



Effect of Nitazoxanide on cardiovascular system, respiratory system and nervous system

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ABSTRACT

Nitazoxanide, a thiazolidine compounds, is an efficient, broad-spectrum anti-parasitic and anti-microbial drug. On the one hand, by measuring breathing rate, breathing extent and electrocardiogram, effect of nitazoxanide on cardiovascular system and respiratory system of anesthetized rats was tested. On the other hand, by observing the activities and behavior change of mice, conducting climbing pole test and muscle fatigue test, the effect of nitazoxanide on nervous system was obtained. Results indicated that in the dose range 100mg-400mg/kg, nitazoxanide affected little to cardiovascular system and respiratory system of anesthetized rats and did not significantly inhibit central nervous system of mice.

Key words: nitazoxanide; safety pharmacology; cardiovascular system; respiratory system; nervous system

INTRODUCTION

Safety pharmacology of drugs is important and irreplaceable in the process of research and development of new drugs[1]. It was reported that adverse drug reactions were the fourth major cause of death in the United States. Whereas, these death can be decreased by safety pharmacology test [2-5]. It is necessary that pre-clinical drugs or new drugs should be studied by using safety pharmacology in order to avoiding drugs to endanger critical systems of body in a relatively short period of time[6-8]. Nitazoxanide, a thiazolidine compounds, is an efficient, broad-spectrum anti-parasitic and anti-microbial drug. Nitazoxanide was approved as the special drug to treat *Cryptosporidium* infection by the FDA and was put to the U.S. market in the formulation of oral suspension[9, 10]. With the in-depth study, nitazoxanide is now widely used to treat diarrhea of AIDS patients caused by the intestinal bacteria or intestinal parasites [11-14]. This study aimed to reveal the effect of nitazoxanide on cardiovascular system and respiratory system of rats and on nervous system of mice and to investigate the safety of clinical drug.

EXPERIMENTAL SECTION

Materials and instruments

Nitazoxanide, with batch number as 20130912 and purity as 99.5, was produced by Qingdao Continental Pharmaceutical Co., Ltd. Heparin sodium, with batch number as 120812, was produced by Shanghai Shanpu Chemical Technology Co., Ltd. Sodium Chloride Injection of 0.9%, with batch number as 120414W, was produced by Shandong Kelun Pharmaceutical Co., Ltd. Sodium pentobarbital, with batch number as 20121216, was produced by China Medicine (Group) Shanghai Chemical Reagent Company. RM6240 multi-channel physiological signal acquisition and processing test system was manufactured by Chengdu Instrument Factory.

Test animals

Sixty-four Wister rats (SPF) with weight of 150~250g and half male and half female were used in test. Forty Wister mice (SPF) with weight of 18~22g and half male and half female were used in test. These rats and mice were all provided by Lukang Pharmaceutical Experimental Animal Center. These animals were used to test after adapting in laboratory for a week.

Methods of investigating effect of nitazoxanide on cardiovascular system and respiratory system of rats

Grouping and administration of the test animals. These rats were divided randomly into four groups by weight, with eight rats in every group and half male and half female. The four groups were control group (CG), high-dose group (HDG), middle-dose group (MDG) and low-dose group (LDG) respectively. Nitazoxanide was mixed with Sodium chloride solution of 0.9% in different ratio to obtain suspension formulation for using in test. And the suspension was administered by gavage. The rats in control group were administered only sodium chloride solution of 0.9% with no drugs. The rats in high-dose group were administered drugs in 400mg/kg. The rats in middle-dose group were administered drugs in 200mg/kg. And the rats in low-dose group were administered drugs in 100mg/kg.

Measurement of breathing rate and breathing extent. Rats were anesthetized by being injected pentobarbital sodium of 40mg/kg. After being anesthetized, rats were supine and fixed on dissecting table. The skin of rats was cut along the center position of two fingers below xiphoid and surgery line was threaded through muscles. The surgery was connected to one end of the sensor of tension transducer which was connected with RM6240 multi-channel physiological signal acquisition and processing test system. Test information, such as breathing rate and extend, was automatically collected and recorded by computer before administering drug and after administering drug at 10、20、30、60、90、120 minutes.

