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Full-Length Research Article

Influence of Seasonal Variation on Anxiety-Like Behaviours in Albino Mice and the Modulatory Role of N-Acetyl Cysteine

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Summary: Anxiety disorders form one of the most common type of mental illness and comprises of separation anxiety, specific phobias, social anxiety disorder, and generalized anxiety disorder. These disorders offer considerable comorbidity with other mental disorders like depression, memory and learning deficit which all show considerable seasonal variance. Seasonal changes have major impact on health of individuals especially mental health. N-Acetylcysteine (NAC) is an acetylated derivative of cysteine, and is considered as an antioxidant precursor to glutathione. It has been shown to ameliorate various types of mental disorders. This study investigated changes in anxiety-like behaviours in albino mice across different seasons in Nigeria and the modulatory role of NAC. The study was conducted across two seasons (cold dry and hot humid seasons) in Nigeria. Forty (40) mice of both sexes (20/season) were used, divided into control (normal saline) and NAC (500 mg/kg body weight) groups, respectively. All administration was done orally for one week and the rectal temperatures were measured. Behavioural test was conducted using Elevated Plus Maze (EPM) and Open Field Test under standard protocols for each season for a period of one week. The parameters for seasonal changes were also recorded. All analyses were done using Mixed ANOVA followed by Tukey's and Bonferroni's test, respectively. The results of the study revealed a significant ($P < 0.05$) increase in anxiety-like behaviours in hot humid season and increased locomotive activity in cold dry season in male mice. Female mice were more susceptible to anxiety in cold dry season, which was ameliorated by NAC. In conclusion, the study revealed that hot humid season have anxiogenic like effects, while cold dry season increased locomotive activities in male mice, demonstrating increase exploratory activity and decrease in level of anxiety. The study establishes the occurrence of anxiogenic-like behaviours in female mice in the cold dry season which was ameliorated by NAC administration.

Keywords: *Anxiety, Seasonal Variation, N-Acetyl Cysteine, Neurobehaviour*
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INTRODUCTION

Physical and mental state of humans is thought to be under the considerable influence of the seasons (Charlson *et al*., 2021). In the mental domain, seasonal changes are thought to have impact on human mood and behaviour hence predisposes people to symptoms of psychiatric illnesses such as depression and anxiety disorders (Fonte *et al*., 2021). Studies have also shown that about 1.5% to 10% of the general population suffer from the impact of seasonality involving serious depressive complaints. A typical illustration of the impact of seasonal changes on mental is a phenomenon described as Seasonal Affective Disorder (SAD) which is more prevalent in the northern latitudes (Meesters *et al*., 2016; Nussbaumer-Streit *et al*., 2021). SAD is considered multifactorial, with its symptoms influence by several mechanisms, including disruption of circadian rhythms, altered melatonin secretions, changes in serotonergic, norepinephrine, dopaminergic neurotransmitter systems, and variation in photoperiodism which has been reported to play crucial roles in the development of its symptoms (Nussbaumer-Streit *et al*., 2021). The prevalence of SAD in North America was said to be two times higher compared to Europe, which was attributed to few hours of daylight in winter, with women being mostly affected than men (Meesters et al., 2016).

There is paucity of information on a comprehensive prevalence rate of seasonal affective disorder and its economic burden in the tropics including Nigeria. However, the prevalence rate of 0.1% and 1.83% of bipolar disorder was reported in Nigeria and Ethiopia, respectively (Esan and Esan, 2016). Despite the low prevalence rate reported in

Nigeria, patients with bipolar disorder thought to be clinically stable had substantial disability in the long run posing serious health and economic burden to their family and the society at large (Adegbaju *et al*., 2013). In another study on Economic burden of non-communicable diseases (NCD) on households in Nigeria from 2018-2019, an average expenditure of ₦ 122,313.60 or $ 398.52 per year on NCD care was reported, representing 24% of household food expenditure, with cancer, mental health problems, and renal diseases contributing the highest rate to the cost of NCD care (Odunyemi et al., 2023). This leads to catastrophic health expenditure (CHE) and impoverishing effects due to out-of-pocket (OOP) health spending.

Anxiety has been defined as a state experienced in response to threats or dangers that are either distal or uncertain involving changes in an individual's subjective state, behaviour and physiology (Kenwood *et al*., 2022). When such experience becomes intense, frequent, or even continuous, it is referred to as anxiety disorders (Staner, 2003). Anxiety disorders (ADs) are highly prevalent in the general population and offer considerable comorbidity with other mental disorders like depression, memory and learning deficit (Vismara *et al.,* 2020; Ter Meulen *et al.,* 2021). The global prevalence of anxiety disorders has been reported to be 4.05% with 2722 cases per 100, 000 population prevalence rates reported in Nigeria (Javaid *et al*., 2023). Peripheral biomarkers related to neurotransmitters such as Serotonin (5-HT), norepinephrine (NE), epinephrine (E), dopamine (DA), and gamma-aminobutyric acid (GABA) which are under considerable influence of seasons are the most investigated neurotransmitters in ADs (Vismara *et al*., 2020; Nussbaumer-Streit *et al*., 2021).

