Screening for Physical Dependence Liability of *Anacardium occidentale* Infusion in Rats

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Abstrak

Telah diteliti kemampuan infusum Anacardium occidentale menimbulkan efek ketergantungan pada tikus. Sebanyak 36 tikus betina strain LMR dibagi menjadi 6 kelompok, masing-masing kelompok mendapat injeksi morfin, morfin, diazepam, dipiron, infusum Anacardium occidentale dan garam faal secara oral selama 36 hari dengan dosis yang terus ditingkatkan setiap 6 hari, kecuali yang mendapat garam faal. Pada hari ke 37 semua pemberian obat dihentikan dan hari berikutnya semua obat diberikan kembali. Pemberian morfin meningkatkan berat badan tikus pada siang hari dengan puncaknya terjadi antara jam 12.00 - 14.00, yang berbeda bermakna dengan berat badan pada jam 08.00 ($p < 0.05$). Bila pemberian morfin dihentikan berat badan tikus menurun tajam. Kelompok tikus yang diberi Anacardium occidentale tidak menunjukkan perubahan pola penurunan berat badan yang berbeda dengan sebelum pemberian obat. Hasil ini menunjukkan bahwa infusum daun Anacardium occidentale tidak terbukti mempunyai kemampuan menimbulkan efek ketergantungan obat tipe morfin.

Abstract

The physical dependence liability effect of an infusion of Anacardium occidentale leaves was studied on rats. Thirty-six female rats (Lembaga Makanan Rakyat strain) were divided into six groups of six. The groups received respectively morphine, diazepam, dipyron, saline, and Anacardium occidentale infusion. Morphine was administered orally and subcutaneously, while the other agents were given orally. These drugs were given continously for 36 days in 5 graded doses except for saline. The doses were increased after each interval of six days. At day 37 all drugs were stopped and on the next day were given again. Prior to drug administration body weight decreased from 8.00 a.m. to 4.00 p.m. After chronic administration of morphine orally or subcutaneously, the pattern of body weight changes showed an opposite diurnal pattern. Morphine increased the body weight, which reached a peak from 12.00 m. to 2.00 p.m. with values which were significantly different from those at 8.00 a.m. ($p < 0.05$). When morphine was stopped, body weight decreased more sharply. The Anacardium occidentale group, however, did not show any alteration from the pretreatment pattern. This result showed that there was no evidence that an infusion of Anacardium occidentale leaves had a morphine type physical dependence liability effect.

Keywords : *Anacardium occidentale; Drug dependency*

INTRODUCTION

After a drug is available on the market, it takes a long time to determine its dependence liability, and so before a drug is known to possess a certain degree of dependence liability it may already have created many addicts as well as a need for prevention and cure. That is why it is important to find a methods for testing substances for dependence liability. Nozaki[1] created a method to evaluate the dependence liability of morphine-like drugs. With this method he examined many drugs suggested to have dependence liability. Santoso and Sukasediati[2] concluded in their study that *Anacardium occidentale* prolonged reaction time to pain stimulus in mice. They suggested that this was an analgesic effect which might be related to morphine-like characteristics. They also suggested that *A. occidentale* might produce dependence like that caused by other morphine-like drugs. So they proposed *A. occidentale* be studied for dependence liability.

The aim of this study was to determine whether *Anacardium occidentale* does have a dependence liability similar to that of morphine-like drugs.

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MATERIALS AND METHODS

Thirty six female rats of *Lembaga Makanan Rakyat* (LMR) strain were randomly divided into six groups, each consisting of six rats. During this study each rat was weighed every two hours from 8 a.m. to 4 p.m. every day. After an acclimatization period of 15 days the administration of drugs was started. Each group received morphine orally or by subcutaneous injection, saline orally, diazepam orally, dipyron orally, or the *Anacardium occidentale* infusion orally in the morning after the first weighing and in the afternoon after the last weighing. The dosage was gradually increased every six days and stopped after 36 days of administration. The dosing scheme is described in Table 1. The pattern of body weight changes was studied throughout the experiment. Food and drink were supplied in the morning and in the afternoon.

For analysis we used Anova two ways statistical method.