Electrocardiogram measurement. Three metal needles were clamped separately by the yellow, red and black alligator clips and were inserted respectively subcutaneous tissue of right forelimb, left hind limb and right hind limb of rats. ECG lead was connected with RM6240 multi-channel physiological signal acquisition and processing test system. Test information, such as the heart rate of ECG, P wave, PR interval, QRS time, QT interval, R-wave, T wave, ST segment, was automatically collected and recorded by computer before administering drug and after administering drug at 10、20、30、60、90、120 minutes.

Methods of investigating effect of nitazoxanide on nervous system of mice

Grouping and administration of the test animals. These mice were divided randomly into four groups by weight, with ten mice in every group and half male and half female. The four groups were control group (CG), high-dose group (HDG), middle-dose group (MDG) and low-dose group (LDG) respectively. Nitazoxanide was mixed with Sodium chloride solution of 0.9% in different ratio to obtain suspension formulation for using in test. And the suspension was administered by gavage. The mice in control group were administered only sodium chloride solution of 0.9% with no drugs. The mice in high-dose group were administered drugs in 400mg/kg. The mice in middle-dose group were administered drugs in 200mg/kg. And the mice in low-dose group were administered drugs in 100mg/kg.

Activities and behavior change of mice. Activities and behavior change of mice in every group were observed regularly every day. The changes of motor function, behavior, coordination function, sensory / motor reflex were recorded. General behavior of mice after administration, such as posture, gait, with or without salivation, muscle trembling, pupillary changes, mouth breathing, were observed directly. Locomotor activity and body coordination of mice after administration were evaluated qualitatively and quantitatively. Activities and behavior change of mice continue to be observed two weeks after the test.

Climbing pole test. A smooth metal rod, with length as 76.2cm and diameter as 1.27cm, was fixed on base. The test time were 10、20、30、60、90、120 minutes before administering drug and after administering drug. Test mice were put on the top of the metal rod at test time and climbed down on its own. These mice were graded and scored according to the following standard. Zero grade: climbing down Step by step; first grade: sliding downward; second grade: can not grasp the metal rod; third grade: righting reflex disappearing. There were 0.5, 1.5 and 2.5 grades between two grades respectively.

Muscle fatigue test. Climbing pole holder was a vertical frame on which glass rod hanged. The diameter of glass rod was 0.8 cm and length was 25cm. The top part of glass rod was fixed on holder and the lower end was suspended approximately 20 cm from the ground. Every mouse was trained for two days so as to assured that it can stay on glass rod for a period of time. Mice were fasting for sixteen hours before test. Mice were put on glass rod at 30 and 60 minutes after administration. Their muscles were in static tension and the time of dropping because of muscle fatigue from glass rod was recorded by stopwatch. The test was end when dropping three times. Cumulative

time of three dropping was treated as climbing pole time so as to determine muscle fatigue.

Positive reflective observation. The tail of mice was seized and the mice face up at 30, 60 and 120 minutes before administration and after administration. The mice was two inches from ground and then it fall freely. If the mice landed with four legs perpendicular to the ground, it was scored one. If the mice landed with side to ground, it was scored two. If the mice landed back to ground, it was scored three. The process was repeated three times and the total score was recorded.

Statistical analysis

Software SPSS was used in statistical analysis and data was show in the form of $\bar{x} \pm SD$. significant difference test was conducted among numbers in different treatments in the same period.

RESULTS AND DISCUSSION

Effect of nitazoxanide on respiratory system of rats

Effect of nitazoxanide on breathing rate of rats was shown in Table 1. The differences of breathing rates at each test time before test and after test were not significant in the four groups ($p > 0.05$). It indicated that nitazoxanide in high dose, middle dose or low dose affected little on breathing rates of rats. Effect of nitazoxanide on breathing extent of rats was shown in Table 2. The difference among high-dose group, middle-dose group and low-dose group was not significant at 20 minutes after administration ($p > 0.05$). The difference between high-dose group and middle-dose group was not significant at 120 minutes after administration ($p > 0.05$), whereas the difference between middle -dose group and low-dose group was significant ($p < 0.05$). At other test time, the difference among the four groups was not significant ($p < 0.05$). It indicated that effect of nitazoxanide on breathing rates of rats was little.

