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Original Article

Effect of Bacopa Monnieri on Ethanol-induced Anxiolysis and Withdrawal Anxiety in Wistar Rats

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Abstract

Background & Objective: *Bacopa Monnieri* (BM) is known to have general anxiolytic properties. However, its possible anxiolytic effect in ethanol withdrawal induced anxiety is not yet identified. This study was done to evaluate the effect of BM on ethanol-induced anxiolysis and withdrawal anxiety in Wistar rats.

Methods: A total of 126 rats were included in the study. Elevated plus maze and bright and dark arena were used to assess the anxiolytic action. Actophotometer paradigm was used to assess the locomotor activity in rats. BM was given orally in doses of 50, 100, 200, 400 mg/kg & ethanol was given in dose 0.5, 1 and 1.5 g/kg to evaluate the anxiolytic effect of single doses of BM and ethanol. Ethanol withdrawal was induced in rats by giving ethanol as the sole drinking solution at 5% solution and gradually increasing to 10% solution for 15 days followed by 3-day withdrawal period. A similar cycle of ethanol withdrawal was repeated to increase the intensity of ethanol withdrawal symptoms.

Results: Anxiolytic action seen with BM 100, 200, 400 mg/kg doses and ethanol 0.5 g/kg dose. Beneficial effect of BM 100, 200, 400 mg/kg seen even in ethanol withdrawal induced anxiety. CNS depressant action was observed with ethanol at doses 1 and 1.5 g/kg, but not with BM 400 mg/kg dose as demonstrated in actophotometer paradigm.

Conclusion: BM in doses 100, 200, 400 mg/kg showed anxiolytic action in ethanol withdrawal induced anxiety in rats with no CNS depressant action even at 400 mg/kg. Ethanol at 1 and 1.5 g/kg doses showed CNS depressant action.

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Introduction

Ethanol consumption in humans for a long period leads to the derangement of the neurotransmitters in the brain, which may play a part in subjective symptoms of stress and anxiety during withdrawal of ethanol and this may be a precipitating factor in relapse of ethanol drinking. The manifestations of ethanol withdrawal include anxiety, insomnia, illusions, hallucination, paranoid ideas, nausea, vomiting, irritability and signs like elevated blood pressure, tachycardia, elevated body temperature, dilated pupils, disorientation, tremor, hyperarousal, and grand-mal-seizure (1). The underactive GABAergic system is involved in ethanol withdrawal-induced anxiety. Anxiety like behaviour during the drug free intervals in the later phase of ethanol withdrawal is decreased or increased by the administration of agents that increase or decrease the activity of benzodiazepine GABA receptors, respectively (2).

Bacopa monnieri (BM), also known as Brahmi is a medicinal Ayurvedic herb, traditionally used in various disorders. Its memory and cognition enhancing properties were demonstrated in various studies. It has also been reported to possess anti- ulcerogenic, anti- oxidant, antidepressant, hepatoprotective, neuroprotective and anti-anxiety properties. BM contains several phytochemicals, the most important being triterpenoidsaponins called as bacosides (3). The mechanisms involved in the beneficial effects of BM include acetylcholinesterase inhibition, choline acetyltransferase activation, β-amyloid reduction, increased cerebral blood flow, and monoamine potentiation (4).

Currently, benzodiazepines are the agents used in the management of ethanol withdrawal induced anxiety. BM is known to have general anxiolytic properties and it may have additional advantages over benzodiazepines as an antianxiety agent in view of side effects like retrograde amnesia and drowsiness associated with benzodiazepines (5). However, a thorough search of the literature did not provide any supporting evidence for the possible beneficial effect of BM in ethanol withdrawal induced anxiety. Hence, this study was planned to elicit the action of BM in ethanol withdrawal-induced anxiety-like behaviour in rats.

Methods

This experimental study was conducted in male Wistar rats weighing 150- 200 grams. A total of 126 rats were included in the study. Animals were procured from the central animal house of Kasturba Medical College, Mangalore. All animals were allowed to acclimatize to the laboratory conditions for five days before starting the study. Animals were housed in standard polycarbonate cages as three per cage during the initial period of acclimatization. Rooms were controlled for temperature and animals were kept at 12 hours each of bright and dark period. Animals were allowed to have free access to food and water (except when ethanol is substituted for water). To reduce the effect of circadian rhythms, tests were performed during the first half of the day. This study was conducted after obtaining the approval from the Institutional Animals Ethics Committee (IAEC). Care of animals was taken as per the guidelines of CPCSEA, Department of Animal Welfare, Government of India.