N-acetylcysteine (NAC) is a mucolytic agent used as an adjunct therapy in patients with Paracetamol overdose and was most recently found to exert beneficial effect on different psychiatric conditions in both preclinical and clinical studies through regulation of several neurotransmitters, oxidative homeostasis, and inflammatory mediators (Smaga *et al*., 2021). The emerging interest in the use of NAC as therapeutic agent in psychiatric disorders in over the years was driven by its physiological benefits including its antioxidant and anti-inflammatory properties and the modulation of neurotransmitters such as glutamate and acetylcholine (Syiem *et al*., 2024). It has been shown to lessen the symptoms of anxiety, enhance anti-depressant properties, stabilize mood and reduces the risk of relapse in bipolar disease through lowering oxidative stress and modifying neurotransmitter networks (Khoja and Erbas, 2024; Syiem *et al*., 2024). In a recent study, some investigators revealed that NAC administration was found to ameliorate methotrexate-induced anxiety and depression-like behaviours, increase antioxidant capacity, reduce oxidative stress and inflammatory response, and regulate AChE activity and BDNF release (Aslanlar *et al*., 2024). Other studies found that NAC ameliorated Tetrahydrocannabinol (THC) induced long-lasting anxiety- and depressive-like phenotypes concomitant with differential neuronal and molecular abnormalities in the shell and core sub-regions of the nucleus accumbens of Male Sprague Dawley rats (De Felice *et al*., 2024).

Since seasonal affective disorder is common to winter, there are greater possibility that seasonal changes could influence anxiety symptoms in Nigeria as cold-dry season is also characterized with both longer night, short period of daylight and cold stress. It has also been reported that Nigeria has the highest rate of suicide and depression in Africa, with current rate of suicide placed at 9.50% out of 100, 000 people and it's still on the rise (Oyetunji *et al*., 2022). Most seasonality studies focus on seasonal changes on symptoms of depression. So far, little or no effort was made to ascertain the effect of seasonal variation on anxiety symptoms especially in the tropics. Therefore, this study investigated the influence of seasonal variations on anxiety-like behaviour in albino mice and modulatory role N-Acetyl cysteine in Gombe, North East Nigeria.

MATERIALS AND METHODS

Study area: The study was conducted at the Department of Human Physiology, Faculty of Basic ad Allied Medical Sciences, College of Medical Sciences, Gombe state university, Tudun wada Gombe, Gombe state, P.M.B 127, Located on latitude $10^0,18^{'},9^{''}$ N and longitude $11^0,10^{'}, 28^{''}$E. The climate of Gombe state is characterized by six months of dry season alternating with the six months of rainy season which starts from May to October (Audu et al., 2019). The average annual rainfall of Gombe is 850 mm and receives its highest amount in the month of August with a mean annual temperature of about 32°C and a relative humidity of 95% (Alhaji et al., 2018). From December to February, the hamattan wind tend to prevail making the weather cooler with mean annual temperature of 26^0C and a relative humidity of 10% (Audu et al., 2019).

Chemicals and drugs: N-Acetyl Cysteine of analytical grade was purchased from Sigma Aldrich (A7250) and used for the study.

Apparatus: Cotton swap, methylated spirit, weighing machine, clinical thermometer for taking rectal temperature, dry/wet bulb thermometer which was mounted in the animal house for assessing ambient temperature, stop watch, mounted camera, intragastric tube, syringe, beaker, sample bottles, Elevated Plus Maze and Open Field models.

Drugs preparations and Administration: N-Acetyl Cysteine (500 mg/kg body weight was dissolved and administered orally (positive control), while normal saline was administered to the negative control groups (Lai *et al*., 2023).

Animals and management: A total of twenty (10 male and 10 female Swiss albino mice, 8-12 weeks of age, and average weight of 22 g were obtained from the Animal House Facility of the Department of Human Physiology, Faculty of Basic and Allied Medical Sciences, Gombe State University, Nigeria and used for this study. The animals were divided into four (4) different groups, each having five (5) males and five (5) female mice, and given free access to animals feed and water. All experimental protocols were carried out in accordance with the Gombe State University Research policy, ethics and regulations, governing the care and use of experimental animals (NIH Publication no. 85-23, revised 1996). Ethical clearance approval was gotten from the Faculty of Basic and Allied Medical Sciences Research and Ethical Committee with an approval number;

GSU/COMS/FBMAS/R&EC/22/P011. The experiments were conducted in a quiet laboratory from 9: 00 h to 16: 00 h, under the natural seasonal photoperiod, which correspond to the local sunrise and sunset times obtainable in Gombe as at the period of conducting the experiment (approximately 11.5-12.5 hours of light, with an average close to 12:12 hour lihgt: dark cycle). The ambient temperature and relative humidity were recorded consecutively thrice daily throughout the research, 0600h in the morning, 1200h in the afternoon, and 0600 in the evening throughout the period of the experiment.

