e-ISSN: 0976-822X, p-ISSN:2961-6042

Available online on http://www.ijcpr.com/

International Journal of Current Pharmaceutical Review and Research 2026; 18(9); 857-862

Original Research Article

Evaluation of Anxiolytic Activity of Vitex negundo Leaf Extract using Vogel's Water-Lick Conflict Test in Rats

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Received: 01-06-2026 / Revised: 15-07-2026 / Accepted: 21-08-2026

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Conflict of interest: Nil

Abstract

Background: The Vogel's water-lick conflict test is an established behavioural model for evaluating anxiolytic-like activity in rodents. It measures the conflict between the motivation to drink water and avoidance of an aversive electrical stimulus. Vitex negundo has been investigated for potential anxiolytic activity in experimental models, but its effects in a conflict-based paradigm warrant further evaluation.

Objective: To evaluate the anxiolytic-like activity of Vitex negundo leaf extract using the Vogel's water-lick conflict test in rats.

Materials and Methods: An experimental, randomized, comparative animal study was conducted in 24 male Wistar albino rats weighing 150–250 g. Animals were divided into four groups of six: control, diazepam 1 mg/kg, V. negundo leaf extract 100 mg/kg (VNE100), and V. negundo leaf extract 200 mg/kg (VNE200). The leaf extract was prepared by exhaustive extraction with 95% methanol using a Soxhlet apparatus and administered orally. Following 24-hour water deprivation, animals were assessed in the Vogel apparatus for 3 minutes. Total water licks and total shocks received were recorded, while urination was documented. Data were expressed as Mean ± SD for continuous variables and number (percentage) for categorical variables. Appropriate statistical tests followed by Dunnett's multiple comparisons were used, with $p<0.05$ considered statistically significant.

Results: Mean water licks were 107.7 ± 18.22 in controls, 188.2 ± 32.31 with diazepam, 156.3 ± 35.25 with VNE100, and 163.8 ± 45.19 with VNE200. Compared with control, significant increases were observed with diazepam ($p=0.0016$) and VNE200 ($p=0.0261$), whereas VNE100 did not reach statistical significance ($p=0.0578$). Mean shocks received were 5.00 ± 1.09, 9.00 ± 1.67, 7.33 ± 1.86 and 7.67 ± 2.34, respectively. Significant increases occurred with diazepam ($p=0.0027$) and VNE200 ($p=0.0472$), but not VNE100 ($p=0.0898$).

Conclusion: V. negundo leaf extract, particularly at 200 mg/kg, demonstrated anxiolytic-like activity in the Vogel conflict test, reflected by increased punished drinking and shock acceptance. Further pharmacological and clinical studies are required to establish its therapeutic potential.

Keyword: Vitex negundo, Vogel's Water-Lick.

DOI: 10.25258/ijcpr.18.9.121

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Introduction

Anxiety-related behaviour in rodents can be investigated using behavioural paradigms based on conflict between approach and avoidance. Such models provide experimental approaches for evaluating compounds with potential anxiolytic-like activity. [1,2]

The Vogel water-lick conflict test is a conditioned conflict model in which a motivated drinking response is suppressed by an aversive electrical stimulus. In the procedure described by Vogel, Beer and Clody, water-deprived rats are allowed access to water while drinking is punished by electrical shock. [3]

The resulting conflict occurs between the motivation to obtain water and avoidance of the aversive stimulus. Anxiolytic drugs can reduce this

avoidance and consequently increase punished drinking responses. [1-3] The number of shocks received is an important outcome in the Vogel paradigm. A greater number of shocks indicates that the animal continued drinking despite punishment and is conventionally interpreted as an anxiolytic-like behavioural response. [1-3] However, interpretation of the test requires consideration of factors other than anxiety. Thirst and motivational state can influence drinking behaviour, while pain sensitivity, shock intensity and learning can influence responses to punishment. Therefore, the Vogel test should be interpreted within the context of its experimental conditions. [1,2]

Recording the total number of water licks provides additional information regarding drinking behaviour during the conflict period. Experimental descriptions of the Vogel test commonly record both total licks and shocks delivered during the test. [4] The lick count therefore provides useful contextual information for interpreting the observed shock response.

