An Extract of Neem Leaves Reduces Anxiety without Causing Motor Side Effects in an Experimental Model

KA Thaxter¹, LE Young¹, RE Young¹, O Parshad¹, J Addae²

ABSTRACT

Anxiety modulation often requires pharmaceutical intervention, and though effective in the short term, benzodiazepines may cause impaired motor function. As a potential alternative, anxiety-modulating effects of a neem leaf (Azadirachta indica, A Juss) extract were investigated using ethological analysis of rat behaviour on an elevated X maze and compared with diazepam treatment. Sexually immature female Sprague-Dawley rats received 0.07 or 7 mg/kg neem leaf steroidal extract, a sham injection, a 1% DMSO/saline vehicle, 2 mg/kg diazepam or no treatment one hour prior to a recorded five-minute exploration of the elevated X maze. Neem matched diazepam in anxiety reduction as both treatments caused a decrease in per cent protected stretched-attend postures (PPSAP). Neem treatment had no effect on closed arm entries or total rears, distinguishing it pharmacologically from diazepam which resulted in a predictable decrease in those locomotor measures. Whereas both neem and diazepam reduced anxiety in complex ethological behavioural indices, only neem produced anxiolysis without motor deficiency.

Keywords: Anxiety, Azadirachta indica, neem, X maze

Un Extracto de Hojas de Neem Reduce la Ansiedad sin Causar Efectos Colaterales Motores en un Modelo Experimental

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RESUMEN

La modulación de la ansiedad requiere a menudo la intervención farmacéutica, y aunque eficaz a corto plazo, las benzodiazepinas pueden afectar la función motora. Como una alternativa potencial, los efectos moduladores de la ansiedad obtenidos a partir de un extracto de la hoja de neem (Azadirachta indica, A Juss), fueron investigados mediante análisis etiológico del comportamiento de ratas en un laberinto x elevado, y comparados con el tratamiento con diazepam. Ratas Sprague-Dawley hembras, sexualmente inmaduras, recibieron 0.07 ó 7 mg/kg de extracto esteroidal de hojas de neem, una inyección simulada, un vehículo salino de DMSO al 1%, 2 mg/kg de diazepam o ningún tratamiento una hora antes de registrarse una exploración de cinco minutos en el laberinto X elevado. El neem igualó al diazepam en la reducción de ansiedad, ya que ambos tratamientos causaron una disminución en las posturas de atención extremada protegida porcentual (PPSAP). El tratamiento de Neem no tuvo efecto sobre las entradas al brazo cerrado o actividades aéreas (con las patas traseras) distinguiéndose así farmacológicamente del diazepam que producía una disminución predecible en esas medidas locomotoras. Si bien tanto el neem como el diazepam reducían la ansiedad en los índices conductuales etológicos complejos, solamente el neem producía ansiolisis sin deficiencias motoras.

Palabras claves: Ansiedad, Azadirachta indica, (neem), laberinto X

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INTRODUCTION

The prevalence rates of anxiety disorders in the Caribbean are not known, partly because their detection rates are low compared to those of developed countries. Nevertheless, anxiety disorders are perceived to be common in the region and get treated with standard psychosocial approaches and medications. The use of benzodiazepines forms part of the treatment regime for anxiety disorders although these drugs have several side effects including impaired motor function. Neem trees (Azadirachta indica, A. Juss) which are common in the region are known for their medicinal properties that have been recognized in India for thousands of years. Neem leaf extracts have been used by practitioners of Ayurveda for their sedative and central nervous system (CNS) modulating properties (1, 2). Anecdotal reports have been supported by scientific investigations of CNS effects of the neem leaf extracts (3, 4). Previous studies in our laboratory using a steroidal extract of neem leaves found a decrease in the spontaneous motor activity of rats (5) but questions remained regarding whether their motionlessness was related to decreased desire (ie anxious freezing) or ability (ie behavioural suppression) to move. Therefore, in this study, we explored the use of neem leaves as a possible treatment for anxiety disorders using an established animal model of anxiety.