Drugs and drinking solution:

Ethanol : The ethanol solutions were prepared from 99.9% ethanol (Manufactured by CHANGSHU YANGYUAN CHEMICALS CHINA).

Study drug:

BM (Brahmi tablets) were obtained from Himalaya Herbals. Tablets were initially crushed to make a powder and then mixed with 0.5 in 10 dilutions (distilled water) of tween 80 solutions and were given by oral route.

Standard drug:

Injection Diazepam

All drugs were administered 60 min (oral) and 15 minutes (IP) prior to the experiment.

Experiment 1: To study the effect of BM on anxiolytic effect of acute administration of ethanol

Animals were divided into various groups with 6 animals in each group as follows :

Group 1: Normal control (Tween 80)

Group 2: Diazepam (1 mg/kg, IP)

Group 3: BM (50 mg/kg, oral)

Group 4: BM (100 mg/kg, oral)

Group 5: BM (200 mg/kg, oral)

Group 6: BM (400 mg/kg, oral)

Group 7: Ethanol (0.5 g/kg, IP)

Group 8: Ethanol (1 g/kg, IP)

Group 9: Ethanol (1.5 g/kg, IP)

Group 10: BM (400 mg/kg, oral) + Ethanol (0.5 g/kg, IP)

A single dose of different strengths/dose levels of BM and ethanol was administered to animals as per their grouping. These animals were subjected to anxiety test one hour after oral administration or 15 minutes after IP administration of the study drugs. Throughout the study, animals were allowed to have free access to food.

Bright and dark arena was used to evaluate anxiety in all the study groups. This apparatus consisted of an open top wooden box, consisting of two distinct chambers, a black chamber (25×35×35 cm, a bright chamber 25×35×35 cm). A 40W white light source was placed at a height of 15 cm above the bright chamber. The two chambers are connected through a small open doorway (7.5×5 cm) situated on the floor level at the center of partition. The apparatus was cleaned before the procedure and every time before evaluating anxiety in a new rat. The animals were kept for overnight fasting before the procedure. Various parameters in Bright and Dark box (number of bright chamber entries, time spent in the bright chamber and number of rears in the bright chamber) were observed for five minutes.

Elevated plus maze was also used for evaluation of anxiety for the experiment no. 1. But, the model did not provide us reliable results.

Experiment 2: To study the effect of BM on ethanol withdrawal induced anxiety

Group 1: Normal control (Tween 80)

Group 2: Diazepam (1 mg/kg, IP)

Group 3: BM (50 mg/kg, oral)

Group 4: BM (100 mg/kg, oral)

Group 5: BM (200 mg/kg, oral)

Animals were housed singly in polypropylene cages. Ethanol 5-10% was given gradually as the sole drinking fluid for 15 days i.e. 5% on the first day, 7.5% on the second day and then maintained on 10% ethanol until the 15th day. For the next 3 days (16th to 18th day) animals were allowed to have free access to drinking fluid free of ethanol. Similarly, another cycle of Ethanol 5-10% was given gradually as the sole drinking fluid for the next 15 days. Animals were allowed to have free access to drinking fluid free of ethanol from the day 49 to day 51. Drugs were administered on the 51st day. These animals were subjected to anxiety test using Bright & dark box arena, one hour after the oral administration or 15 minutes after the IP administration of drugs. Throughout the study, animals were allowed to have free access to food.

Experiment 3: To study the CNS depressant action of best effective dose of BM as well as ethanol (locomotor activity):

Group 1: Normal control (Tween 80)

Group 2: Diazepam (1 mg/kg, IP)

Group 3: BM (400 mg/kg, oral)

Group 4: Ethanol (0.5 g/kg, IP)

Group 5: Ethanol (1 g/kg, IP)

Group 6: Ethanol (1.5 g/kg, IP)

Animals were administered a single dose of the best effective dose of *BM monnieri* and ethanol as per their grouping. These animals were subjected to spontaneous motor activity test in actophotometer, one hour after the oral administration and 15 minutes after the IP administration of the drugs.

