

The Role of Mast Cell Depletion On the Acute Toxicity of Morphine In Mice

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SUMMARY

LD₅₀ dose of morphine sulphate shows much varying differences, which was reported upon the experiments carried out on rats. When it is given 40-200 mg/kg, LD₅₀ dose, although, is stable, death rates increases above the dose of 200 mg/kg.

Morphine sulphate shows its acute toxicity through the histamine liberation but its LD₅₀ dose falls as low as 30 mg/kg if it is given following acute injection of 48/80 compound which a powerful histamine liberator. On the other hand, by chronic 48/80 compound injection, in mast cells depleted rats, its LD₅₀ dose increased as high as 795 mg/kg.

Close relation in the effectiveness of both morphine sulphate and 48/80 compound showed that histamine liberation has very important role in acute toxic effect of morphine sulphate. The indifference effect between aminopyrine, a central effective analgesic but not causing any histamine liberation, and 48/80 compound also proved our experiment result.

INTRODUCTION

In 1933, *Schmidt* and *Livingston*¹¹ suggested that in the decerebrated and anaesthetised cats and in the unanaesthetised dogs, the depressing effect of morphine on the arterial blood pressure may be related to the release of histamine. But he later dismissed this possibility. Later, *McIntosh* and *Paton*⁶ showed that the opium alkaloids also have the properties of releasing histamine. Subsequently, *Nasmyth* and *Stewart*⁸, *Feldberg* and *Paton*⁴ demonstrated more clearly that morphine causes release of morphine in the cat's skin and the perfused gastrocnemius muscle. Furthermore they stated that the release of histamine was reduced if the injection of morphine was repeated.

It is known that morphine exhibits its analgesic effects through its own receptor located in the central nervous system. Furthermore, if it is administered in subtoxic doses, it also produces some non-selective effects^{1,2}. In

1952, *Evans et al*³ demonstrated that the effects of morphine on the circulation, when injected i.v. to the cat, dog, and rat, were related to the released histamine.

In the literature, the lethal dose of morphine varies greatly from one investigator to the other, departing from the hypothesis in which different factors may play a role in the acute toxicity. Therefore, with the aim of determining whether or not these differences were related to the histamine releasing effect of morphine, we decided to investigate the influence of compound 48/80, a potent histamine releaser, on the acute toxicity of morphine. Although the mechanism of histamine releasing activity of compound 48/80 is different from that of other histamine releasers, as exposed by the work of *Rotschild*¹⁰, *Yamaski* and *Ende*¹⁴, their effects on the target organ during the histamine release must be similar.

MATERIAL and METHOD

Swiss albino mice of both sexes weighing 24 ± 7 grams were used throughout the experiments. All injections were made subcutaneously to the animals kept fasting 24 hours prior to experiments. The deaths seen within 48 hours were evaluated for determination of LD_{50} . The calculation of LD_{50} was made according to the graphic method described by *Miller and Tainter*⁷. Percentage of death was indicated as probit on the ordinate, and log dose recorded on the abscissa of the graph. The LD_{50} of morphine was found on the abscissa corresponding to the value of probit 5 on the ordinate. LD_{50} of morphine determinations were carried out on 350 mice divided into seven groups of 50. Each group received five different doses of morphine, so that each point on the graph. Consisted of seven different death percentage values which enabled us to evaluate the doses statistically.

The second series of LD_{50} estimations was made on 200 mice, and dealt with the influence of compound 48/80 on LD_{50} of morphine as determined in the first group. 200 mice were divided into four groups: in the first group of 50, 10 mg/kg of 48/80 was injected together with the 5 different doses of morphine, each dose given to 10 mice: in the second groups of 50, 10 mg/kg of 48/80 was injected 30 minutes before the administration of the 5 doses of morphine in the third and fourth groups of 50, the mice received 10 mg/kg of 48/80 60 and 120 minutes before morphine, respectively.