Diazepam was included as the standard pharmacological comparator. Benzodiazepines have been used as reference anxiolytic agents in conflict-based animal models and can increase punished drinking behaviour. [1,2]

Vitex negundo L. has previously been investigated for potential anxiolytic-like activity in experimental animals. Adnaik et al. reported anxiolytic activity of Vitex negundo extract in experimental models of anxiety. [5] These findings provide a rationale for further evaluation of Vitex negundo, particularly using a conflict-based behavioural model involving an aversive consequence. [6-10]

The present study therefore evaluated the effect of Vitex negundo leaf extract at 100 and 200 mg/kg in the Vogel water-lick conflict test in rats.

Total water licks, total shocks received and urination response were considered to provide a broader description of behavioural responses during the conflict procedure. Diazepam was included as the standard comparator.

Materials and Methods

An experimental, randomized, comparative animal study was conducted using male Wistar albino rats weighing 150–250 g. The study was commenced after IAEC approval. The animals were divided into four groups of six rats each: control, diazepam, VNE100 and VNE200. The control group received double-distilled water (0.5 mL), while VNE100 and VNE200 groups received Vitex negundo leaf extract at 100 and 200 mg/kg, respectively. Diazepam 1 mg/kg was administered to the standard drug group. The Vitex negundo leaves were shade-dried, powdered and exhaustively extracted with 95% methanol using a Soxhlet apparatus. The concentrated extract was used for oral administration. The elevated plus maze (EPM) is an unconditioned behavioural conflict model used to assess anxiety-like behaviour in rodents. It consists of two open and two enclosed arms elevated above the floor and is based on the natural aversion of rodents to elevated, exposed spaces. Reduced avoidance of the open arms, reflected by increased open-arm entries and time spent in open arms, is generally interpreted as an anxiolytic-like response.[1–3]

For the Vogel water-lick conflict test, rats were deprived of water for 24 hours before testing. Each animal was placed individually in the Vogel apparatus, consisting of a plexiglass cage fitted with a water bottle, lickometer and shocker. During the 3-minute observation period, drinking responses were recorded, with an electrical shock of 0.5 mA for 1 second delivered according to the experimental protocol after drinking contacts. The total number of water licks and total number of shocks received were recorded as behavioural outcomes. Urination during the test was also documented. Data were expressed as Mean ± SD for continuous variables and number (percentage) for categorical variables. Statistical analysis was performed using the appropriate parametric or non-parametric test followed by Dunnett's or Dunn's multiple comparisons, as applicable; Fisher's exact test was used for urination response. A p value <0.05 was considered statistically significant.

Result

Table 1: Vogel's Water-Lick Conflict Test: Total Number of Shocks Received

Group	Mean ± SD	Change vs Control (%)
Control	5.00 ± 1.09	Reference
Diazepam	9.00 ± 1.67	+80.00
VNE100	7.33 ± 1.86	+46.60
VNE200	7.67 ± 2.34	+53.40

Percentage change was calculated from group means using Control as the reference.

Table 2: Vogel's Water-Lick Conflict Test: Total Number of Water Licks

Group	Mean ± SD	Change vs. Control (%)
Control	107.7 ± 18.22	—
Diazepam	188.2 ± 32.31	+74.75
VNE100	156.3 ± 35.25	+45.26
VNE200	163.8 ± 45.19	+52.09

Table 3: Urination Response during the Behavioural Test

Group	Yes, n (%)	No, n (%)	Total
Control	4 (66.7%)	2 (33.3%)	6
Diazepam	2 (50.0%)	2 (50.0%)	4
VNE100	3 (50.0%)	3 (50.0%)	6
VNE200	3 (50.0%)	3 (50.0%)	6