The Actophotometer test apparatus consisted of a square box with a closed lid. Inside the box are the arrays of light that fall on the photo electric cells. Once the rat is placed inside the box, the locomotion of the rat cuts each beam of light that falls on photoelectric cell and that will be recorded digitally as a count. The apparatus was cleaned before the procedure and every time before evaluating spontaneous motor activity in a new rat. The animals were kept for overnight fasting before the procedure. The number of counts was observed for each rat for a period of five minutes.

Statistical analysis:

The data obtained were analyzed using one-way analysis of variance (ANOVA) followed by Post- Hoc LSD test. $p<0.05$ was considered statistically significant.

Results

The Table I shows the effects of BM on the anxiolytic effect of ethanol in the bright and dark arena. Diazepam 1 mg/kg (P = 0.001), BM 100, 200, 400 mg/kg (p<0.05), alcohol 0.5 g/kg (p<0.001) and BM 400 mg/kg + alcohol 0.5 g/kg showed increased number of bright arm entries as compared to normal control (p<0.001). Diazepam 1 mg/kg group showed increased number of bright arm entries compared to BM 50 mg/kg and alcohol 1.5 g/kg (p<0.05). Ethanol 0.5 g/kg group showed increased number of bright arm entries compared to BM 50 (p<0.001), 100, 200 mg/kg (p<0.05), ethanol 1 g/kg (p<0.05), 1.5 g/kg (p<0.001). BM 400 mg/kg + ethanol 0.5 g/kg showed increased number of bright arm entries compared to diazepam 1 mg/kg (p<0.05), *BM* 50, 100, 200, 400 (p<0.001), ethanol 1 g/kg, 1.5 g/kg (p<0.001). Almost similar findings were seen with regard to the analyses of other behavioural parameters such as time spent in the bright chamber and the number of rears in the bright arm, as shown in Table I.

Table II shows the effect of BM on the anxiolytic effect of ethanol in elevated plus maze. Antianxiety effects of diazepam as well as different doses of BM could not be elicited in this model. Only BM 400 + ethanol 0.5 g/kg group showed a significant antianxiety effects with regard to all the behavioural parameters assessed in this model. There was a statistically significant increase in the number of open arm entries, increased time spent in open arm and increased number of rears in the open arm with BM 400 + ethanol 0.5 g/kg group when compared to the control group whereas ethanol 0.5 g/kg showed a statistically significant increase in the duration spent

TABLE I: Effect of BM on the anxiolytic effects of ethanol (bright and dark arena test).

Groups	*Number of bright arm entries*	*Time spent in bright arm*	*Number of rears in bright arm*
Normal control	1.67±0.21	10.33±1.28	0.33±.21
Diazepam (1 mg/kg), ip	4.50±0.22$^{*\&@}$	99.50±12.61$^{*@}$	7.17±1.40$^{*@}$
BM 50 mg/kg, oral	2.17±0.31$^{\$\#\&}$	38.00±5.35$^{\$\#\&\wedge}$	5.33±0.72$^{*\#@}$
BM 100 mg/kg, oral	3.50±0.56$^{*\#\&}$	65.17±17.76$^{*\#}$	7.0±1.92$^{*\#@}$
BM 200 mg/kg, oral	3.67±0.803$^{*\#\&}$	79.17±15.02$^{*\#}$	9.17±2.21$^{*@}$
BM 400 mg/kg, oral	3.33±0.62$^{*\&}$	97.33±5.36$^{*@}$	9.67±1.05$^{*@}$
Ethanol 0.5 g/kg, ip	5.33±0.67$^{*@}$	129.67±11.04$^{*@}$	11.5±2.62$^{*@}$
Ethanol 1 g/kg, ip	3.17±0.60$^{\#\&}$	67.17±23.11$^{*\#}$	7.17±2.27$^{*@}$
Ethanol 1.5 g/kg, ip	2.17±0.60	44.33±31.27	0.00±0.00
BM 400 mg/kg, oral + Ethanol 0.5 g/kg, ip	6.83±0.87$^{*@}$	103±4.03$^{*@}$	9.67±0.21$^{*@}$

ANOVA
All values expressed as Mean±SEM
*p<0.05 compared to control
$^{\#}$p<0.05 compared to ethanol 0.5 g/kg
&p<0.05 compared to BM 400 mg/kg + ethanol 0.5 g/kg
$^{\$}$p<0.05 compared to diazepam
$^{@}$p<0.05 compared to ethanol 1.5 g/kg
^p<0.05 compared to BM 400 mg/kg

TABLE II: The effect of BM on anxiolytic effects of ethanol in elevated plus maze.