Experimental Design: The mice were allowed to acclimatized for two weeks before the commence of the experiment and divided into the following groups;
Group I: 5 male mice received normal saline (10 ml/kg)
Group II: 5 male mice received NAC (500 mg/kg)
Group III: 5 female mice received normal saline (10 ml/kg)
Group IV: 5 Female mice received NAC (500 mg/kg)
The rectal temperature was measured and recorded daily at 0800 hours before administration throughout the period of administration of NAC and normal saline. To minimize impact of stress on the experimental animals, an interval of 30 minutes was given after the rectal temperature measurement before administration. Neurobehavioural evaluation was conducted for a period of one week after the last administration in a neurobehavioural room equipped with a red light, with little or no distraction to make sure nothing external serves as cue to alter the behaviour. The following tests were conducted using standard protocols.

Neurobehavioural Assessments:
Elevated Plus Maze Test (EPM): The EPM was used for the assessments of anxiety-like behaviours as described by Lister (1987). The maze was composed of black wood; comprising two open arms (29 x 5 cm) and two closed arms (29 x 5 15 cm) crossed at an open centre (2.5 x 2.5 cm) in plus shape. The maze was supported 15 cm high above the floor. The behavioural assessment was conducted in a red illuminated room (60 lx). Throughout the experiment, the observer stayed in the same room 1 m away from the maze. At the start of each trial, animal was placed on the central platform facing the open arm (Kraeuter et al., 2019). The mice on each occasion were allowed to move freely round the EPM. A digital camera was mounted and the frequency and duration of 8 behavioural parameters were scored during a 5-min test for each mouse after which it was removed from the maze to its cage. The maze was then cleaned with a solution of 70 % ethyl alcohol and permitted to dry between tests to avoid any olfactory cue.

All video clips were analyzed after which the following behaviours were recorded for each mouse:

1. Open arms entries (OAE): the frequency with which the animal entered the open arms with all four paws
2. Closed arm entries (CAE): the frequency with which the animal entered the open arms with all four paws.
3. Open arm duration (OAD): Length of time the animal spent in the open arms.
4. Closed arm duration (CAD): Length of time the animal spent in the open arms.
5. Head dipping (HD): Frequency with which the animal lowered its head over the side if the open arm towards the floor.
6. Rearing (R): Frequency with which the animal stands on hind limbs or leans against walls of the maze with front paws.
7. Defecation (D): Number of fecal boli produced.

Index of open arm avoidance: The Index of open arm avoidance could be drawn from the Trullas and Skolnick, (1993) formula:

$$\text{IOAA} = \frac{100 - (\%\text{time on open arms} + \%\text{ entries into the open arms})}{2}$$