- In the third series of experiments, LD_{50} of morphine was estimated in 50 previously mast cell depleted mice by injecting compound 48/80 in doses of 10 mg/kg three consecutive days, twice a day. In the last group of fifty mice, the influence of compound 48/80 on the LD_{50} of aminopyrine, which is not a mast cell depletor and which causes convulsions before death, was tested. This group served as a control for showing that compound 48/80 has no effect on LD_{50} of aminopyrine.
- Statistical analyses were made by *Student T* test, and all graphs were drawn on the basis of probit versus log. dose.

The estimation of LD_{50} of morphine in intact naive mice :

LD_{50} of morphine was estimated on 350 mice divided into seven groups. Each group received morphine in doses starting from 40 mg/kg and reaching, in the last groups, 1600 mg/kg. The results are summarized in table 1/A and 1/B. Death percentages for doses of 40 mg/kg was 10; for 100 mg/kg was 10 and for 200 mg/kg was 10. It is obvious that the death percentages of these each three doses were the same this indicates that death percentages are not dose-dependent in these doses, whereas after 400 mg/kg, death rate increased according the dose administered to the mice. 1600 mg/kg was found as LD_{100} for morphine. The calculation of LD_{50} made by applying the method of *Miller Tainter*⁷, taking LD_1 as 40 mg and LD_{100} as 1600 mg/kg, showed that LD_{50} for morphine is 502 mg/kg. If the LD_1 is taken as 200 mg/kg and LD_{100} 1600 mg/kg the results showed that LD_{50} is 795 mg/kg. In our experiment, 795 mg/kg was accepted as LD_{50} of morphine, because between 40-200 mg/kg the death rate is not dose-dependent.

Table 1/A. Morphine Sulphate LD_{50} values obtained in the first group.

Dose (mg/kg)	Log. dose (X)	% Percentage of death (Y)	Probit	LD_{50} (mg/kg)
100	2.00	10	3.72	550
200	2.30	20	4.16	
400	2.60	30	4.48	
600	2.77	50	5	
800	2.90	90	6.28	
1000	3.00	100	+∞	

Table 1/B. Morphine sulphate LD₅₀ values obtained in the second group.

Dose (mg/kg)	Log. dose (x)	% Percentage of death (Y)	Probit	LD ₅₀ (mg/kg)	
40	1.60	10	3.72		
100	2.00	10	3.72		
200	2.30	10	3.72		
400	2.60	20	4.16	795	502
800	2.90	40	4.75		
1200	3.07	60	5.25		
1600	3.20	100	+∞		

The acute influence of 48/80 on the LD₅₀ of morphine :

As indicated in Table 2, if morphine is administered with 10 mg/kg of 48/80, which is not lethal, LD₅₀ of morphine is diminished. In other words, the LD₅₀ curve shifts to the left. The combination of morphine with the non-toxic dose shifts of 48/80 LD₅₀ decreased from 795 mg/kg to 37 mg/kg. The shift to the left was even more when morphine doses were administered 30 minutes after the administration of 10 mg/kg of 48/80. Thus the LD₅₀ became 22.5 mg/kg.

If the time between 48/80 and morphine is increased, the LD curve of morphine gradually shifts from the extreme left toward the LD curve estimated in control animals as illustrated in Fig. 1.

LD₅₀ of morphine in previously mast cell depleted animals :

The mast cells of the mice were depleted prior to the toxicity test by injection of 10 mg/kg of compound 48/80 twice a day for three consecutive days. The depleted animals were subjected to the toxicity test 24 hours after the completion of mast cell depletion. The results of such experiments are tabulated in Table 3. In these groups of animals, LD₅₀ of morphine was calculated as 880 mg/kg.

The difference between 790 and 880 mg/kg is significant statistically. The LD curve is also shifted to the right which can be seen in Figure 1.

The control experiments were carried out with aminopyrine, which is not a histamin liberator, and its LD₅₀ is fixed.

