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Original Article

# Anxiolytic Effects of the Pozol a Mexican Ancestral Beverage

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## ABSTRACT

**Background:** Pozol is a non-alcoholic, non-fermented pre-hispanic beverage prepared with nixtamalized maize and cocoa paste, mixed with water. This refreshing drink is nowadays yet highly consumed in some of the Mexican southwest states with the popular putative aim to improve excitement and mood. **Objectives:** The Aim of the study to ascertain whether or not pozol is endowed with anxiolytic effects in mice. **Materials and Methods:** Putative pozol anxiolytic effects were evaluated in mice using models to assess anxiety such as the hole-board, light/dark box, and the exploratory cylinder tests. **Results:** The results of this paper suggest that pozol has indeed anxiolytic-like effects in all the animal models studied. **Conclusion:** Our studies show that this ancestral drink has an anxiolytic effect in animal models and that it is possible that this activity contributes to its popular consumption.

**Keywords:** Pozol, Anxiolytic Effect, Ancestral Drink, Cocoa, Maize.

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## INTRODUCTION

Pre-hispanic natives of Mesoamerica traditionally consumed cocoa-based (*Theobroma cacao*) beverages, among which pozol was particularly appreciated (Ulloa *et al*., 1987). These drinks however were only available to an elite and were considered of a sacred nature, "drink of the gods" said the Nahuas (Ulloa *et al*., 1987; Verna, 2013). Under this background, Carl Linnaeus named the cacao plant *Theobroma cacao*, from the Latin name Theobroma, literally "food of the gods" (Montagna *et al*., 2019).

With the arrival of the Spanish, they lose their exclusivity and sacredness, and consumption was exposed to the common population (Gage, 1982). Pozol survived the Spanish conquest and is currently a popular non-fermented food and refreshing drink in the states of Chiapas, Tabasco, Campeche, and Yucatan, Mexico (Figure 1) (Ulloa *et al*., 1987).

Pozol (from the Nahuatl, pozolli, foamy) can be prepared as either a non-alcoholic, non-fermented or as a fermented beverage (Álvarez-Ríos *et al*., 2022). Maize (*Zea mays*) nixtamalized in the masa is mixed with toasted cacao paste and water being it thus available for consumption (Cañas-Urbina *et al*., 1993).

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The consumption of cocoa-rich products may, according to a traditional view relax the state of excitement and to improve mood (Dallard *et al*., 2001; Yamada *et al*., 2009). In this work, we study whether pozol is endowed with anxiolytic effects using mouse models of anxiety justifying in this way the widespread consumption of pozol in some Mexican states such as Chiapas and Tabasco.

## MATERIALS AND METHODS

### Animals

Animal experiments were approved by the Research Ethics Committee of Chiapas Medicinal Plants Research Institute, Benemerite Autonomous University of Chiapas, Mexico. (UNACH/IIPMECH/ CEI/0021). Male ICR mice weighing 20-25 g were used in this work. Mice were housed in a controlled environment (temperature 23±2ºC; lights on 07:00-19:00 hr) with water and food (Purina, Mexico) available ad libitum. Animals were donated by the bioterio from Laboratorio Estatal de Salud, Chiapas, Mexico.

### Drugs

#### *Diazepam was purchased from Roche Mexico.*

#### *Pozol preparation*

Pozol was made according to the traditional way by cooking the maize in a lime solution of approximately 1% (w/v) for 30 min at boiling temperature. The cooking solution was discarded and the resulting nixtamal was washed four times with tap water to

remove excess of lime (Palacios-Pola *et al.*, 2008). Cooked maize grains were washed with water, and ground to make a dough known as "nixtamal", which was mixed with roasted ground cocoa in a ratio of 3:1 respectively, forming dough balls. This is mixed with water to produce fine porridge (atole) (Ulloa *et al.*, 1987).

### Behavioral evaluation

Behavioral experiments were carried out in a sound-attenuated room. The room was dimly illuminated and was equipped with video-recording facilities. The behavioral evaluation was carried out in a manual way. All evaluations were conducted between 10:00 and 18:00 hr. and were carried out in a Plexiglas box (30 x 11 x 12 cm each). The apparatuses used for all behavioral tests were cleaned with detergent and dried before each trial. In all experiments, animals were assigned to each group at random. Animals were used only once.