Table 1. Effect of nitazoxanide on breathing rate of rats (times/min, $\bar{X} \pm SD$, n=8)

Groups	Before administration	After administration (min)					
		10	20	30	60	90	120
CG	92.25±20.97 a	92.37±24.48 a	85.62±27.81 a	84.87±23.22 a	77.37±32.77 a	85.50±28.98 a	79.25±27.08 a
LDG	187.87±192.13a	168.12±215.29a	99.87±25.63a	104.50±35.61 a	99.62±27.24 a	103.37±22.61 a	104.62±34.22 a
MDG	104.62±40.13 a	93.50±12.60 a	139.50±112.79 a	93.25±29.86 a	114.12±58.57a	89.25±32.68 a	103.00±33.41 a
HDG	97.37±18.40 a	97.87±19.92 a	98.87±22.09 a	104.87±24.16a	107.12±27.95a	162.50±158.78a	95.62±36.69 a

Note: If subscripts were different lowercase letters in the same column values, it indicted significant differences. If subscripts were different capital letters, it indicted extremely significant differences. If subscripts were same, it indicated not significant differences. It was same in the following tables.

Table 2. Effect of nitazoxanide on breathing extent of rats (g, $\bar{X} \pm SD$, n=8)

Groups	Before administration	After administration (min)					
		10	20	30	60	90	120
CG	3.63±1.86 aA	3.43±1.58 a	3.94±1.62 aA	4.05±1.30 a	3.24±1.57 a	2.43±1.0 8 a	3.74±2.55abA
LDG	3.12±2.23abA	3.00±1.97 a	2.51±1.02bA	3.26±1.90 a	3.91±2.18 a	4.63±3.12 a	5.02±3.10 aA
MDG	1.89±0.68bA	2.08±0.68 a	2.12±1.30bA	2.72±1.59 a	2.78±1.83 a	3.19±1.68 a	2.37±0.98 bA
HDG	2.26±0.98abA	2.35±1.06 a	2.79±1.29abA	3.03±2.00 a	4.02±2.87 a	3.97±3.25 a	3.90±2.77 abA

Effect of nitazoxanide on cardiovascular system of rats

Effect of nitazoxanide on heart rate of rats was shown in Table 3. As can be seen from the Table, at some test points, the differences between certain two groups were significant, such as at 10 minutes point between high-dose group and control group, or at 120 minutes point between high-dose group and middle-dose group. In most cases, the differences between two groups were not significant. It indicated that effect of nitazoxanide on heart rate of rats was little in most cases.

Table 3. Effect of nitazoxanide on heart rate of rats (times/min, $\bar{X} \pm SD$, n=8)

Groups	Before administration	After administration (min)					
		10	20	30	60	90	120
CG	260.78±76.23bB	303.62±71.45bB	310.48±85.10bB	290.16±83.00bB	331.87±115.72a	344.59±114.81a	319.37±132.67abA
LDG	406.93±80.02aA	413.18±101.59aA	380.90±96.37aAB	390.82±82.11abAB	369.62±91.94a	438.78±299.84a	331.45±69.06abA
MDG	392.29±66.29aA	394.94±74.25aAB	377.96±72.80aAB	374.29±84.57abAB	366.52±99.67a	329.79±85.70a	314.25±80.20 bA
HDG	387.65±54.71aA	377.63±64.05abAB	424.00±68.06a A	420.52±147.44a A	393.36±209.40a	401.44±87.55a	422.36±111.77 aA

Effects of nitazoxanide on P-wave of rats were shown in Table 4. It can be seen from the Table that in many cases, he differences between high-dose groups and control group or between middle-dose groups and control group were significant, which indicated that nitazoxanide of high dose and middle dose have a certain impact on P-wave of rats.

Table 4. Effect of nitazoxanide on P-wave of rats (mv, $\bar{X} \pm SD$, n=8)

Groups	Before administration	After administration (min)					
		10	20	30	60	90	120
CG	120.49 ± 67.22 a	109.40 ± 61.02 ab	73.82 ± 35.94 b	80.20 ± 41.07 ab	90.00 ± 52.11 ab	77.36 ± 36.04 b	62.07 ± 31.86 b
LDG	73.52 ± 41.17 a	83.09 ± 65.33 ab	85.11 ± 51.87 b	88.65 ± 75.21 ab	77.39 ± 47.21 b	97.05 ± 52.80 b	86.12 ± 32.94 b
MDG	130.94 ± 87.68 a	127.55 ± 68.08 a	136.07 ± 55.05 a	134.48 ± 66.65 a	177.87 ± 109.67 a	159.79 ± 63.65 a	122.74 ± 28.19 a
HDG	75.75 ± 37.84 a	64.61 ± 35.08 b	75.79 ± 39.19 b	54.69 ± 24.59 b	113.73 ± 52.33 ab	92.05 ± 35.00 b	77.34 ± 46.14 b