Fisher's exact test was used in the source results; no statistically significant difference was reported between groups. Percentages are calculated within each group. Figure 1. VOGEL'S TEST: Total Number of Water Licks

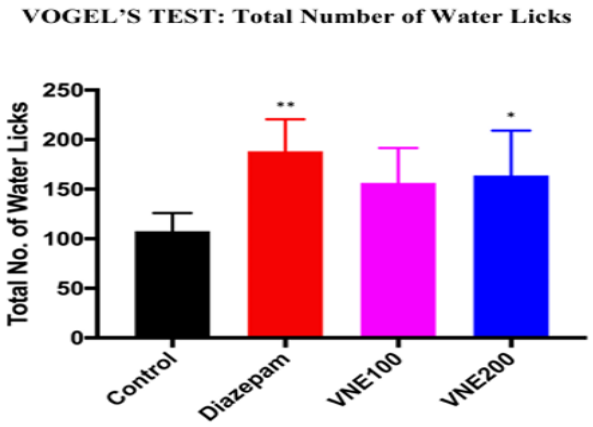

Figure 1:

Table 4:

Group	Control	Diazepam	VNE100	VNE200
Mean ± SD	107.7 ± 18.22	188.2 ± 32.31	156.3 ± 35.25	163.8 ± 45.19

Dunnett's multiple comparisons test

Dunnett's multiple comparisons test	Mean difference	Significant?	Adjusted P Value
Control vs. Diazepam	−80.5	Yes	0.0016
Control vs. VNE100	−48.67	NS	0.0578
Control vs. VNE200	−56.17	Yes	0.0261

p value < 0.05, ** p value < 0.01, NS = Not significant.

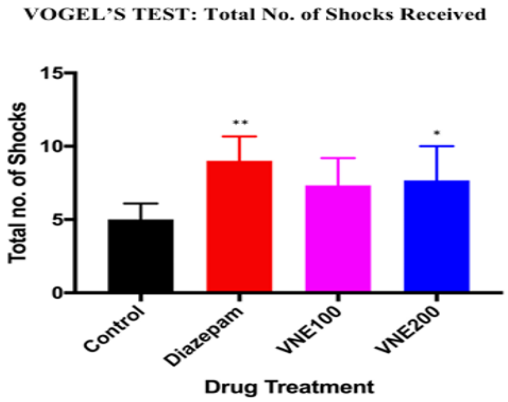

Figure 2: Vogel's Test: Total Number of Shocks Received

Table 5:

Group	Control	Diazepam	VNE100	VNE200
Mean ± SD	5 ± 1.09	9 ± 1.67	7.33 ± 1.86	7.67 ± 2.34

Dunnett's multiple comparisons test

Dunnett's multiple comparisons test	Mean difference	Significant?	Adjusted P Value
Control vs. Diazepam	−4	Yes	0.0027
Control vs. VNE100	−2.333	NS	0.0898
Control vs. VNE200	−2.667	Yes	0.0472

p value < 0.05, ** p value < 0.01, NS = Not significant.

The total number of water licks was 107.7 ± 18.22 in the control group, 188.2 ± 32.31 in the diazepam group, 156.3 ± 35.25 in the VNE100 group, and 163.8 ± 45.19 in the VNE200 group. Compared with control, water licking increased by 74.75% with diazepam, 45.26% with VNE100, and 52.09% with VNE200. Dunnett's multiple comparisons test demonstrated a statistically significant increase in water licks in the diazepam group compared with control (mean difference −80.50, adjusted P = 0.0016) and in the VNE200 group (mean difference −56.17, adjusted P = 0.0261). The increase observed with VNE100 was not statistically significant (mean difference −48.67, adjusted P = 0.0578). Thus, VNE200 produced a significant increase in punished water-licking behaviour, whereas VNE100 showed a numerical but statistically non-significant increase.