Table 2. Morphine Sulphate LD₅₀ values 0, 30, 60, 120, minutes after compound 48/80 injection.

Time lapse between 48/80 and morphine injection (min.)	Dose (mg/kg)	Log. dose (x)	% Percentage of death	Probit	LD ₅₀ (mg/kg)
0'	10	1	0	-∞	37
	25	1.39	20	4.16	
	40	1.60	60	5.25	
	50	1.69	90	6.28	
	75	1.87	100	—	
30'	10	1	10	3.72	22.5
	20	1.30	40	4.75	
	30	1.47	60	5.25	
	40	1.60	80	5.84	
	50	1.69	100	+∞	
60'	25	1.39	10	3.72	64
	50	1.69	40	4.75	
	100	2.00	70	5.52	
	150	2.17	90	6.28	
	200	2.30	100	+∞	
120'	50	1.69	10	3.72	210
	100	2.00	30	4.48	
	250	2.39	60	5.25	
	300	2.47	80	5.84	
	400	2.60	100	+∞	

Table 3. Morphine injection following chronic 48/80 depletion.

Dose (mg/kg)	Log. dose (x)	% Percentage of death (Y)	Probit	LD ₅₀ (mg/kg)
525	2.72	0	-∞	880
700	2.84	20	4.16	
800	2.90	60	5.25	
1000	3.00	70	5.52	
1200	3.07	100	+∞	