Table 1. Scheme of administration of drugs

Drugs	Doses during each six day period					
	1	2	3	4	5	6
Morphine orally	20	40	60	80	100	100
Morphine by injection	10	20	40	80	100	100
Diazepam orally	1	2.5	5.0	7.5	10	10
Dipyron orally	300	500	700	900	900	900
Saline orally	1.5	1.5	1.5	1.5	1.5	1.5
A. occid. orally	100	200	300	400	500	500

Note : doses in mg/kg BW for drugs.
Saline in ml/100 g of body weight.

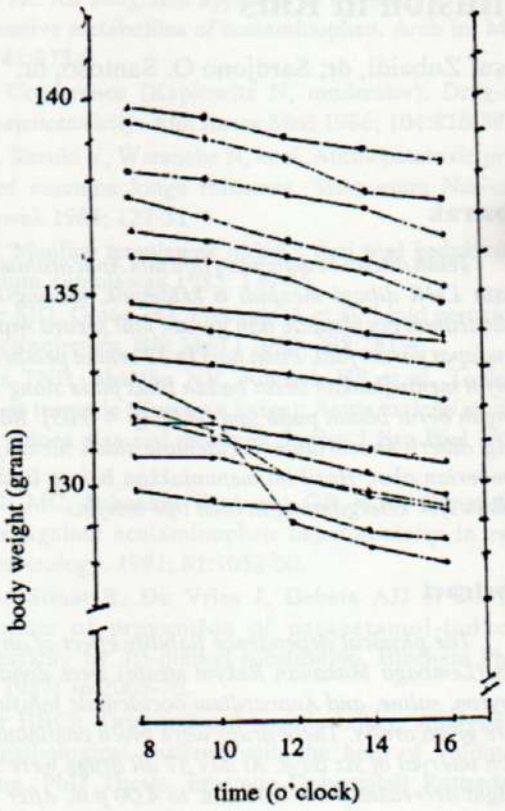


Figure 1. Changes of body weight of rats during the acclimatization period of 15 days (n = 40).

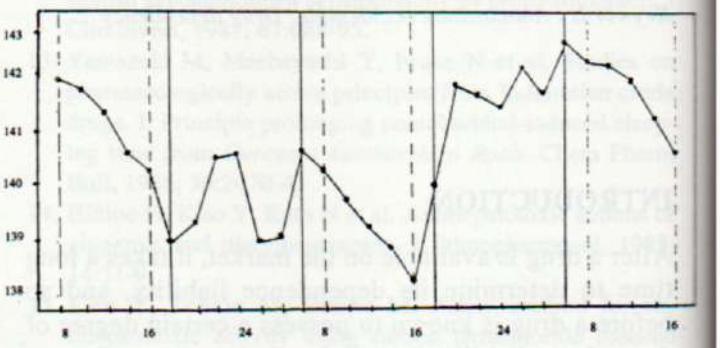


Figure 2. Changes of body weight of rats when continously weighed for 56 hours.

RESULTS

During acclimatization period

During the acclimatization period of 15 days an hour by hour decrease in body weight was found in the day time from 8 a.m. to 4 p.m. as described in Figure 1. Usually after 4 p.m. or 6 p.m. there was an increase in body weight until 8 a.m. or 6 a.m. followed again by a decrease, as described during continous weighing for 56 hours (see Figure 2).

The day before administration of drugs

The body weight changes of all rats before administration of drugs were similar to the body weight changes during acclimatization period as described in Table 2 and Figure 3.

Table 2. Changes of body weight of rats on the day before administration of drug

Group	Mean body weight of rats (grams) at times :				
	08.00	10.00	12.00	14.00	16.00
morphine (oral)	148,30 ± 10,46	148,25 ± 10,39	148,00 ± 10,43	147,63 ± 10,43	147,67 ± 10,27
morphine (inj.)	144,98 ± 13,67	144,90 ± 13,68	144,22 ± 13,49	143,75 ± 13,02	144,08 ± 12,41
diazepam	145,73 ± 10,43	145,68 ± 10,83	145,38 ± 10,93	145,08 ± 11,13	145,25 ± 10,54
dipyron	146,85 ± 12,19	146,80 ± 12,27	146,58 ± 12,14	146,12 ± 12,36	145,83 ± 12,18
saline	146,00 ± 14,36	145,83 ± 14,12	145,72 ± 14,10	145,37 ± 13,98	144,95 ± 13,12
A. occid.	146,58 ± 10,52	146,13 ± 10,35	145,75 ± 10,10	145,47 ± 9,93	144,47 ± 13,12