The mean number of shocks received was 5.00 ± 1.09 in the control group, 9.00 ± 1.67 in the diazepam group, 7.33 ± 1.86 in the VNE100 group, and 7.67 ± 2.34 in the VNE200 group. Compared with control, shocks received increased by 80.00% with diazepam, 46.60% with VNE100, and 53.40% with VNE200. Dunnett's test showed a statistically significant increase in shocks received with diazepam (mean difference −4.00, adjusted P = 0.0027) and VNE200 (mean difference −2.667, adjusted P = 0.0472), whereas the increase with VNE100 was not statistically significant (mean difference −2.333, adjusted P = 0.0898).

The findings therefore showed a similar pattern to the water-licking results, with VNE200 demonstrating significant effects on both major behavioural parameters.

Urination behaviour was observed in 4/6 animals (66.7%) in the control group, 2/4 animals (50.0%) in the diazepam group, and 3/6 animals (50.0%) in both VNE100 and VNE200 groups. Fisher's exact test showed no statistically significant difference among the groups.

Thus, urination behaviour did not demonstrate a significant treatment-related change in the present study. When all three parameters were considered together, VNE200 produced the clearest behavioural response in the Vogel conflict test. It significantly increased both total water licks and total shocks received compared with control, whereas VNE100 produced numerical increases in both parameters that did not reach statistical significance. In the Vogel paradigm, drinking is suppressed by punishment, creating an approach–avoidance conflict between the motivation to obtain water and avoidance of the aversive shock. Increased punished drinking is commonly interpreted as an anxiolytic-like response. The significant increase in water licking with VNE200 indicates that animals continued the drinking response despite the punishment. The corresponding significant increase in shocks received provides a complementary finding, because continued drinking under the punishment schedule necessarily increases the opportunity to receive shocks. Thus, the simultaneous increase in water licks and shocks received at 200 mg/kg is consistent with increased punished responding and supports an anxiolytic-like effect of VNE200 in this model. However, interpretation of the Vogel test should consider other factors that can influence punished drinking, including thirst, motivation, nociception, learning and memory.

The diazepam group showed significant increases in both water licks and shocks received, providing the expected positive-control response. The similarity in the direction of the VNE200 response to that observed with diazepam supports the interpretation that VNE200 produced anxiolytic-like behavioural activity in the present experimental model. Nevertheless, this finding does not establish that VNE200 has an effect equivalent to diazepam.

Although the mean number of water licks and shocks received was higher with VNE200 than with VNE100, the present analysis did not include a direct statistical comparison between the two VNE doses. Therefore, the findings suggest greater anxiolytic-like activity at 200 mg/kg than at 100 mg/kg, but they do not establish a statistically confirmed dose-response relationship.

The absence of a significant difference in urination further indicates that this parameter did not contribute to the observed treatment-related pattern. Overall, the findings demonstrate that Vitex negundo leaf extract, particularly at 200 mg/kg,

increased punished drinking behaviour in the Vogel's water-lick conflict test. The significant increases in both water licks and shocks received provide convergent behavioural evidence of anxiolytic-like activity, while the effect at 100 mg/kg remains suggestive but statistically inconclusive.

Discussion

The present study evaluated the anxiolytic-like activity of Vitex negundo leaf extract using Vogel's water-lick conflict test, a learned approach–avoidance paradigm in which drinking is paired with an aversive electric shock. In this model, suppression of punished drinking reflects avoidance, whereas increased punished responding following an anxiolytic treatment is interpreted as an anxiolytic-like effect. [1,2] The Vogel conflict test has been widely used for evaluating potential anxiolytic agents and is particularly useful because it measures drug effects on a conditioned conflict response. [1,2]

In the present study, diazepam produced a significant increase in the total number of water licks compared with the control group. The mean number of water licks increased from 107.7 ± 18.22 in the control group to 188.2 ± 32.31 in the diazepam group (P = 0.0016). This finding provides the expected positive-control response and supports the responsiveness of the experimental procedure to a known anxiolytic agent.