Effects of nitazoxanide on P-R interval of rats were shown in Table 5. These data showed that the differences between each administration group and control group were significant ($p < 0.05$), whereas the differences between any two administration groups were not significant ($p > 0.05$). These results indicated that nitazoxanide can affect P-R interval of rats, but there was no dose-effect relationship.

Table 5. Effect of nitazoxanide on P-R interval of rats (ms, $\bar{X} \pm SD$, n=8)

Groups	Before administration	After administration (min)					
		10	20	30	60	90	120
CG	22.87 ± 8.32 b	19.87 ± 4.82 b	19.00 ± 4.24 b	18.50 ± 4.44 b	20.37 ± 6.09 b	20.50 ± 2.82 b	23.62 ± 11.13 b
LDG	34.62 ± 4.95 b	38.37 ± 8.48 a	38.00 ± 5.65 a	45.37 ± 22.42 a	43.62 ± 25.03 a	64.62 ± 44.11 a	64.62 ± 44.51 a
MDG	49.87 ± 12.12 a	47.75 ± 13.56 a	44.50 ± 17.91 a	49.50 ± 17.64 a	58.75 ± 26.35 a	57.25 ± 21.82 a	56.87 ± 23.83 a
HDG	39.87 ± 9.77 a	41.25 ± 7.90 a	38.12 ± 10.61 a	41.12 ± 15.69 a	53.12 ± 24.40 a	40.50 ± 11.77 a	40.50 ± 13.45 a

Effects of nitazoxanide on QRS time of rats were shown in Table 6. As can be seen from the Table, only the difference between middle-dose group and low-dose group at 10 minutes after administration was significant ($p < 0.05$), the differences among the other groups were not significant ($p > 0.05$). Results indicated that nitazoxanide didn't affect QRS time of rats.

Table 6. Effect of nitazoxanide on QRS time of rats (ms, $\bar{X} \pm SD$, n=8)

Groups	Before administration	After administration (min)					
		10	20	30	60	90	120
CG	34.50 ± 11.33 a	31.37 ± 8.63 b	40.25 ± 21.19 a	40.62 ± 19.50 a	42.87 ± 19.62 a	50.00 ± 18.67 a	56.75 ± 28.83 a
LDG	33.00 ± 5.20 a	31.50 ± 8.22 b	39.00 ± 14.37 a	38.62 ± 18.25 a	44.75 ± 20.35 a	49.00 ± 29.73 a	53.62 ± 24.87 a
MDG	38.12 ± 16.41 a	40.87 ± 12.34 a	40.75 ± 9.76 a	46.87 ± 17.53 a	49.62 ± 20.47 a	48.12 ± 12.00 a	48.00 ± 19.35 a
HDG	32.25 ± 2.91 a	34.50 ± 4.07 a	36.50 ± 2.07 a	46.25 ± 22.48 a	52.62 ± 30.71 a	43.00 ± 10.51 a	36.37 ± 6.75 a

Effects of nitazoxanide on Q-T interval of rats were shown in Table 7. As can be seen from the Table, only the difference between every administration group and control group at 10 minutes and the difference between middle-dose group and control group at 10 minutes after administration were significant ($p < 0.05$), the differences among the other groups were not significant ($p > 0.05$). It indicated that nitazoxanide has a little influence on Q-T interval of rats in 30 minutes after administration. However, the influence disappeared after administration 60 minutes.