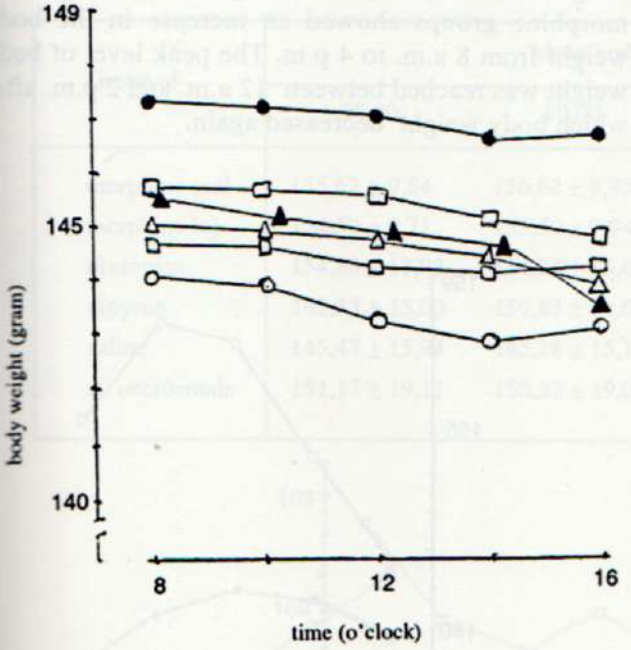
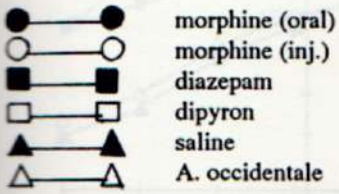


Figure 3. Changes of body weight of rats on the day before administration of drugs.



The first day of administration of drugs

The changes of the body weight during the first day of administration of drugs are described in the Table 3 and Figure 4. They show a slight change of body weight in the groups receiving morphine orally and by injection, while the others showed no changes at all. At the end of the first dose period the changes in the body weight were similar to those at the beginning of the first administration of the drugs, as described in Figure 5.