Groups	*Open arm entries*	*Time spent in open arms*	*Rears in open arms*	*% ratio of open/ total arm entries*
Normal control	4.17±0.75	62.33±11.17	0.83±0.40	0.53±0.05
Diazepam (1 mg/kg),ip	6.00±0.89^	110.67±18.03&	2.50±0.22&	0.61±0.03@
BM 50 mg/kg, oral	3.33±0.42$&	48.00±7.35$&#	0.83±0.31&#@	0.58±0.03@
BM 100 mg/kg, oral	4.17±0.48$&	74.17±13.69&	1.33±0.21&#@	0.56±0.04
BM 200 mg/kg, oral	5.33±0.42^	84.17±12.14&	2.17±0.31&#^	0.57±0.05@
BM 400 mg/kg, oral	2.50±0.96&	78.33±13.08&	1.33±0.49&#	0.53±0.06@
Ethanol 0.5 g/kg, ip	5.17±0.75^	125.50±32.52*	4.83±1.49*	0.57±0.04@
Ethanol 1 g/kg, ip	4.00±1.16&	110.33±44.46&	4.67±2.22*^	0.42±0.11
Ethanol 1.5 g/kg, ip	5.00±0.89^	50.33±12.95$&#	0.17±0.17&#@	0.69±0.04*@^
BM 400 mg/kg, oral + Ethanol 0.5 g/kg, ip	6.83±0.31*^	172.17±15.88*	6.33±0.49*$	0.69±0.02*@^

ANOVA
All values expressed as Mean±SEM
*p<0.05 compared to control
$p<0.05 compared to diazepam
#p<0.05 compared to ethanol 0.5g/kg
@p<0.05 compared to ethanol 1 g/kg
&p<0.05 compared to BM 400 mg/kg + ethanol 0.5 g/kg
^p<0.05 compared to BM 400 mg/kg @p<0.05 compared to ethanol 1.5 g/kg
&p<0.05 compared to BM 400 mg/kg + ethanol 0.5 g/kg
^p<0.05 compared to BM 400 mg/kg

TABLE III: The effect of BM on ethanol withdrawal induced anxiety.

Groups	*Number of bright arm entries*	*Time spent in bright arm (seconds)*	*Number of rears in bright arm*
Control (drug naïve ethanol withdrawal)	1.17±0.17	4.50±0.62	0.00±0.00
Diazepam (1mg/kg), ip	3.50±0.34*	79.0±7.64*	8.33±0.88*
BM 100mg/kg, oral	3.83±0.48*	90. 67±15.80*	6.00±1.21*
BM 200mg/kg, oral	4.17±0.31*	76.83±13.23*	4.83±0.83*$^
BM 400mg/kg, oral	3.17±0.48*	70.67±7.02*	7.5±0.88*

ANOVA
All values expressed as Mean±SEM
*p<0.05 compared to control
$p<0.05 compared to diazepam
^p<0.05 compared to BM 400 mg/kg

in the open arm and increased number of rears in the open arm when compared to the control group. Ethanol 1 g/kg showed statistically significant increase in the number of rears in the open arm when compared to the control group. All four doses of BM, diazepam and ethanol 1.5 g/kg did not show significant results with regard to the behaviours such as open arm entries, time spent in open arms and number of rears in the open arm in elevated plus maze.

BM 400 + ethanol 0.5 g/kg showed increased number of open arm entries compared to BM 50, 100, ethanol 1 g/kg, BM 400. BM 400 showed decreased number of open arm entries, time spent in open arms, rears compared to BM 200, diazepam, ethanol 0.5 g/kg and ethanol 1.5 g/kg groups. Diazepam showed increased time spent in open arm when compared to BM 50 ($p<0.05$), ethanol l.5 g/kg, BM 400 + ethanol 0.5 g/kg.Ethanol 0.5 g/kg showed increased time spent in open arm compared to BM 50 ($p<0.05$), ethanol 1.5 g/kg ($p<0.05$).