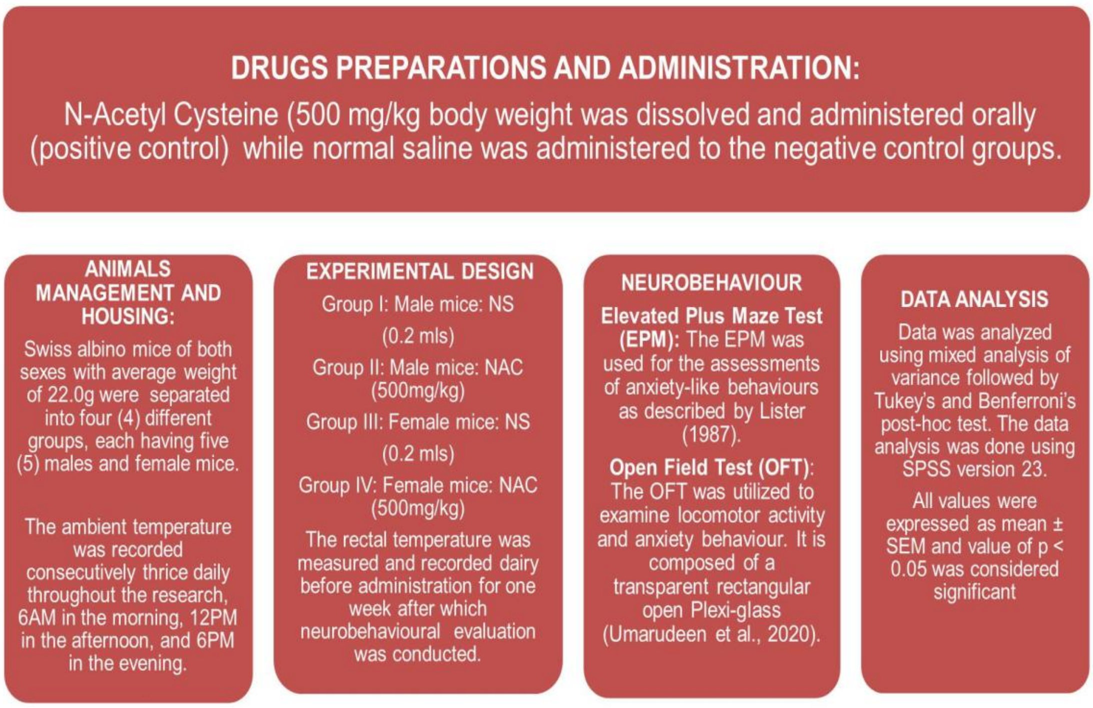

Figure 1
Scheme of preparation and administration of drugs

Open Field Test (OPT): The OFT was utilized to examine locomotor activity as well as anxious behaviour. It is composed of a transparent rectangular open Plexi-glass (Umarudeen *et al*., 2020). Each apparatus is 40 cm in height, 60 x 40 cm at the top and 58 x 38 cm at the base. The floor is divided into 4 rolls of 6 squares each, with the central 4 squares designated as the centre zone while the rest of the squares constitute the peripheral zone. The open-field apparatus was supported on 50-cm high flat-topped stands. Each mouse was gently placed in the center square of the open field and was allowed to explore the arena freely for 5 minutes. Between each animal the maze was thoroughly cleaned with ethanol solution and dried afterward (Scholl *et al.,* 2019). All experiments were carried out by the same experimenter in order to reduce data variability were recorded using phone camera.

All video tapes clips were analyzed after which the following behaviours were recorded for each mouse:

1. Line crossing frequency (LCF): The number of lines crossed by the mice over 5 minutes interval
2. Rearing (R): The frequency of mice standing on their hind limbs
3. Grooming (G): the rapid cleaning movements of the fore paws towards the face and/or the body.
4. Defecation (D): The number of fecal pellets excreted by each individual mouse.
5. Central square duration (CSD): the time spent at the central part of the apparatus (i.e. the four squares in the center of the apparatus).
6. Central square entry (CSE): the frequency of entry into the central square.
7. Stretched attend posture (SAP): the number of times a mouse attends full stretch prior to movement.

Statistical Analysis:

Data was analyzed with SPSS version 22 using mixed analysis of variance (Mixed ANOVA) followed by Tukey's and Bonferroni's post hoc test. All values were expressed as mean ± standard error of mean (SEM). Level of significance for P-values was $P \leq 0.05$.

RESULTS

Metrological data

Thermal Environmental Data during the Cold-Dry and Hot-Season: The results for thermal environmental data during the cold-dry and hot-humid seasons were presented in table 1. A significant statistical decrease ($P < 0.05$) in dry bulb temperature was observed in the 6:00 hours (25.04 ± 0.45 ºC) during the cold dry season when compared to the 18:00 hours (27.89 ± 0.58 ºC).

The relative humidity significantly increased in the 6:00 hours (48.67 ± 1.42 %) of the cold dry season, compared to the 12:00 (43.73 ± 1.77 %) and 18:00 (39.23 ± 1.3 %) hours, respectively.There was significant statistical decrease in Heat Index (HI) in 6:00 (25.02 ± 0.53) hours, compared to 18:00 (27.48 ± 0.49) hours.

Thermal environmental data during the Hot Humid Season: The results for thermal environmental data during the hot humid season were presented in table 1. There was no significant statistical difference in dry-bulb temperature, relative humidity and heat index in the 6:00, 18:00 and 12:00 hours, respectively, in hot humid season.

However, the relative humidity (%) in the 6:00h (86.79 ± 5.35), (48.67 ± 1.42); 12:00h (80.69 ± 4.59), (43.73 ± 1.77), and 18:00h (85.95 ± 1.20) (39.23 ± 1.3) and the heat indices in the 6:00 (28.19 ± 0.69) (25.02 ± 0.53); 12:00 (30.65 ± 1.09), (25.76 ± 0.44); 18:00 (29.26 ± 0.61), (27.48 ± 0.49) significantly increased ($p < 0.05$) in the hot humid season, compared to the cold dry season, respectively (Table 1).