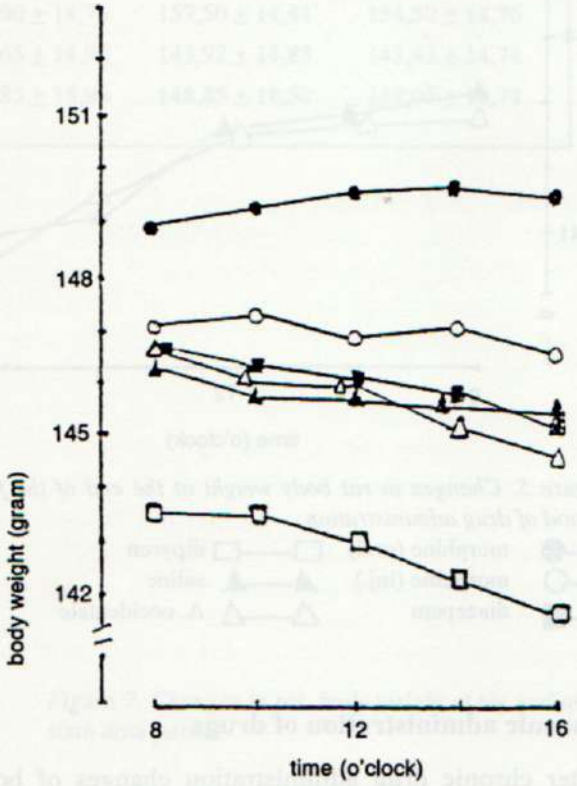


Figure 4. Changes in rat body weight on the first day of drug administration

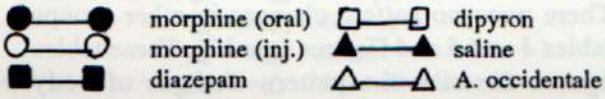


Table 3. Changes of body weight in rat on the first day of drug administration

Group	Mean body weight of rats (grams) at time :				
	08.00	10.00	12.00	14.00	16.00
morphine oral	148,92 ± 10,51	149,27 ± 10,19	149,58 ± 10,18	149,67 ± 10,26	149,30 ± 10,56
morphine inj.	147,05 ± 13,95	147,33 ± 14,38	146,92 ± 14,46	147,08 ± 14,15	146,62 ± 13,64
diazepam	146,30 ± 10,63	145,80 ± 10,43	145,80 ± 10,40	145,63 ± 10,40	145,43 ± 10,43
dipyron	146,62 ± 12,38	146,18 ± 12,07	146,00 ± 12,14	145,33 ± 12,05	144,58 ± 11,88
saline	143,53 ± 13,58	143,52 ± 13,42	143,00 ± 13,42	142,28 ± 13,17	141,75 ± 13,37
A. occidentale	146,58 ± 9,96	146,33 ± 10,40	146,10 ± 10,63	145,73 ± 10,43	145,32 ± 10,07

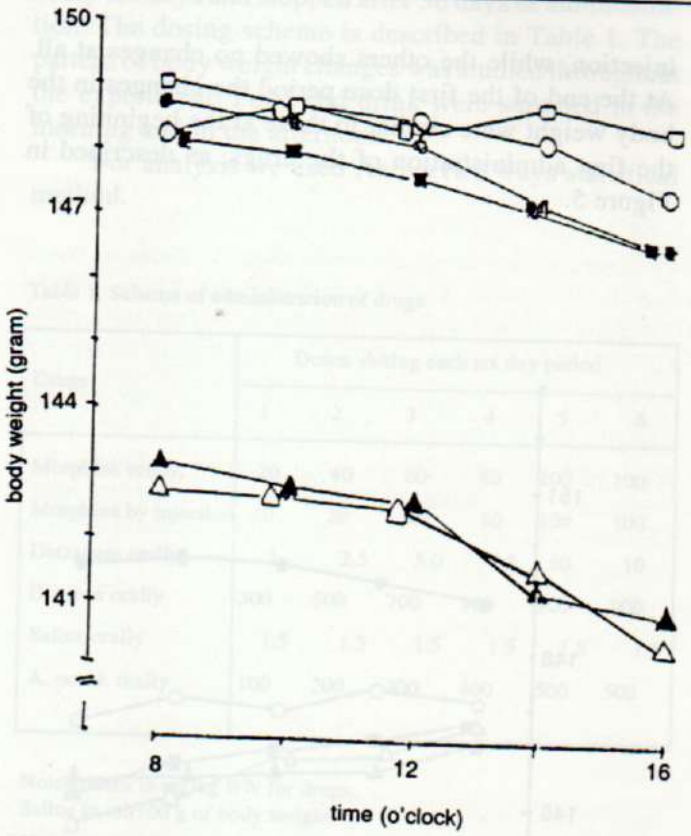


Figure 5. Changes in rat body weight at the end of the first period of drug administration

morphine (oral)
morphine (inj.)
diazepam
dipyron
saline
A. occidentale

Chronic administration of drugs

After chronic drug administration changes of body weight pattern from 8 a.m. to 4 p.m. were clearly seen in the groups receiving morphine orally and by injection. There were no pattern changes in other groups (see Tables 4 and 5 and Figures 6 and 7). These tables and figures describe the pattern changes of body weight after the third and sixth levels of dosage. The morphine groups showed an increase in the body weight from 8 a.m. to 4 p.m. The peak level of body weight was reached between 12 a.m. and 2 p.m. after which body weight decreased again.

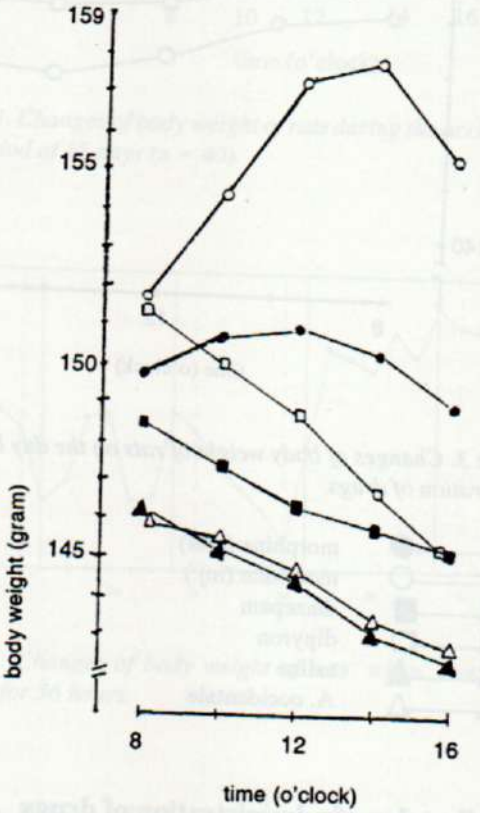


Figure 6. Changes in rat body weight at the end of third dose period.