Table 7. Effect of nitazoxanide on Q-T interval of rats (ms, $\bar{X} \pm SD$, n=8)

Groups	Before administration	After administration (min)					
		10	20	30	60	90	120
CG	72.75 ± 20.88 b	70.87 ± 15.73 c	85.25 ± 35.09 a	82.00 ± 29.44 b	86.50 ± 28.19 a	97.62 ± 34.08 a	108.00 ± 46.16 a
LDG	86.87 ± 5.84 ab	87.12 ± 7.16 ab	96.75 ± 20.33 a	104.37 ± 29.60 ab	113.87 ± 37.58 a	123.37 ± 58.10 a	133.25 ± 47.55 a
MDG	103.00 ± 23.64 a	103.25 ± 20.54 a	106.37 ± 18.66 a	110.37 ± 19.22 a	113.50 ± 16.50 a	118.12 ± 19.20 a	127.12 ± 36.81 a
HDG	90.25 ± 12.56 ab	90.75 ± 11.78 ab	93.12 ± 7.52 a	107.50 ± 28.58 ab	114.87 ± 38.94 a	102.12 ± 15.37 a	95.50 ± 12.16 a

Effect of nitazoxanide on R-wave of rats was shown in Table 8. There were no significant differences between each administration group and control group, which indicated that the three doses had little influence on R-wave of rats.

Table 8. Effect of nitazoxanide on R-wave of rats (mv, $\bar{X} \pm SD$, n=8)

Groups	Before administration	After administration (min)					
		10	20	30	60	90	120
CG	632.20 ± 405.33 a	612.78 ± 369.02 a	559.29 ± 272.27 a	540.70 ± 267.78 ab	627.10 ± 273.31 ab	597.83 ± 119.89 a	562.79 ± 219.67 ab
LDG	492.76 ± 411.59 a	505.18 ± 430.15 a	543.16 ± 416.06 a	573.64 ± 488.56 ab	530.17 ± 302.05 ab	455.58 ± 195.19 a	479.61 ± 204.23 ab
MDG	553.02 ± 247.22 a	564.66 ± 217.35 a	619.42 ± 315.10 a	706.54 ± 326.99 a	726.44 ± 462.63 a	729.89 ± 453.82 a	623.27 ± 171.73 a
HDG	356.97 ± 153.92 a	358.50 ± 118.75 a	384.52 ± 207.57 a	352.46 ± 70.34 b	358.77 ± 59.98 b	461.87 ± 213.49 a	372.86 ± 158.42 b

Effect of nitazoxanide on T-wave of rats was shown in Table 9. As can be seen from the Table, only the difference between high-dose group and control group at 10 minutes and 20 minutes and the difference between each administration group and control group at 30 minutes after administration were significant ($p < 0.05$), the differences

among the other groups were not significant ($p>0.05$). It indicated that nitazoxanide has a little influence on T-wave of rats in 30 minutes after administration. However, the influence disappeared after administration 60 minutes.

Table 9. Effect of nitazoxanide on T-wave of rats (mv, $\bar{X} \pm SD$, n=8)

Groups	Before administration	After administration (min)					
		10	20	30	60	90	120
CG	41.18±15.91 aA	50.95±29.30 aA	62.86±25.95 aA	69.89±32.88 aA	42.71±27.81 a	44.93±37.69 a	46.08±32.63 a
LDG	23.50±18.48aAB	20.07±13.76bA	29.98±233aAB	28.42±20.78bB	38.37±25.92a	38.22±17.50 a	39.04±29.45 a
MDG	15.09±8.92bB	35.25±30.49abA	44.32±52.39abAB	33.44±23.62bB	28.29±17.49 a	34.41±35.20 a	28.45±11.77 a
HDG	14.90±11.63bB	19.56±18.99bA	14.55±9.37bB	23.49±19.80bB	22.09±12.71 a	21.11±12.54 a	24.84±14.77 a

Effect of nitazoxanide on ST segment of rats was shown in Table 10. It can be seen from the Table that there was no significant difference between every two groups. It indicated that nitazoxanide in these three doses has no influence on ST segment of rats.

Table 10. Effect of nitazoxanide on ST segment of rats (ms, $\bar{X} \pm SD$, n=8)

Groups	Before administration	After administration (min)					
		10	20	30	60	90	120
CG	10.75±5.44 a	11.00±3.92 a	13.87±6.72 a	13.12±6.83 a	13.87±7.03 a	11.62±6.65 a	11.87±3.09 a
LDG	12.62±4.30 a	12.37±3.81 a	16.25±9.89a	15.50±6.56 a	18.00±11.28 a	18.25±16.49 a	18.00±14.15 a
MDG	15.87±10.90 a	15.25±12.62 a	13.62±8.83 a	16.25±8.37 a	14.75±4.13 a	12.62±4.27 a	13.25±3.88 a
HDG	10.00±3.58 a	12.50±4.65 a	11.62±4.13 a	18.50±9.22 a	19.87±16.12 a	13.37±3.96 a	15.37±5.78 a