Rectal Temperature Evaluation

Effects of Seasonal Variation on Rectal Temperature of Mice

Test between Group, Sex and Time: There was a significant statistical difference observed [$F(1, 22) = 14.281$; $P=0.001$] in the rectal temperature (°C) of male mice between Normal Saline (36.40 ± 0.33) and NAC (34.63± 0.33) treatment groups on the 1st and 6th days of the study; Normal Saline (36.42± 0.85) and NAC (31.76± 0.85) in cold dry season [$F(1, 22) = 15.054$; $P=0.001$], respectively (Table 2). In Normal saline group, there was no significant change between male and female mice in rectal temperature. However, in NAC treatment group, a significant statistical difference was observed between male (34.63± 0.33) and female (36.43±0.43) mice on the 1st [$F(1, 22) = 11.120$; $P=0.003$] and 6th days [$F(1, 22) = 6.393$; $P=0.019$]; male (31.76± 0.85) and female (35.27±1.10) of the study, respectively.

Table 1:
Thermal environmental data during the hot humid period (Mean ± SEM)

	Cold Dry Season			Hot Humid Season		
Time (h)	**Dry Bulb Temperature (ºC)**	**Relative Humidity (%)**	**Heat Index (HI)**	**Dry Bulb Temperature (ºC)**	**Relative Humidity (%)**	**Heat Index (HI)**
6:00	25.04 ± 0.45*	48.67 ± 1.42*	25.02 ± 0.53*	26.04 ± 1.18	86.79 ± 5.35[a]	28.19 ± 0.69 [a]
	(23-27)	(42.94-56.65)	(22.6-27.2)	(24-28)	(78.47-96.00)	(24.9-33)
12:00	25.85 ± 0.37	43.73 ± 1.77	25.76 ± 0.44	27.63 ± 0.90	80.69 ± 4.59 [a]	30.65 ± 1.09 [a]
	(24-27)	(32.65-51.39)	(23.60-27.20)	(25-36)	(36.66-92.24)	(26-38.1)
18:00	27.89 ± 0.58	39.23 ± 1.3	27.48 ± 0.49	26.54 ± 0.22	85.95 ± 1.20 [a]	29.26 ± 0.61 [a]
	(26-31)	(30.89-45.43)	(25.7-29.8)	(25.50-36.00)	(78.04-92.40)	(26.8-33)
Overall Mean	26.72 ± 0.33	84.55 ± 1.65	26.04 ± 0.33	26.22 ± 0.34	44.04 ± 1.11	29.33 ± 0.49

*Values in parentheses are minimum – maximum. * = mean values with superscript letters indicate significantly ($P < 0.05$) statistical difference between the three-time intervals. [a] = mean values with superscript letters indicate significantly ($P < 0.05$) statistical difference between hot-humid and cold-dry season*

Table 2:
Diurnal Rectal Temperature of Mice rats treated with N-Acetyl Cysteine during the Cold dry and Hot-Humid Season

Season	Group	Sex	RT1 (ºC)	RT2(ºC)	RT3(ºC)	RT4(ºC)	RT5(ºC)	RT6(ºC)	RT7(ºC)
Cold-Dry	Control	Male	36.40 ± 0.33	34.50 ± 0.47	35.56± 0.42	35.52±0.32	36.30± 0.39	36.42± 0.85	35.16± 0.39
		Female	33.40± 0.43	33.80±0.61	35.53±0.54	35.80±0.41	36.47±0.50	35.47±1.10	34.87±0.50
	NAC	Male	34.63± 0.33[a]	35.10±0.47	36.32±0.42	36.06± 0.32	36.50± 0.39	31.76± 0.85[a]	34.46±0.39
		Female	36.43±0.43*	34.77±0.61	35.90± 0.54	35.33± 0.41	37.70± 0.50	35.27±1.10*	35.10±0.50
Hot-Humid	Control	Male	35.63±0.37	36.00±0.53#	34.68±0.47	34.95±0.36	35.55±0.43	35.43±0.45	35.50±0.44
		Female	36.13±0.43	36.80±0.61[#]	35.43±0.54	36.37±0.41*	35.20±0.50	35.10±1.10	35.70±0.50
	NAC	Male	35.55±0.37	35.40± 0.53	34.48±0.47#	35.33±0.36	35.10±0.43[#]	34.90±0.95[#]	34.23±0.44[b]
		Female	35.43±0.43	36.17±0.61	34.73±0.54	35.97±0.41	35.30±0.50[#]	34.80±1.10	34.63± 0.50

[a] = Indicate significant statistical difference between NS and NAC in Cold-dry Season in males mice on the 1st and 6th day ($P < 0.05$) = 0.001
[b] = Indicate significant statistical difference NS and NAC in Hot-humid Season in Male on the 7th day ($P < 0.05$) = 0.05
[*] = Indicate significant statistical difference between Male mice and Female mice in the NS and NAC groups, respectively ($P < 0.05$)
[#] = Indicate significant statistical difference between Cold-dry and Hot-humid Seasons on both Male and Female mice, respectively ($P < 0.05$)
NS= Normal Saline, NAC = N-Acetyl Cysteine, RT = Rectal Temperature