morphine (oral)
morphine (inj.)
diazepam
dipyron
saline
A. occidentale

Table 4. Changes in body weight at the end of the third dose period

Group	Mean body weight of rats (grams) at time :				
	08.00	10.00	12.00	14.00	16.00
morphine oral	151,92 ± 11,24	152,58 ± 11,45	153,73 ± 11,59	152,92 ± 10,98	151,43 ± 10,72
morphine inj.	152,60 ± 13,86	153,27 ± 13,74	156,17 ± 14,59	155,62 ± 14,91	154,3 ± 14,73
diazepam	151,95 ± 14,18	151,25 ± 13,89	150,08 ± 13,51	149,13 ± 13,53	148,95 ± 13,45
dipyron	153,43 ± 11,49	152,22 ± 11,26	151,05 ± 11,34	149,70 ± 11,70	148,78 ± 11,99
saline	146,87 ± 13,36	146,03 ± 13,32	145,23 ± 13,21	144,28 ± 13,02	142,92 ± 14,01
A. occidentale	152,07 ± 16,34	151,37 ± 16,44	150,62 ± 16,44	148,62 ± 15,51	147,30 ± 14,82

Table 5. Changes in rat body weight at the end of sixth dose

Group of	Mean body weight of rats (grams) at time :				
	08.00	10.00	12.00	14.00	16.00
morphine oral	155,62 ± 9,84	156,62 ± 9,95	157,97 ± 9,64	157,25 ± 9,67	156,33 ± 9,90
morphine inj.	154,50 ± 9,71	155,50 ± 9,94	158,83 ± 9,97	160,00 ± 10,30	158,73 ± 10,33
diazepam	154,50 ± 13,92	154,25 ± 13,61	153,68 ± 13,67	153,25 ± 13,70	152,55 ± 13,57
dipyron	162,33 ± 15,00	159,83 ± 14,68	158,90 ± 14,76	157,50 ± 14,41	154,50 ± 14,76
saline	145,47 ± 15,94	145,28 ± 15,16	144,65 ± 14,96	143,92 ± 14,83	143,43 ± 14,74
A. occidentale	151,17 ± 19,11	150,32 ± 19,02	149,85 ± 18,96	148,85 ± 18,50	148,05 ± 18,74