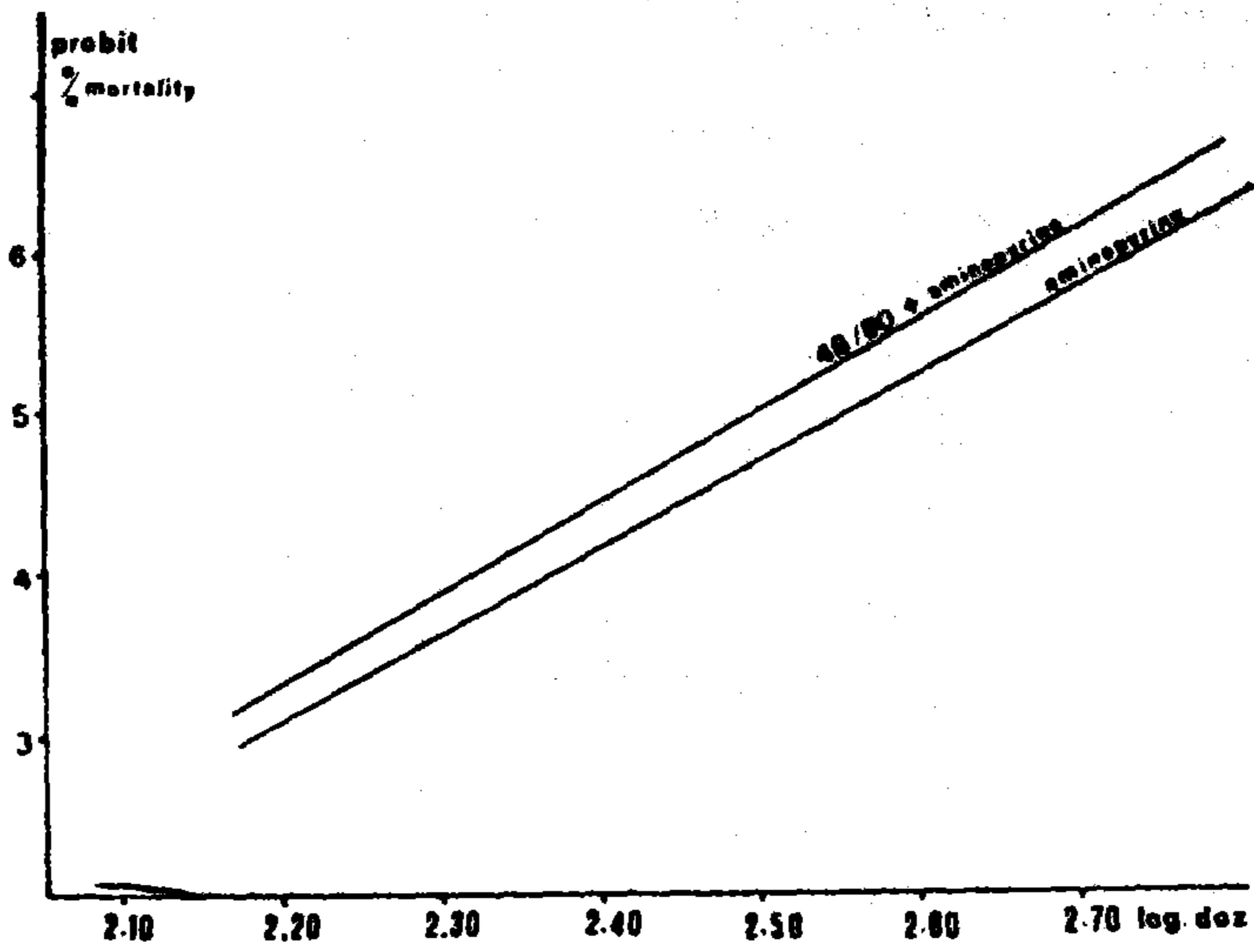


Figure 1

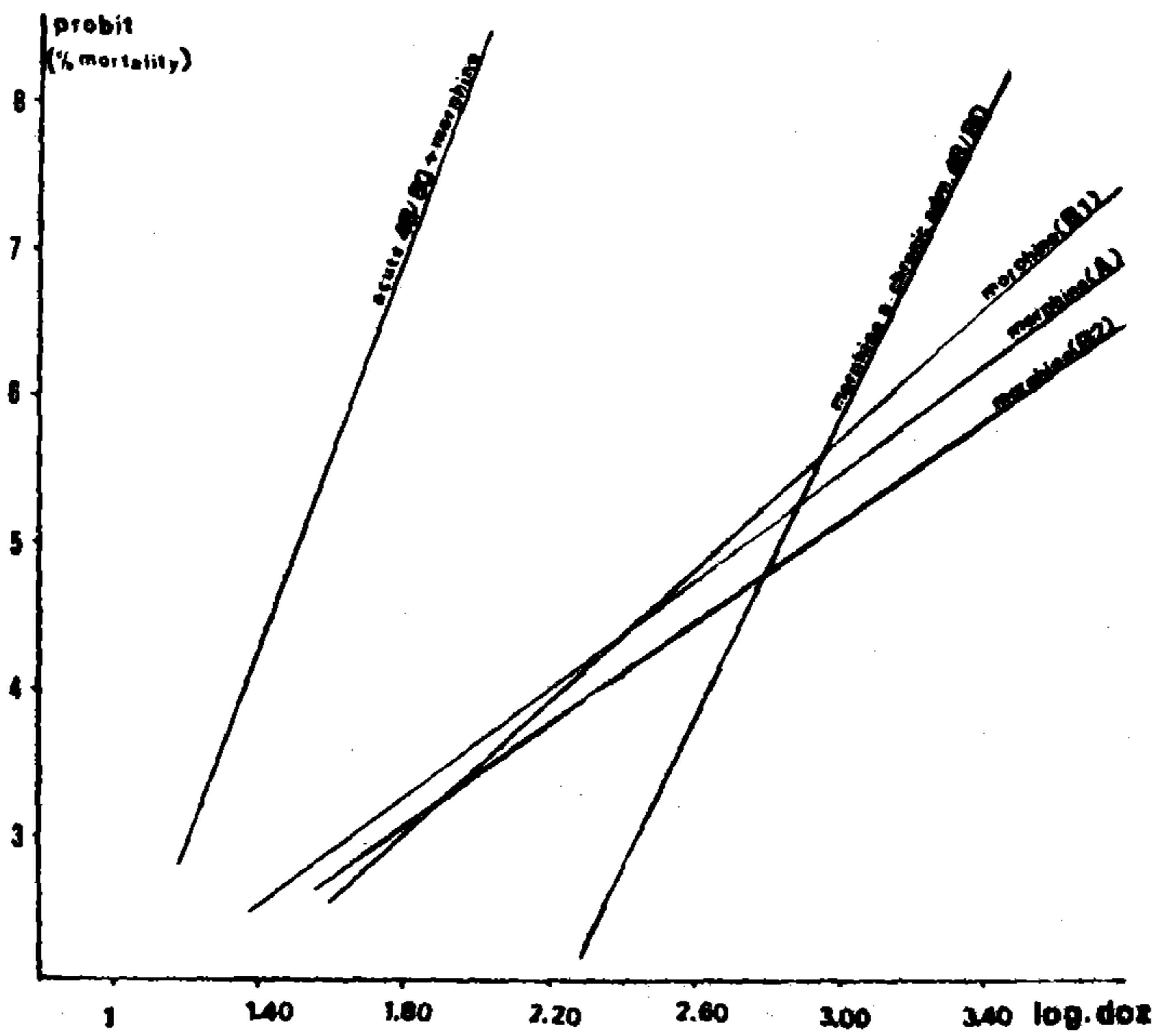


Figure 2

The first LD₅₀ of aminopyrine was determined in our laboratory conditions. LD₅₀ is 364 mg/kg. If it is administered together with 10 mg/kg, of 48/80, the LD₅₀ is found to be 330 mg/kg. The difference is not significant. The results of aminopyrine experiments can be seen in the Table 4 and 5.

Table 4. Aminopyrine LD₅₀ value.

Dose (mg/kg)	Log. dose (x)	% Percentage of death (Y)	Probit	LD ₅₀ (mg/kg)
200	2.30	10	3.72	364
300	2.47	30	4.48	
400	2.60	60	5.25	
500	2.69	80	5.84	
600	2.77	100	+∞	

n = 5

Table 5. The value aminopyrine LD₅₀ following acute 48/80 depletion

Dose (mg/kg)	Log. dose (x)	% Percentage of death (Y)	Probit	LD ₅₀ (mg/kg)
200	2.30	10	3.72	330
300	2.47	40	4.75	
400	2.60	70	5.52	
500	2.69	90	6.28	
600	2.77	100	+∞	

n = 5

DISCUSSION

In literature, the LD₅₀ values for morphine sulphate reported vary greatly from investigator to another in mice, it was determined as 200 mg/kg by *Starkenstein*¹²; 200-400 mg/kg by *Zunz*¹⁵; 500 mg/kg by *Merck Index*¹³; and 600 mg/kg by *Blozovski* and *Jacop*². In our experiments we also found different results for the LD₅₀ of morphine sulphate. A result of 550 mg/kg was obtained in breeding A, and values of 502 mg/kg and 795 mg/kg were found in breeding B, two rather different results.

As seen in Table 1/B, the percentage of deaths at doses ranging from 40 to 200 mg/kg, remain at a constant value of 10%. When a dose of 40 mg/kg is considered as the LD₁ value and that of 1600 mg/kg as the LD₁₀₀ value, the LD₅₀ value rises to 795 mg/kg. The fact that the percentage of deaths produced by doses ranging from 40 to 200 mg/kg is not dose-dependent, only becoming dose dependent when over 200 mg/kg, supports the hypothesis that the lethal dose of morphine sulphate in mice is caused by more than one factor. In order to find this out, a subtoxic dose of compound 48/80, a mast cell depletor and potent histamine-releasing substance, was administered with different doses of morphine sulphate. It was then observed that the LD₅₀ value morphine sulphate was lowered to a values as low as 35 mg/kg that is, morphine's toxicity is greatly increased. This finding demonstrates that compound 48/80 and morphine sulphate affected the same target organe. It is known that compound 48/80 has no major effect on the central nervous system comparable to morphine.

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