Ethology is the study of animal behaviour with emphasis on the behavioural patterns that occur in natural environments. These behavioural patterns are specific to the animal involved, and tell stories of emotional states in great detail. The elevated X maze, recognized as the 'gold-standard' animal model of anxiety-related behaviour, is widely used in the search for novel agents and is sensitive to the actions of both anxiolytic and anxiogenic agents (6-8). The maze consists of two opposed arms enclosed by walls except at the central area. These 'closed' arms are perpendicular to two open arms of equal dimension and the whole apparatus is elevated above the ground (6). Rodents spontaneously explore the elevated X maze environment in the absence of explicit reward or consummatory behaviour and display increased anxiety in exposed areas. The level of naturally induced anxiety is indicated by recognized 'ethological' actions and postures (9, 10).

The specific objectives of this study were to compare the effect of a steroidal extract of dried neem leaves with that of the robust anxiolytic diazepam on rats exploring an elevated X maze, utilizing ethological analysis of specific maze behaviours to distinguish between locomotion-related and anxiety-related outcomes.

SUBJECTS AND METHODS

Experiments were performed according to the guidelines set out by the Bioethics Committee of the University of the West Indies (UWI), St Augustine Campus. Sexually immature female Sprague-Dawley rats, aged 5–8 weeks and weighing 100–150 g, were obtained from the Animal House of the Veterinary School of the UWI, St. Augustine. The animals were housed in groups of 10–15, had access to food and water *ad libitum* and were exposed to a light:dark cycle of 10:14 hours.

A neem leaf steroidal extract was prepared according to the method previously published (5) and dissolved in a 1% DMSO/saline vehicle. Two dilutions were made so that administration of a constant 1 ml per 100 g rat body weight provided doses of 7 mg/kg (high dose) and 0.07 mg/kg (low dose) of crude extract, respectively.

The rats were randomly assigned to one of six treatment groups (n = 8 for each group). All compounds were injected intraperitoneally (*ip*) one hour prior to maze exposure. Group 1 animals received no treatment. Group 2 was given a single *ip* needle prick (sham injection) and were controls for the diazepam treated group. Group 3 received a low dose (0.07 mg/kg,) of the neem leaf steroidal extract and Group 4 received a high dose (7 mg/kg). Group 5 received the 1% DMSO/saline vehicle and was a control for the two neem treated groups. Group 6 was given 2 mg/kg diazepam.

A wooden elevated X maze with arm dimensions 50 cm x 10 cm, closed arm side walls 10 cm high and a central platform of 10 cm² (11) was used. The maze was raised to a height of 1 metre off the ground and was housed within a sound proof room. The maze was enclosed by 4 'walls' of opaque black cloth to eliminate visual cues to the rats from the rest of the room. Experiments were conducted under dim white light of intensity 88–175 lux. All test sessions were conducted in the dark phase of the light:dark cycle (1400–1600 h). The maze was thoroughly cleaned in between each rat exploration.

Each rat was placed in the centre of the elevated X maze, facing an open arm, and its exploratory activity recorded for 5 minutes using a video camera mounted one metre above the maze. Each animal was tested only once.

The parameters shown in the Table were collected from the videotaped explorations and analysed. These parameters

Table: Definitions of parameters scored on the elevated X maze

Anxiety-related measures	Motor-related measures
<i>Open arm entries:</i> The number of times the rat entered an open arm with all 4 paws in	<i>Total arm entries:</i> The number of times the rat entered an arm with all 4 paws in
<i>Total stretched-attend postures (TSAP):</i> The rat stretches forward and retracts to its original position without forward locomotion	<i>Closed arm entries:</i> Number of entries into a closed arm
% protected SAP (PPSAP): SAP performed within a closed arm, or in the centre square	<i>Total rears:</i> Balancing on the hind legs. This includes rising up on to the hind legs when supported by the wall of a closed
<i>Grooming duration:</i> Time spent licking, scratching and washing the head and body	arm

represent anxiety, locomotion, risk assessment, vertical activity and exploration as verified by factor analysis (12). Data were analysed by single factor ANOVA or ANOVA on Ranks, with post-hoc analysis via the Tukey test.

RESULTS

In general, rats were less active after 0.07 mg/kg neem and diazepam treatments, compared to after treatment with 7 mg/kg neem leaf extract. There was no significant difference in anxiety and locomotor measures between the untreated, sham injected and 1% DMSO/saline vehicle controls.

Rats treated with 0.07 mg/kg neem made significantly more (p = 0.018) entries into the open arms of the elevated X maze than did rats treated with 7 mg/kg neem. The 0.07 mg/kg group also tended to spend more time on the open arms (p = 0.059), although this was not significant (Fig. 1A).