The Table III shows the effect of BM on ethanol withdrawal induced anxiety. Compared to disease control group (ethanol withdrawal), ethanol withdrawal+diazepam 1 mg/kg, ethanol withdrawal+ BM 100, ethanol withdrawal + BM 200, ethanol withdrawal + BM 400 group showed significantly increased number of bright chamber entries,

increased time spent in the bright chamber and increased number of rears in the bright chamber. Ethanol withdrawal - diazepam showed increased number of rears in the bright chamber compared to ethanol withdrawal + BM 200 ($p<0.05$). Ethanol withdrawal + BM 400 showed increased number of rears in bright chamber compared to ethanol withdrawal + BM 200 ($p<0.05$).

Table IV shows the effect of BM and ethanol on spontaneous motor activity. Diazepam 1 mg/kg ($p<0.001$), ethanol 1 g/kg ($p<0.05$), ethanol 1.5 g/kg ($P = 0.001$) showed decreased number of counts in actophotometer compared to normal control group. BM 400 showed increased counts than diazepam ($p<0.05$). Ethanol 0.5 g showed increased counts when compared to diazepam ($p<0.001$), BM 400 ($p<0.05$), ethanol 1 g/kg and ethanol 1.5 g/kg ($p<0.001$).

TABLE IV: The effect of BM and ethanol on spontaneous motor activity (Number of counts in actophotometer).

Groups	*Number of counts*
Normal control	160.40±15.00
Diazepam (1 mg/kg), ip	41.94±10.47*#
BM 400 mg/kg, oral	114.0±12.98$#
Ethanol 0.5 g/kg, ip	201.16±37.16$
Ethanol 1 g/kg, ip	89.56±6.45*#
Ethanol 1.5 g/kg, ip	61.94±11.34*#

ANOVA
All values expressed as Mean±SEM
*$p<0.05$ compared to control
$$p<0.05$ compared to diazepam
#$p<0.05$ compared to ethanol 0.5 g/kg

Discussion

The present study was done to assess the effect of BM on anxiolytic effects of acute administration of ethanol as well as to evaluate its effect on ethanol withdrawal induced anxiety in Wistar rats. The anxiolytic effect was evaluated by using bright and dark arena and elevated plus maze.

In the bright and dark arena, the different doses of BM (100, 200, 400 mg/kg) showed a significant anxiolytic effect compared to normal control, but there were no statistically significant differences between the different doses of BM. Ethanol at a low dose (0.5 g/kg) showed a significant anxiolytic effect compared to higher doses of ethanol (1 and 1.5 g/kg). This could be explained by the sedative effect of ethanol at a higher dose that would have masked the anxiolytic effect. Ethanol 0.5 g/kg also showed a better anxiolytic effect than BM 200 mg/kg. BM 400 mg/kg + ethanol 0.5 g/kg showed a better anxiolytic effect compared to all doses of BM. Hence, it can be deduced that ethanol at low dose potentiates the anxiolytic effect of BM. Anxiolytic effect was insignificant at the lower dose of BM (50 mg/kg).

Elevated plus maze paradigm did not provide us reliable results as we could not appreciate the antianxiety effect of different doses of BM. Paradoxically, antianxiety effects were not seen even in the diazepam treated group. In this model, a significant anxiolytic effect was observed with BM 400 mg/kg + ethanol 0.5 g/kg group, which was consistent in all the parameters assessed such as open arm entries, time spent in open arms, number of rears in the open arm and the percentage of the ratio of open/total arm entries. Ethanol 0.5 g/kg ($p<0.05$) showed a significant anxiolytic effect compared to normal control, but the effects were inconsistent as significant readings were seen only with regard to time spent in open arm and the number of rears in the open arm. Ethanol 1.5 g/kg showed a significant anxiolytic effect as assessed by its increased preference to open arms. But, again these findings were inconsistent, as analysis of other behaviours did not support for the significant anxiolytic effect. Paradoxically, BM 400 mg/kg showed a significant decrease in anxiolytic effect compared to BM 200, diazepam, ethanol 0.5 g/kg, ethanol 1.5 g/kg, BM 400 + ethanol 0.5 g/kg. Higher doses of BM may have some sedative effect which would have affected the locomotion of the animals, there by masking the possible anxiolytic effect. A previous study has demonstrated the CNS depressant effect with BM 500 mg/kg dose in rodents (6). Thus, our observations in this model suggest that elevated plus maze may not be a suitable model to elicit the anxiolytic effect of BM.