Pozol was administered orally at a dose of 0.1 mL / 10 g. 1 hr after administration, open field, hole-board, dark-light box, and exploratory cylinder tests were performed. Diazepam was used as a positive control (1 mg/kg; i.p.).

### Open field test

This method has been used to evaluate the locomotor activity of rodents (Archer, 1973). The test was performed in a glass box (with transparent walls and a floor of 48 x 48 x 30 cm). The floor was divided with black painted lines forming squares of 12 x 12 cm (de Sousa and de Almeida, 2005). The mouse was placed in the center of the box and locomotion was registered for a period of 5 min. An observer, blind to the treatments, registered both the number of lines crossed (horizontal locomotor activity) and the number of rearings made by the animals (vertical locomotor activity) (Vasconcelos *et al.*, 2004).

### Hole-board test

For this test, a gray perspex platform (40 x 40 cm and 2.2 cm thick) was used, which had 16 holes of 3 cm in diameter, distributed equidistantly on its surface. The platform was placed on a support at a height of 15 cm from the ground (File and Pellos, 1985; Takeda, Tsuji, and Matsumiya, 1998). The test was performed by placing the animal in the center of the platform and recording the head-dipping; the number of times the animal explored the holes for 5 min. Only those examinations in which the mouse inserted its head into the holes up to the level of the ears were considered positive.

### Light/dark box test

In this method, a 44 x 21 x 21 cm propylene chamber darkened with black paint deposited on one-third of its surface was used. Darkened and non-darkened areas were separated by a propylene wall containing a 13 x 5 cm opening. The non-darkened area was brightly illuminated with a 22 W fluorescent lamp. At the start of the test, the animal was placed on the lighted side of the box, and its behavior was observed, and recording it over a period of 10 min. During this period, both the number of transitions (the times the mouse entered the dark side and returned to the illuminated area of the chamber) and the time spent in each of the chamber compartments were quantified (Crawley and Goodwin, 1980).

### Exploratory cylinder test

The acrylic cylinder (45 cm in height, 20 cm in diameter, with a wall of 3 mm) was placed over a flat surface covered with filter paper. Naive mice were individually placed inside the cylinder and the number of rearings over 5 min was recorded (Hiller and Zetler, 1996). The exploratory cylinder test is related to the aversion that mice must be enclosed by surrounding walls. Reduced exploratory rearing showed by naive mice after placement in an unfamiliar environment reveals a mild anxiolytic-like effect (Hiller and Zetler, 1996; Alonso-Castro *et al.*, 2020).

### Statistical analysis

Results are presented as the Mean±SEM of 6 mice per group. The statistical analysis of the results was carried out by using a one-way ANOVA followed by the Newman-Keuls test for multiple comparisons when required. An alpha value of $p<0.05$ was considered statistically significant. Statistical parameters were computed using GraphPad Prism statistical software (GraphPad Software, Inc).

## RESULTS

In the hole-board test, pozol significantly increased the number of spontaneous head-dips ($F_{2,15}=10.81$; $p<0.05$) as compared to the vehicle-treated control. The administration of diazepam (1mg/kg) used as a positive control induced similar effects to those observed with pozol-group (Figure 2).

Both oral pozol administration and diazepam i.p. injection induced a decrease in the rearing activity in mice ($F_{2,15}=27.07$; $p<0.05$) compared with controls in the exploratory cylinder test (Figure 3).

In the light/dark box test there was a significant main effect on the duration of time spent in the light compartment, ($F_{2,15}=10.35$; $p<0.05$) with both pozol and diazepam-treated animals spending significantly more time in the light compartment (Figure 4).

On the other hand, although pozol administration induced as Diazepam an increased number of transitions between darkened and non-darkened compartments of the light/dark test apparatus only Diazepan effects were statistically significant ($F_{2,15}=4.825$; $p<0.05$) (Table 1).