Table 3.
Result showing the effect of seasonal variation and NAC on anxiety behaviour of mice using EPM essay (Group and time comparison)

Group	Parameters	TIME: Cold dry season Mean±SEM	TIME: Hot humid season Mean±SEM
NS	% Close arm entry	86.44±5.13	83.10±5.02
NAC		84.66±5.13	83.20±5.02
NS	% Open arm entry	13.56±5.13	16.90±5.02
NAC		15.34±5.13	16.80±5.02
NS	Close arm duration	224.63±11.43[#]	196.96±17.43
NAC		186.63±11.43	198.79±17.43
NS	Open arm duration	89.62±4.07	87.83±4.30
NAC		88.80±4.07	85.41±4.30
NS	Head dipping	16.83±2.84	16.29±3.49
NAC		24.54±2.84	19.04±3.49
NS	Rearing	18.13±2.87	16.71±2.91
NAC		17.08±2.87	22.21±2.91
NS	Defecation	2.13±0.61	2.86±0.59
NAC		1.36±0.61	1.13±0.59

Result presented as Mean ± SEM; NS: Normal saline; NAC: N-acetylcysteine
 # $P < 0.05$, indicate significant statistical difference between normal saline and N-acetylcysteine and applies to the entire table; NS: Normal saline; NAS: N-acetylcysteine

Table 4
Result showing the effect of seasonal variation and NAC on anxiety-like behaviour of mice using EPM essay

			Cold dry season	Hot humid season
GROUP	**PARAMETERS**	**SEX**	**MEAN±SEM**	**MEAN±SEM**
NS	% Close arm entry	M	74.96±6.72[a]	91.42±6.57
		F	97.92±7.76	74.77±7.59
NAC	% Close arm entry	M	84.21±6.72	87.22±6.57
		F	85.11±7.76	79.18±7.59
NS	% Open arm entry	M	25.04±6.72[a]	8.58±6.57
		F	2.08±7.76	25.23±7.58
NAC	% Open arm entry	M	15.80±6.72	25.23±6.57
		F	14.89±7.76	25.23±7.59
NS	Close arm duration	M	176.25±14.97	208.25±22.82
		F	273.00±17.28	185.67±26.35
NAC	Close arm duration	M	177.25±14.96	228.25±22.82
		F	196.00±17.28	169.33±26.35
NS	Open arm duration	M	80.56±5.33[a]	93.67±5.63
		F	98.68±6.15	82.00±6.50
NAC	Open arm duration	M	87.94±5.33	90.40±5.63
		F	89.67±6.15	80.42±6.50
NS	Head dipping	M	26.00±3.72*[a]	11.25±4.57
		F	7.67±4.29# *	21.33±5.28
NAC	Head dipping	M	20.75±3.72	13.75±4.57
		F	28.33±4.29	24.33±5.28
NS	Rearing	M	21.25±3.76	16.75±3.81
		F	15.00±4.35	16.67±4.40
NAC	Rearing	M	15.50±3.76	20.75±3.81
		F	18.67±4.35	23.67±4.40
NS	Defecation	M	2.25±0.80	1.75±0.77
		F	2.00±0.92	4.00±0.89 #
NAC	Defecation	M	1.75±0.80	1.25±0.77
		F	1.00±0.92	1.00±0.89

Result presented as Mean ± SEM; NS: Normal saline; NAC: N-acetylcysteine
** P < 0.05, indicate significant statistical difference between cold dry season and hot humid season throughout the entire table.*
 # P < 0.05, indicate significant statistical difference between normal saline and N-acetylcysteine and applies to the entire table.
[a] P < 0.05, indicate significant statistical difference between male and female mice throughout the entire table

Table 5:
Result showing the effect of seasonal variation and NAC on anxiety behaviour of mice using EPM essay

		TIME	
		Cold dry season	Hot humid season
Group	**Parameter**	**Mean±SEM**	**Mean±SEM**
NS	IOAA	89.62±4.07	87.83±4.30
NAC		88.80±4.07	85.41±4.30

In the hot-humid season, a significant statistical difference was observed in the rectal temperature (°C) of male mice between Normal Saline (35.50±0.44) and NAC (34.23±0.44) treatment groups [F (1, 22) = 4.261; P=0.051] on the 7th day of the study. Furthermore, in the control group, there was a significant statistical difference was observed in the rectal temperature (°C) between male (34.95±0.36) and female (36.37±0.41) mice [F (1, 22) = 6.772; P=0.016] on the 4th day of the study.