Vitex negundo leaf extract at 200 mg/kg produced a statistically significant increase in total water licks, from 107.7 ± 18.22 in controls to 163.8 ± 45.19 (P = 0.0261), corresponding to a 52.09% increase. VNE100 also increased water licking to 156.3 ± 35.25, representing a 45.26% increase, but this difference was not statistically significant (P = 0.0578). Thus, the water-licking findings suggest an anxiolytic-like effect of VNE200, whereas the response at VNE100 was numerically increased but statistically inconclusive.

A similar pattern was observed for the total number of shocks received. Diazepam significantly increased shocks received from 5.00 ± 1.09 in controls to 9.00 ± 1.67 (P = 0.0027). VNE200 also significantly increased the number of shocks received to 7.67 ± 2.34 (P = 0.0472), while VNE100 produced a numerical increase to 7.33 ± 1.86 that did not reach statistical significance (P = 0.0898). In the Vogel paradigm, an increase in shocks received occurs when the animal continues drinking despite punishment and is therefore considered increased punished responding. [1,2,3] The concordant increase in both water licks and shocks received with VNE200 therefore provides consistent behavioural evidence of anxiolytic-like activity.

The findings are also supported by previous experimental work on Vitex negundo. Adnaik et al. reported anxiolytic-like activity of Vitex negundo extract at 100 and 200 mg/kg in mice using the elevated plus maze and light–dark exploration tests.[4] Their study demonstrated increased exploration of anxiogenic areas following treatment, supporting the potential of Vitex negundo to produce behavioural effects relevant to anxiety. However, their study used a root extract and different animal models; therefore, the findings provide supportive rather than direct evidence for the present Vogel-test results. [4]

The stronger response observed with VNE200 compared with VNE100 is noteworthy. Both water licks and shocks received were numerically higher at 200 mg/kg than at 100 mg/kg. However, because the present statistical analysis compared each VNE dose with the control group and did not include a direct statistical comparison between VNE100 and VNE200, a definitive dose-response relationship cannot be established. The findings may therefore be described as suggesting greater anxiolytic-like activity at 200 mg/kg, rather than proving a statistically significant dose-dependent effect.

Urination behaviour did not differ significantly among the experimental groups. Although increased urination can sometimes accompany anxiety-related responses in rodent behavioural assays, [2] the absence of a significant difference in the present study indicates that urination did not show a treatment-related change and does not provide additional evidence for the anxiolytic-like effect of the extract.

An important consideration in interpreting the present findings is that the Vogel test is influenced by factors other than anxiety, including thirst, motivation to drink, nociception, learning and the intensity of the aversive stimulus.[2] Therefore, increased punished drinking should be interpreted as anxiolytic-like behavioural activity rather than as definitive proof of reduced anxiety. Use of complementary behavioural models and mechanistic investigations would provide stronger evidence regarding the pharmacological action of Vitex negundo.

Overall, the present findings demonstrate that Vitex negundo leaf extract, particularly at 200 mg/kg, increased punished drinking behaviour in the Vogel water-lick conflict test. The significant increases in both total water licks and total shocks received with VNE200, together with the positive-control response to diazepam, support the presence of anxiolytic-like activity of the extract in this experimental model. Further studies involving additional validated anxiety models, larger sample sizes, direct dose-to-dose comparisons and mechanistic investigations are warranted.

Conclusion

Vitex negundo leaf extract demonstrated anxiolytic-like activity in the Vogel's water-lick conflict test, with the 200 mg/kg dose producing significant increases in both total water licks and total shocks received compared with control. The 100 mg/kg dose showed numerical increases in both parameters but did not reach statistical significance. Vogel's water-lick conflict test is used for eveluating anticipatory anxiety which is a correlate of panic disorder in humans.

Therefore, Vitex negundo certainly shows potential as an anxiolytic drug either standalone or addon drug for anticipatory anxiety and panic disorder in humans.

The findings provide preliminary experimental support for the potential of Vitex negundo as a source of an anxiolytic agent and justify further pharmacological, mechanistic and safety studies before therapeutic application can be considered.

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