No significant differences were observed in an open field on the number of lines crossed ($F_{4,25}=0.2249$; $p>0.05$) and on the number

of rearings ($F_{4,25}$=0.7309; $p$>0.05) between the pozol-treated mice and both the vehicle and diazepam control groups (Table 2).

## DISCUSSION

The main finding of this work was that pozol, a highly consumed Mexican traditional beverage including maize and cocoa as their principal components is endowed with anxiolytic effects in mice. The hole-board and the light/dark box tests are valid models for evaluating anxiety in rodents (File and Pellos, 1985; Crawley and Goodwin, 1980). In the hole-board test high frequency of head-dipping was usually linked with novelty seeking, and the low one with increased anxiety (Takeda *et al.*, 1998). In this work, both pozol, and diazepam-treated mice, as was already described after the use of benzodiazepines elicited an increased head-dipping behavior suggesting the induction of anxiolytic effects (File and Pellos, 1985; Takeda *et al.*, 1998; Pyrzanowska *et al.*, 2021).

In line with this, an enhancement of the time spent by the mice in the illuminated compartment of the light/dark test, which has been traditionally considered in this anxiety model as the main behavior indicating lower levels of anxiety (Crawley and Goodwin, 1980; Bourin and Hascoët, 2003; Serchov, Van, and Biber, 2016) was observed following both pozol and diazepam administration. However, despite the above, an apparent dissociation of the effects of diazepam and pozol on the number of transitions between the light and dark chambers was perceived in this work. Thus, although both pozol and Diazepam treatment increased the number of transitions between darkened or non-darkened compartments, which have been associated with anxiogenic effects (Crawley and Goodwin, 1980), only diazepam effects on the number of transitions resulted in statistically significant effects. The reason for these differences is not clear but it may be related to differences in the dose of the drugs employed and to the fact that transitions between darkened and non-darkened compartments in this test are more related to activity/exploration rather than to anxiety (Bourin and Hascoët, 2003; Serchov *et al.*, 2016).

As expected from the above results indicating that pozol as Diazepam administration elicited anxiolytic effects in mice a decrease in the number of rearings was observed in the exploratory cylinder test, which has been reported to be associated with anxiolytic effects after the administration of both compounds (Hiller and Zetler, 1996; Alonso-Castro *et al.*, 2020).

The mechanism involved in the anxiolytic effects of pozol described in this work is unknown. It is also not entirely clear why pozol instead induce anxiogenic effects (Lee *et al.*, 1985; Smith, 2002; Domschke *et al.*, 2011) as because of potential high caffeine concentration (Zoumas *et al.*, 2006), its drinking gives instead rise to anxiolytic actions. However, it is likely that the anxiolytic effects induced by pozol in these anxiety models are linked to its cocoa content and that its anxiolytic effects may be consequently related to the high polyphenol content of the cocoa beans which are used in its processing, and in particular to its richness of catethins and proanthocyanidins (Andújar *et al.*, 2012; Wollgast and Anklam, 2000), which have been described to elicit both potent anxiolytic and antidepressant effects (Messaoudi *et al.*, 2008; Xu *et al.*, 2005; Dias *et al.*, 2012; Bouayed *et al.*, 2007). In support of this, it has been demonstrated that (-)-Epigallocatethin-3-O-gallate has been shown to prevent caffeine-induced anxiogenic-like actions (Park *et al.*, 2010).

Taking together, the results of this paper show that pozol drinking triggers anxiolytic effects that may at least partially explain its widespread consumption in some Mexican southeast regions such as Tabasco and Chiapas. Further studies aimed to evaluate both its clinical effects on a large people population and to disclose the nature of the putative substances involved in the pozol anxiolytic effects, as well as their mechanism of action should be undertaken.

