 0.05	1.30 ± 0.04	1.22 ± 0.06
Testis	1.60 ± 0.13	1.64 ± 0.02	1.77 ± 0.04
n	8	8	7

a: A group was given 1,000 mg/kg daily for 14 days

b: A satellite group was given 1,000 mg/kg daily for 14 days followed by no treatment for 14 days

Values are expressed as mean ± S.E.M.

Significantly different from control: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 25. Hematological values of rats in the subacute toxicity study of PNQ-4482

Group	Control (5% Acacia)	PNQ-4482 (1,000 mg/kg)[a]	PNQ-4482 (1,000 mg/kg)[b]
Female			
HGB	15.36 ± 0.51	14.98 ± 0.44	13.69 ± 0.26**
HCT	43.30 ± 1.28	41.98 ± 1.24	38.38 ± 0.71**
MCV	56.28 ± 0.37	56.21 ± 0.52	54.53 ± 0.21**
MCH	18.70 ±1.31	20.05 ± 0.17	19.45 ± 0.11
MCHC	35.45 ± 0.17	35.65 ± 0.23	35.65 ± 0.17
n	8	7	8
Male			
HGB	15.03 ± 0.07	13.64 ± 0.52*	15.00 ± 0.11
HCT	45.13 ± 0.40	41.09 ± 1.60*	44.84 ± 0.58
MCV	73.75 ± 14.76	105.54 ± 34.31	115.97 ± 21.75
MCH	21.79 ± 5.24	28.14 ± 5.58	40.43 ± 7.61*
MCHC	41.03 ± 7.34	48.44 ± 9.60	74.66 ± 14.14*
n	8	8	7

a: A group was given 1,000 mg/kg daily for 14 days

b: A satellite group was given 1,000 mg/kg daily for 14 days followed by no treatment for 14 days

Values are expressed as mean ± S.E.M.

Significantly different from control: * $P<0.05$, ** $P < 0.01$.

Table 26. Differential white blood cell counts of rats in the subacute toxicity study of PNQ-4482

Group	Control (5% Acacia)	PNQ-4482 (1,000 mg/kg)[a]	PNQ-4482 (1,000 mg/kg)[b]
Female			
Neutrophil	1.56 ± 0.20	2.39 ± 0.63	1.41 ± 0.17
Eosinophil	94.60 ± 0.70	94.31 ± 0.94	95.84 ± 0.40
Monocyte	3.75 ± 0.51	3.03 ± 0.37	2.65 ± 0.27
Lymphocyte	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
n	8	7	8
Male			
Neutrophil	1.66 ± 0.35	1.55 ± 0.33	2.41 ± 0.16
Eosinophil	94.83 ± 0.35	94.64 ± 0.91	94.19 ± 0.56
Monocyte	3.01 ± 0.49	3.81 ± 0.60	3.43 ± 0.39
Lymphocyte	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
n	8	8	7

a: A group was given 1,000 mg/kg daily for 14 days

b: A satellite group was given 1,000 mg/kg daily for 14 days followed by no treatment for 14 days

Values are expressed as mean ± S.E.M.

Table 27. Blood chemistry values of female rats in the subacute toxicity study of PNQ-4482

Group	Control (5% Acacia)	PNQ-4482 (1,000 mg/kg)[a]	PNQ-4482 (1,000 mg/kg)[b]
Female			
Glucose (mg/dl)	161.00 ± 3.12	167.29 ± 12.18	184.25 ± 7.90
BUN (mg/dl)	27.13 ± 1.75	25.57 ± 1.11	24.50 ± 0.89
Creatinine (mg/dl)	0.50 ± 0.03	0.50 ± 0.04	0.56 ± 0.02
Total protein (g/dl)	5.83 ± 0.10	5.06 ± 0.51	5.20 ± 0.11
Albumin (g/dl)	1.74 ± 0.02	1.69 ± 0.03	1.48 ± 0.04*
Total bilirubin (mg/dl)	0.20 ± 0.03	0.24 ± 0.03	0.14 ± 0.03
Direct bilirubin (mg/dl)	0.01 ± 0.01	0.03 ± 0.02	0.03 ± 0.02
SGOT (IU/L)	91.13 ± 4.08	84.57 ± 6.88	97.88 ± 4.41
SGPT (IU/L)	36.00 ± 2.18	39.71 ± 5.22	38.25 ± 0.90
Alk-P	136.88 ± 8.05	151.00 ± 15.35	141.38 ± 6.89
n	8	7	8

a: A group was given 1,000 mg/kg daily for 14 days

b: A satellite group was given 1,000 mg/kg daily for 14 days followed by no treatment for 14 days

Values are expressed as mean ± S.E.M.

Significantly different from control: * $P < 0.001$.

Table 28. Blood chemistry values of male rats in the subacute toxicity study of PNQ-4482

Group	Control (5% Acacia)	PNQ-4482 (1,000 mg/kg)[a]	PNQ-4482 (1,000 mg/kg)[b]
Male			
Glucose (mg/dl)	150.25 ± 9.06	155.13 ± 11.44	157.71 ± 6.39
BUN (mg/dl)	23.88 ± 1.01	23.25 ± 1.32	24.29 ± 2.04
Creatinine (mg/dl)	0.41 ± 0.02	0.40 ± 0.12	0.59 ± 0.03**
Total protein (g/dl)	5.49 ± 0.07	5.53 ± 0.10	5.76 ± 0.17
Albumin (g/dl)	1.51 ± 0.02	1.49 ± 0.04	1.43 ± 0.02*
Total bilirubin (mg/dl)	0.26 ± 0.05	0.26 ± 0.04	0.21 ± 0.03
Direct bilirubin (mg/dl)	0.01 ± 0.01	0.03 ± 0.02	0.04 ± 0.02
SGOT (IU/L)	111.63 ± 5.32	100.25 ± 16.25	101.57 ± 6.16
SGPT (IU/L)	55.63 ± 7.07	58.63 ± 14.28	36.71 ±1.30
Alk-P	237.13 ± 15.51	204.38 +14.25	99.14 ± 4.22**
n	8	8	7

a: A group was given 1,000 mg/kg daily for 14 days

b: A satellite group was given 1,000 mg/kg daily for 14 days followed by no treatment for 14 days

Values are expressed as mean ± S.E.M.

Significantly different from control: * $P<0.05$, ** $P < 0.001$.