Fig 1: (A) The effect of neem on arm entry measures of anxiety. Values are presented as means \pm SE, * p < 0.05 compared to 0.07 mg/kg neem.

(B) The effect of diazepam on arm entry measures of anxiety. Values are presented as means \pm SE, * p < 0.05 compared to sham injection.

Diazepam treatment significantly increased both the percentage of open entries (p < 0.05) and time spent (p < 0.05), compared to animals given a sham injection (Fig. 1B).

There was a remarkable trend toward an increase in TSAP performed by rats in both neem treatment groups but this did not reach statistical significance. Predictably, diazepam significantly reduced the TSAP score (p < 0.05) compared to sham injection.

Whereas neither neem treatment had any effect on total stretched-attend postures (TSAP), both the high and low dose neem treated groups and the diazepam treated group had lower PPSAP scores than the vehicle and sham injected controls, respectively (Figs. 2A and 2B). However, only the



Fig. 2: (A) The effect of neem on ethological measures of anxiety: PSAP Values are presented as Means \pm SE * p < 0.05 compared to vehicle

(B) The effect of diazepam on ethological measures of anxiety: PSAP Values are presented as Means \pm SE * p < 0.05 compared to sham injection

scores of the high dose neem treated (p < 0.05) and diazepam (p < 0.05) groups reached significance.

Neither high nor low dose neem treatment caused any significant change in total or closed arm entries. In contrast, diazepam treatment significantly reduced closed arm entries, compared to the sham injection (p = 0.029) while showing no significant effect on total entries.

Neem treatment, at both doses, caused a significant increase in rearing (p < 0.05) over vehicle treatment (Fig. 3A). In contrast, diazepam treatment significantly reduced rearing (p < 0.05) compared to the sham injected group (Fig. 3B).



Fig. 3: (A) The effect of neem on ethological measures of locomotion: Total rears. Values are presented as Means \pm SE * p < 0.05 compared to vehicle

(B) The effect of diazepam on ethological measures of locomotion: Total rears. Values are presented as Means \pm SE * p < 0.05 compared to sham injection.

DISCUSSION

Anxiety disorders frequently affect the general population and have a worldwide lifetime prevalence ranging from 13.6% to 28.8% (13). Whereas cognitive-behavioural therapy provides cutting-edge non-pharmaceutical options for specific patients, the search continues for the 'ideal' drug, which will provide predictable and consistent anxiolysis without undesirable side effects.

The usefulness of the elevated X maze has recently extended to elucidating the biological basis of various subtypes of anxiety disorders, as well as to defining various brain areas involved in fear and anxiety (14). The popularity of the test has been attributed to its numerous advantages, including economy, rapidity, simplicity of design, bidirectional drug sensitivity and its ability to produce and reproduce an unconditioned or ethological repertoire of behaviours.

Rats in both neem treatment groups conducted a significantly larger proportion of their orienting and investigating activity on the open arms, indicating a shift in risk assessment from protected to unprotected areas of the maze. This is evident in the decrease in per cent protected SAP, an indicator of anxiolysis. The stretched-attend posture is considered an indicator not only of anxiety but also of emotionality. It is considered to be an information-gathering behaviour displayed in potentially threatening situations, the function of which is to devise the most optimal behavioural strategy. Risk assessment measures have proven extremely valuable in identifying anxiolytic-like actions of drugs not detected by such conventional X maze scoring methods as open arm entries and time (15).

Spontaneous motor activity is in fact the equivalent of total and closed arm entries on the elevated X maze (16). The absence of effect on total and closed arm entries confirms the proposal that the neem extract did not reduce the rats' ability to move. This was verified by the accompanying increase in total rears after treatment with both doses of the neem leaf extract. In contrast, diazepam caused a significant reduction in closed arm entries and rears, demonstrating, predictably, benzodiazepine-induced locomotory deficiency.

A criterion for stating that a drug has specific effects on anxiety-like behaviours in the elevated X maze is that general locomotory activity was not altered by the drug (17). Our results demonstrate that neem treatment caused an anxiolytic pattern of behaviour on the elevated X maze. This pattern was accompanied by increased exploratory activity, creating an overall profile which was different from the classical anxiolysis and motor deficit observed after diazepam treatment.

This work leaves scope for further investigation of the anxiety-modulating and potential pharmacological activity of constituents of the neem leaf.

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