## CONCLUSION

Pozol is a refreshing non-alcoholic drink, consumed in southeastern Mexico since pre-Hispanic times as an important part of the daily diet of different ethnic groups. Our results indicate that this ancestral drink has an anxiolytic effect on animal

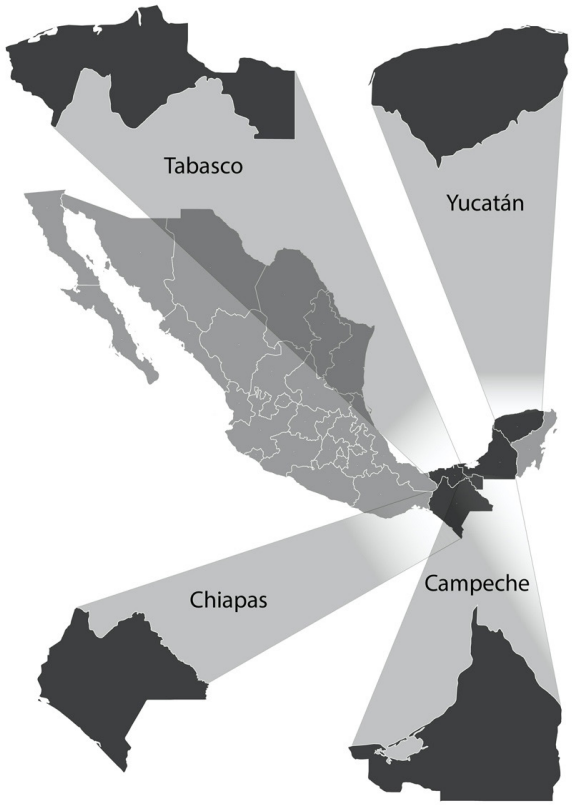


**Figure 1:** Pozol is a refreshing drink, consumed in southeastern Mexico in the states of Chiapas, Tabasco, Campeche, and Yucatan, Mexico.

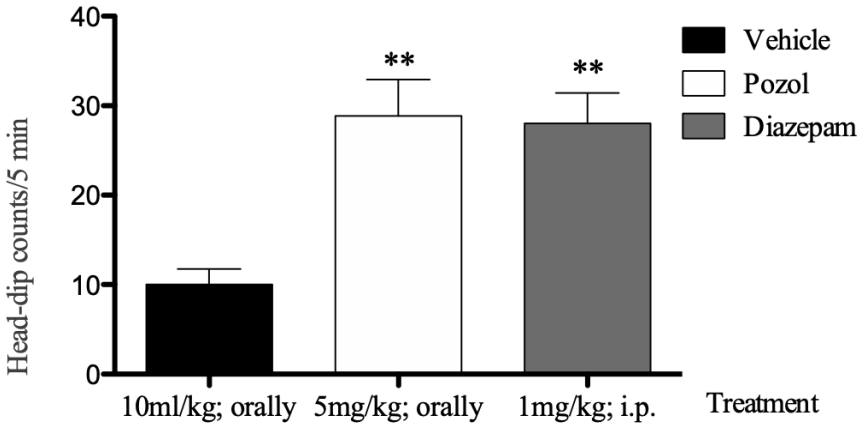


**Figure 2:** The effects of treatments with pozol and diazepam (positive control) over the number of spontaneous head-dips in the hole-board test. ANOVA was followed by the Newman-Keuls test for multiple comparisons. *n*=6 for each group. Means±SEM. ***p*<0.01 vs Vehicle.

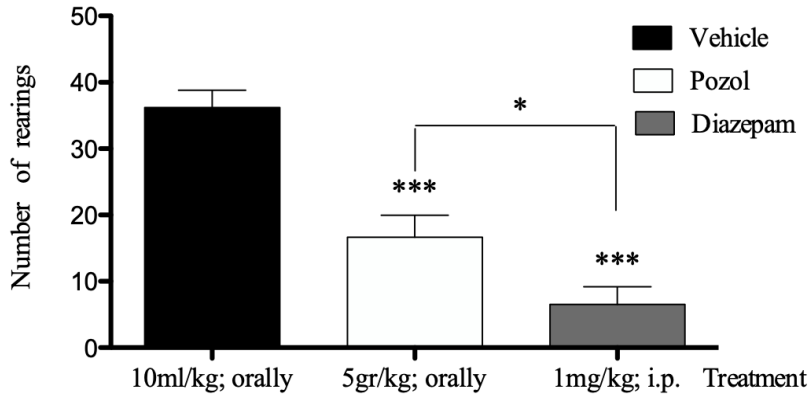


**Figure 3:** The effects of treatments with pozol and diazepam (positive control) over the number of spontaneous rearings in the glass cylinder test. ANOVA followed by Newman-Keuls test for multiple comparisons. *n*=6 for each group. Means±SEM. **p*<0.05 pozol vs Diazepam; ****p*<0.001 either pozol or diazepam vs Vehicle.