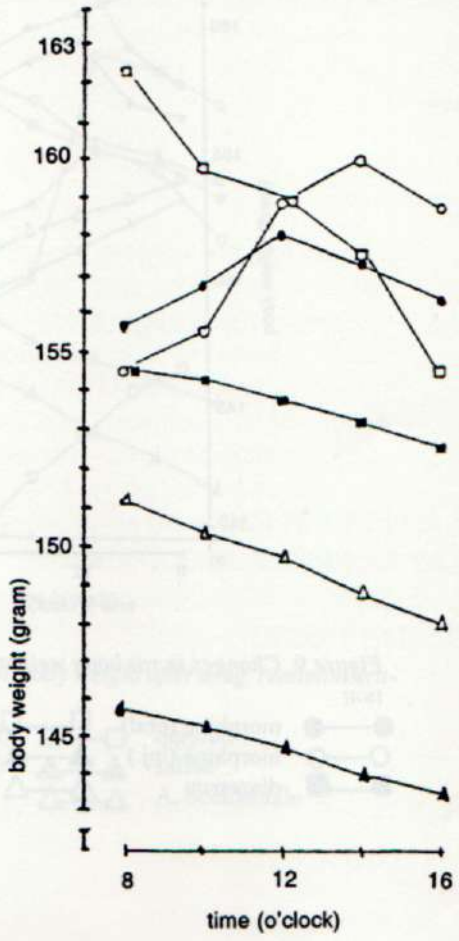


Figure 7. Changes in rat body weight at the end of the sixth dose period

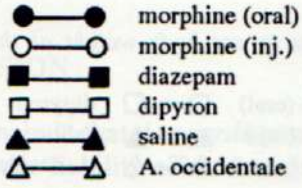


Table 6. Changes in rat body weight at the day of withdrawal

Group	Mean body weight of rats (grams) at times :				
	08.00	10.00	12.00	14.00	16.00
morphine oral	152,35 ± 10,06	151,25 ± 9,99	150,25 ± 10,11	149,30 ± 10,32	148,00 ± 10,47
morphine inj.	152,62 ± 11,72	151,42 ± 11,26	150,17 ± 11,19	148,62 ± 10,94	147,92 ± 10,56
diazepam	153,37 ± 12,57	153,33 ± 12,67	152,17 ± 12,81	150,88 ± 12,65	149,95 ± 12,56
dipyron	161,53 ± 17,52	160,00 ± 17,09	158,83 ± 16,82	156,17 ± 16,56	154,03 ± 16,34
saline	145,48 ± 14,90	144,37 ± 14,46	143,35 ± 14,32	142,77 ± 14,44	142,07 ± 14,41
A. occidentale	150,02 ± 18,23	149,17 ± 18,51	148,30 ± 18,43	147,45 ± 18,54	146,62 ± 18,34

Withdrawal of drug administration

After the administration of drugs was stopped, there were pattern changes of body weight in the morphine groups and no changes in the other groups (see Table 6 and Figure 8). In the groups receiving morphine, the body weight of rats from 8 a.m. to 4 p.m. decreased similar to the others.

Readministration of drugs

After one day of withdrawal, all drugs were administered again. The pattern of body weight changes was again similar to that in chronic administration (see Figure 9). When morphine injection was administered to the group formerly receiving oral morphine while oral morphine was given to the group formerly receiving morphine by injection, the pattern of body weight changes were reversed (see Figures 10 and 11).