Effect of nitazoxanide on nervous system of mice

Effect of nitazoxanide on ability of climbing pole of mice was shown in Table 11. As can be seen from the Table, at 10 minutes after administration, the differences among these groups were not significant. While at other test time, the differences between certain administration group and control group were significant. The certain administration group may be HDG, MDG or LDG. It depended. All these results indicated that nitazoxanide can influence the ability of climbing pole of mice, but there was no dose-effect relationship.

Table 11. Effect of nitazoxanide on ability of climbing pole of mice (grade, $\bar{X} \pm SD$, n=10)

Groups	Before administration	After administration (min)							
		10	20	30	60	90	120	150	180
CG	0±0bB	0±0 a	0±0 bA	0±0 bB	0±0bB	0±0bB	0±0bB	0±0bB	0±0 bB
LDG	0±0bB	0±0 a	0.35±0.41aA	0.35±0.34aA	0.20±0.25acA	0.35±0.41aA	0.35±0.41 aA	0.45±0.36aA	0.50±0.40 aA
MDG	0.15±0 aA	0±0.24 a	0.10±0.21 aA	0.30±0.26a A	0.25±0.26aA	0.30±0.25aA	0.20±0.25 abA	0.20±0.25bAB	0.45±0.36 aA
HDG	0±0.03bB	0±0.13 a	0.75±1.51 aA	0.05±0.16 bAB	0.05±0.15bcA	0.05±0.15b A	0.05±0.15bA	0.05±0.15bB	0.05±0.15bB

Effect of nitazoxanide on positive reflection of mice was shown in Table 12. In the process of trial, all of these mice landed with four legs perpendicular to the ground and it was same in the three repeats. It indicated that nitazoxanide had no influence on positive reflection of mice.

Table 12. Effect of nitazoxanide on positive reflection of mice (scores, $\bar{X} \pm SD$, n=10)

Groups	Before administration	After administration (min)		
		30	60	120
CG	4±0	4±0	4±0	4±0
LDG	4±0	4±0	4±0	4±0
MDG	4±0	4±0	4±0	4±0
HDG	4±0	4±0	4±0	4±0

Table 13. Effect of nitazoxanide on muscles fatigue of mice (s, $\bar{X} \pm SD$, n=10)

Groups	After administration (min)	
	30	60
CG	47.90±35.71 abA	38.50±21.79 bA
LDG	37.30±20.39 bA	130.70±174.43 aA
MDG	82.70±69.21 aA	103.70±85.57 abA
HDG	40.30±26.07 bA	48.60±27.38 abA

Effect of nitazoxanide on muscles fatigue of mice was shown in Table 13. In most cases, the differences were not significant, only at 60 minutes after administration, the difference between LDG and CG was significant. The stay

time of mice in LDG was longer than that in CG. The result indicated that nitazoxanide can't increase the muscles fatigue of mice.

At each test time, the mice didn't show abnormal performance, posture and gait and had no abnormal changes such as Salivating, muscle tremors, pupil change, mouth breathing, convulsions, vertical hair, diarrhea. Within two weeks after the test, these abnormal performance, posture, gait and changes still hadn't occurred. And spirit, appetite, desire to drink of these mice were all normal. There were no mice to die.

CONCLUSION

According to technical guidelines of drug safety pharmacology studies, on the one hand, effect of nitazoxanide on cardiovascular system and respiratory system of anesthetized rats was tested. By this method, the interference of stress response and surgery to related physiological indices of experimental was reduced effectively and it was ensured that the various physiological indicators can reflect more accurately the true state of experimental animals. On the other hand, the effect of nitazoxanide on nervous system was obtained by testing locomotor activity of mice. Results indicated that in the dose range 100mg-400mg/kg, nitazoxanide affected little to cardiovascular system and respiratory system of anesthetized rats and did not significantly inhibit central nervous system of mice. All above-mentioned information indicated that the clinical guidance dosage investigated in the test was safe. It should be investigated further more whether nitazoxanide can affect clinical effect in larger dosage.

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