The anxiolytic effect of ethanol is due to increase in GABA mediated inhibition in the brain as well as a decrease in glutamate mediated excitation in the brain. The exact mechanism of anxiolytic action of BM is unknown. Previous studies indicate possible mechanisms as GABA modulation, antioxidant properties, modulation of brain stress hormones, serotonin agonism, inhibition of inflammatory cytokines in the brain (3, 4). We did not observe significant differences between the different doses of BM in contrast to a previous study that had demonstrated dose dependent anxiolytic effect with the smaller doses of BM 5, 10 and 20 mg/kg (7). Previous studies have also shown the anxiolytic effect of BM in rats and humans (7-10).

Our study also evaluated the effect of BM on ethanol withdrawal induced anxiety. Both diazepam and BM (100, 200, 400 mg/kg doses) were effective in reducing the ethanol withdrawal induced anxiety. But, there was no significant differences between the different doses of BM. Ethanol consumption and stress increase the brain levels of known innate immune molecules that are responsible for the change in the behaviour, anxiety and subsequent development of ethanol use disorders (11). Ethanol withdrawal induced anxiety is due to an imbalance of GABA & glutamate levels in the brain, leading to decreased GABA activity due to the down regulation of GABA receptors and increased glutamate activity due to the up regulation of NMDA receptors on chronic exposure to alcohol. Also specific changes in 5HT2c- receptor signalling in the brain leads to hyper-excitability and stress (12, 13). Chronic intermittent exposure to ethanol up regulates 5HT2c-receptor signalling, leading to increased excitability which may be responsible for the increased anxiety-like behavior during ethanol withdrawal (12).

The possible mechanisms for the anxiolytic effect of BM in ethanol withdrawal induced anxiety could be by reversing the imbalance between GABA and glutamate levels in the brain, regulation of serotonin or by decreasing inflammatory cytokines in the brain or by neuroprotective action (8, 14, 15).

As we presumed that sedative effect might be responsible for the absence of appreciable antianxiety effect of higher doses of ethanol and variable antianxiety effect of BM especially at a higher dose in elevated plus maze, we evaluated the effect of these agents on spontaneous motor activity by using actophotometer. A significant decrease in locomotion was observed with diazepam, ethanol 1 g/kg & 1.5 g/kg, indicating that higher doses of ethanol have caused CNS depression (sedation). However, there was no significant decrease in locomotor activity even at a higher dose of BM (400 mg/kg), indicating that BM has not caused CNS depression or sedation. Paradoxically, BM 400 mg/kg showed a significant increase in the locomotor activity compared to diazepam treated group, indicating that BM is not a CNS depressant like diazepam. This observation is contradictory to the previous study which shows CNS depressant effect with higher doses of BM (7). Ethanol 0.5 g showed a significant increase in locomotor activity compared to other groups, suggesting that this dose of ethanol has not produced sedation.

The limitations of this study should be considered. Though we planned to use two models for the evaluation of the anxiety, we did not get reliable results in the elevated plus maze. Further, we have included only one species of animals in the present study. Incorporating many models as well as more than one animal species may be helpful to draw more authentic conclusions. We did not perform phytochemical analysis to identify the active principle present in the BM. Our study did not plan to evaluate the possible mechanism of action of BM in anxiety including ethanol withdrawal induced anxiety. All these aspects can be incorporated in future studies, thereby providing a valuable evidence to plan human studies with BM in ethanol withdrawal induced anxiety.

Conclusion

BM in doses (100, 200, 400 mg/kg) showed an anxiolytic effect in general as well as in ethanol withdrawal induced anxiety in rats and its antianxiety effects were comparable with diazepam. CNS depressant action was observed with ethanol 1 and 1.5 g/kg, but not with BM 400 mg/kg. The

possible mechanism of BM in ethanol withdrawal induced anxiety could be by correcting the neurotransmitter imbalance in the brain of ethanol withdrawal rats.

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