Table 6:
Result showing the effect of seasonal variation and NAC on anxiety behaviour of mice using EPM essay

Groups	Parameters	SEX	Cold dry season	Hot humid season
			Mean±SEM	**Mean±SEM**
NS	IOAA	M	80.56±5.33	93.67±5.63
		F	98.68±6.15[a]	82.00±6.50
NAC	IOAA	M	87.94±5.33	90.40±5.62
		F	89.67±6.15	80.42±6.50

[a] P < 0.05, indicate significant statistical difference between male and female mice

In Normal saline group, there was a significant statistical difference on the 2nd day between cold dry (34.50 ± 0.47) and hot-humid (36.00±0.53) seasons in male mice [F (1, 22) = 4.469; P=0.049]. On the same vein, a significant statistical difference was also observed between cold dry (33.80±0.61) and hot-humid (36.80±0.61) seasons in female mice [F (1, 22) = 12.067; P=0.002], on the 2nd day of the study.

In the NAC treatment group, there was a significant statistical difference observed between cold dry (36.32±0.42) and hot-humid (34.48±0.47) seasons on the 3rd; [F (1, 22) = 8.715; P=0.007], 5th [F (1, 22) = 5.008; P=0.036]; cold-dry (36.50± 0.39), hot-humid (35.10±0.43), and 6th [F (1, 22) = 6.075; P=0.049]; cold-dry (31.76± 0.85), hot-humid (34.90±0.95) days of the studies in male mice. In female mice, there was also a significant statistical difference observed between cold dry (37.70± 0.50) and hot-humid (35.30±0.50) seasons on the 5th day of the study; [F (1, 22) = 11.522; P=0.003.

Neurobehavioural Evaluation

Effect of Seasons on anxiety-like behaviour in mice using Elevated Plus maze

Tests between Group and Time: There was a significant statistical difference ($p < 0.05$) observed in close arm duration (CAD) between normal saline (224.63±11.43) and NAC (186.63±11.43) in cold dry season [F (1, 10)] = 5.52; P= 0.04 (Table 3). However, no significant change was observed ($P < 0.05$) in hot-humid seasons [F (1, 10)] = 0.006; P = 0.92].

Test between Group, Sex and Time

A significant statistical difference in percentage Open Arm Entry (OAE) and Close Arm Entry (CAE) was observed between male (25.04 ± 6.72), (74.96 ± 6.72) and female mice (2.08 ± 7.76), (97.92 ± 7.76) respectively, administered with normal saline in cold dry season [F (1, 10)] = 5.00; p=0.04, demonstrating significant interaction between group, sex and time (Table 4).

Also, a significant statistical difference in CAD was observed between Normal saline (273.00±17.28) and NAC (196.00±17.28) in female mice in cold dry season [F (1, 10)] = 9.92; p=0.01, while no change was observed in male mice ($p > 0.05$). There was also no significant statistical difference ($P > 0.05$) between normal saline and NAC in hot humid season in both male and female mice.

There was also a significant statistical difference in CAD observed between cold dry (273.00±17.28) and hot humid season (185.67±26.35) in female mice administered Normal saline [F (1, 10)] = 6.90; p=0.02],

A significant statistical difference in CAD was also observed between male (176.25±14.97) and female mice (273.00±17.28) administered with normal saline in cold dry season [F (1, 10)] = 17.9; p=0.002], demonstrating significant interaction between group, sex and time.

A significant statistical difference was also observed in OAD between male (80.56±5.33) and female mice (98.68±6.15) administered with normal saline in cold dry season [F (1, 10)] = 4.954; p=0.05], demonstrating significant interaction between group, sex and time. A significant statistical difference in head dip was observed between Normal saline (7.67±4.29) and NAC (28.33±4.29) in female mice in cold dry season [F (1, 10)] = 11.605; p=0.007.

A significant statistical difference in head dip was observed between cold dry and hot humid seasons in both male (26.00±3.72), (11.25±4.57) [F (1, 10)] =7.77; p =0.01, and female (7.67±4.29), (21.33±5.28), [F (1, 10)] = 5.006; p=0.04, mice administered Normal saline, respectively, while no significant change was observed in mice administered with NAC.

A significant statistical difference in head dip was observed between male (26.00±3.72) and female mice (7.67±4.29) administered with normal saline in cold dry season [F (1, 10)] = 10.437; p=0.009, demonstrating significant interaction between group, sex and time.

A significant statistical difference in defecation frequency was observed between Normal saline (4.00±0.89) and NAC (1.00±0.89) in female mice in hot humid season [F (1, 10)] = 5.74; p=0.04], while no change was observed in male mice ($p > 0.05$).

Index of Open Arm Avoidance (IOAA)

Test between Group and Time: There was no a significant statistical difference ($p < 0.05$) observed in IOAA between normal saline and NAC in cold dry season [F (1, 10)] = 0.02; P = 0.89 and hot wet season [F (1, 10)] = 0.159; P=0.69 (Table 5).