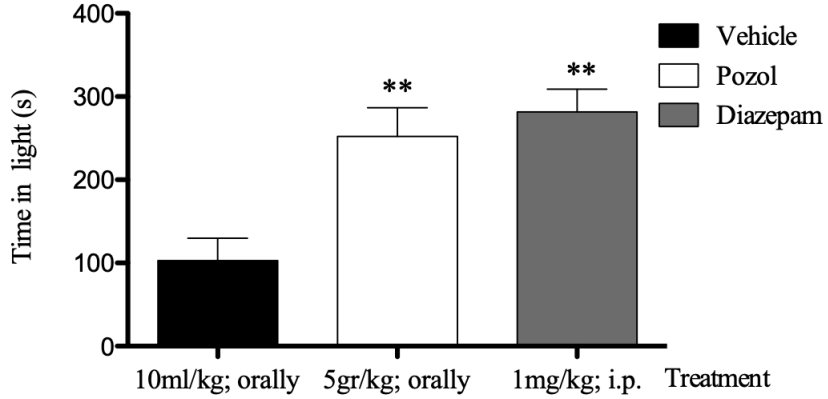


**Figure 4:** The effect of treatments with pozol and diazepam over time spent in the light compartment of the light/dark test. Results are expressed as means±SEM. Both pozol and diazepam, used as a positive control, increased the time spent by the mice in the light compartment compared to the vehicle group. ** *p*<0.01 versus vehicle. ANOVA followed by Newman- Keuls test for multiple comparisons. *n*=6 for each group.

**Table 1: Effects of the oral administration of pozol on the transitions between darkened and non-darkened compartments in the light/dark box test**

| Treatment | Number of transitions |
|---|---|
| Vehicle | 5.8±1.32 |
| Pozol | 8.1±2.42 |
| Diazepam | 14.5±2.20* |

Results are expressed as Means±SME. *$p$<0.05 versus vehicle group. ANOVA followed by Newman-Keuls test for multiple comparisons, $n$=6 for each group.

**Table 2: Effects of the oral administration of pozol on the locomotion of mice in the open field test.**

| Treatment | Number of line crossings | Number of rearings |
|---|---|---|
| Vehicle | 111.9±10.63 | 21.71±4.69 |
| Pozol | 114.6±16.25 | 19.57±3.68 |
| Diazepam | 159.3±15.36 | 16.83±3.51 |

Results are expressed as Means±SME, $n$=6-7 for each group.

models and that it is possible that this activity contributes to its popular consumption. Although it is suggestive, it is not yet clear, whether the anxiolytic activity of the pozol preparations is solely attributable to their high polyphenol concentration provided by their cocoa content.

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## ABBREVIATIONS

**UNACH:** Autonomous University of Chiapas; **IIPMECH:** Chiapas Medicinal Plants Research Institute; **CEI:** Research Ethics Committee; **i.p.:** intraperitoneal; **w/v:** Weight/Volume.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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## SUMMARY

This study evaluated the anxiolytic effects of pozol, a traditional non-alcoholic beverage made from nixtamalized maize and roasted cacao, commonly consumed in southeastern Mexico. Using established behavioral models of anxiety in mice (open field, hole-board, light/dark box, and exploratory cylinder tests), oral administration of pozol significantly increased exploratory behavior and reduced anxiety-related responses, showing effects comparable to those of diazepam. While the exact mechanism remains unclear, the anxiolytic action is likely associated with the high polyphenol content of cacao, particularly catechins and proanthocyanidins. These findings may explain the cultural and widespread consumption of pozol in regions like Chiapas and Tabasco. Further clinical studies are needed to validate these results and identify the active compounds responsible for its anxiolytic properties.

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