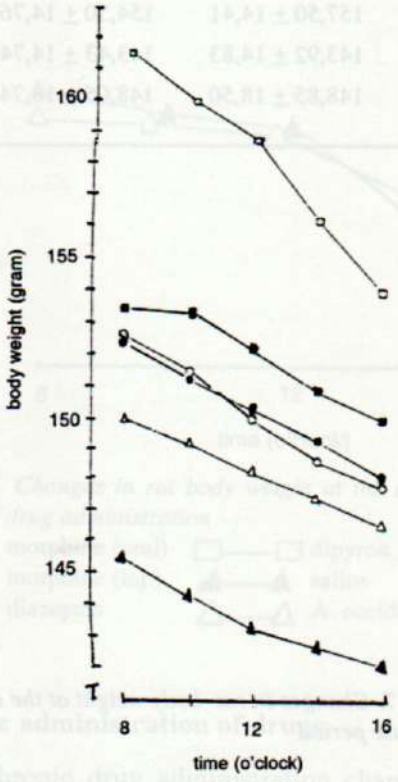


Figure 8. Changes in rat body weight at the one day of withdrawal

morphine (oral) — dipyron
morphine (inj.) — saline
diazepam — A. occidentale

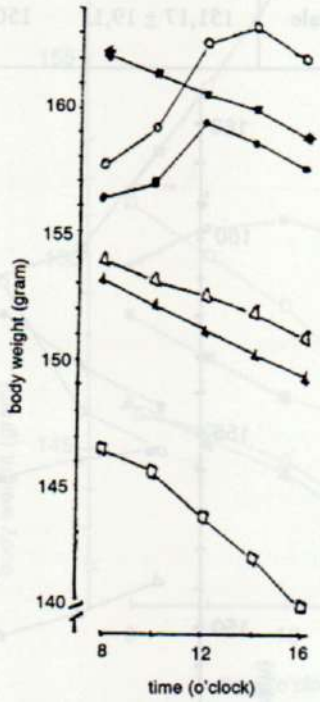


Figure 9. Changes in rat body weight after drug readministration

morphine (oral) — dipyron
morphine (inj.) — saline
diazepam — A. occidentale

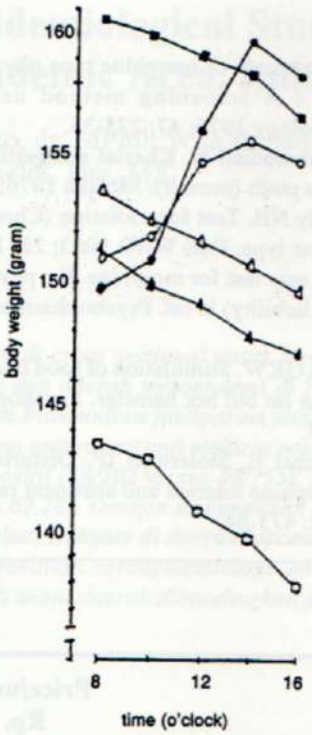


Figure 10. Changes in rat body weight if oral morphine was replaced with morphine by injection (and vise-versa)

morphine (oral) — dipyron
morphine (inj.) — saline
diazepam — A. occidentale

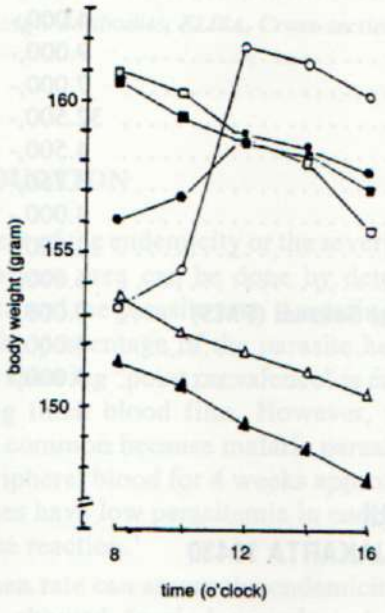


Figure 11. Changes in rat body weight after drug readministration

morphine (oral) — dipyron
morphine (inj.) — saline
diazepam — A. occidentale

DISCUSSION

During the acclimatization period of 15 days, body weight increased, however the pattern of body weight changes during the day time from 8 a.m. to 4 p.m. each day was always the same, it showed a decrease. This pattern was also found by Nozaki[1] in his study using Donryo strain rats. Similar pattern of body weight changes was also seen when rats were continuously weighed for 56 hours. Our conclusion is that the pattern of body weight changes of LMR strain rats is similar to that of Donryo strain rats.

There are many methods for testing dependence liability, using different animals and different parameters. Evaluation of results was more subjective than objective, such as conducted by Halbach[3] and Bekett.[4] They used a scoring system for some symptoms of drug dependency that appeared in animals, which were difficult to measure objectively. The method of measuring dependence liability continuously developed. Martin et al[5] found that morphine dependent rats showed an increase in motor activity and eating behavior. The alteration in these behaviors were measured objectively by Kumar et al[6] using suitable instruments. In his study, Kumar concluded that the motor activity and eating behavior of morphine dependent rats were greater in the morning than in the evening or night, although morphine was injected in the morning and also in the evening. The changes in eating and drinking behaviors altered the pattern of body weight changes from morning to the afternoon. These changes of body weight could be measured objectively. This method was developed by Nozaki to evaluate the dependence liability by measuring body weight from 8 a.m. to 4 p.m. Using this method, Nozaki[1] had examined many drugs suggested to have dependence liability.

During the first dose period of our study, there was hardly any changes in the body weight pattern of rats from 8 a.m. to 4 p.m. However after chronic administration of the drugs, we found alteration in the body weight pattern that occured only in groups receiving morphine orally or parenterally. While in general, the saline group, the dipyrone group, the diazepam group and the *Anacardium occidentale* group did not show any change in body weight pattern from 8 a.m. to 4 p.m.

CONCLUSION

We can conclude that *Anacardium occidentale* is free of dependence liability effect found in morphine like

drugs. It is also concluded that dipyron and diazepam have effects similar to those of *Anacardium occidentale* on the pattern of body weight changes in rats.

The pattern of body weight changes of LMR strain rats showed a consistent decrease from 8 a.m. to 4 p.m.

Chronic administration of morphine either orally or parenterally, increases the body weight during the day time from 8 a.m. to 4 p.m. while dipyron, diazepam, saline and *Anacardium occidentale* infuse do not.

Based on this study *Anacardium occidentale* infusion has no dependence liability effect such as found in morphine-like drugs.

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