Test between Group, Sex and Time: There was no significant statistical difference ($P > 0.05$) in IOAA observed between Normal saline and NAC in both male and female mice in cold dry and hot humid seasons, respectively (Table 6).

There was no significant statistical difference ($P > 0.05$) in IOAA observed between cold dry and hot humid season in both male and female mice administered Normal saline and NAC, respectively.

A significant statistical difference in IOAA was observed between male (80.56±5.33) and female (98.68±6.15) mice administered with normal saline in cold dry season [F (1, 10)] = 4.954; p=0.05, demonstrating significant interaction between group, sex and time

Effects of Seasons on Anxiety-Like Behaviour in Mice Using Open Field Test (OFT)

Test between Group and Time: The results obtained shows that there was a significant statistical difference in grooming between cold dry season (2.33±0.46) and hot-humid season (5.33±0.92) in mice administrated with normal saline [F [(1, 10)] =9.434; P=0.01], while no significant change was observed in mice administered with NAC [F [(1, 10)] =0.657; P=0.43 respectively (Table 7).

There was a significant statistical difference in rearing between cold dry season (42.08±4.54) and hot-humid season (28.80±4.81) in mice administrated with normal saline [F [(1, 10)] =5.120; P=0.04, while no significant change was observed in mice administered with NAC [F [(1, 10)] =3.119; P=0.10], respectively

Test between Group, Sex and Time: A significant statistical difference was observed in grooming between cold dry (2.00±0.61) and hot humid season (6.00±1.21) in male mice administered Normal saline [F (1, 10)] = 9.783; p=0.01 (Table 8),

The frequency of defecation of female mice significantly increased in the Normal saline group (6.67±1.04) when compared to the NAC group (2.33±1.04) in hot humid seasons [F (1, 10)] = 8.711; P = 0.01), respectively. Also, significant statistical increase was observed in defecation frequency in cold dry season (6.33±1.02) when compared to hot humid season (2.33±1.04) in female mice administered NAC [F (1, 10)] = 4.915; p=0.05],

A significant decrease defecation frequency was observed in male mice (3.00±0.90), compared to female mice (6.67±1.04) administered with normal saline in hot humid seasons [F (1, 10)] = 7.128; p= 0.02]. The frequency of rearing significantly increased in cold dry season (59.67±6.87), compared to hot humid season in female mice (35.67±7.28) administered NAC [F (1, 10)] = 0.7.30; p=0.02. A significant decrease in rearing was observed in male (26.75±5.95), compared to female mice (59.67±6.87) administered with NAC in cold dry season [F (1, 10) = 13.126; p= 0.005].

Table 7:
Result showing the effect of seasonal variation and NAC on anxiety behaviour of mice using OFT essay (Group, and time comparison).

		TIME	
		Cold dry season	Hot wet season
Group	Parameters	Mean±SEM	Mean±SEM
NS	Central square duration	15.54±4.09	11.42±2.88
NAC		16.04±4.09	15.08±2.88
NS	Central square entry	6.71±1.12	8.58±1.85
NAC		6.50±1.12	6.00±1.85
NS	Stretch attend posture	1.29±0.65	1.46±0.40
NAC		3.29±0.65	2.21±0.40
NS	Line crossing frequency	92.71±9.86	82.38±8.50
NAC		79.00±9.86	77.00±8.50
NS	Rearing	42.08±4.54*	28.80±4.81
NAC		43.21±4.54	32.83±4.81
NS	Grooming	2.33±0.46*	5.33±0.92
NAC		2.00±0.46	2.79±0.92
NS	Defecation	5.17±0.68	4.83±0.69
NAC		5.17±0.68*	2.92±0.69

Result presented as Mean ± SEM; NS: Normal saline; NAC: N-acetylcysteine
 # P < 0.05, indicate significant statistical difference between NS and NAC group cold dry season.
**P < 0.05, indicate significant statistical difference between cold dry season and hot wet season*

The LCF was significant increased (P < 0.05) in the Normal saline group (101.75±12.91), compared to NAC group (62.00±12.91) in male mice in the cold dry season [F (1, 10)] = 4.742; p=0.05], with no significant change observed in hot humid season and in female mice, respectively.

There was a significant decrease (P < 0.05) observed in SAP in Normal saline group (1.25±0.85), compared to the NAC group (5.25±0.85) in male mice in cold dry season [F (1, 10)] = 11.098; P = 0.01

DISCUSSION

The aim of our study was to assess the effects of seasonal variation on anxiety-like behaviours across different genders of mice as we all to ascertain the modulatory role of N-Acetylcysteine using Elevated Plus Maze (EPM) Test and Open Field Test (OFT).

Table 8.
Result showing the effect of seasonal variation and NAC on anxiety behaviour of mice using OFT essay (Group, sex and time comparison)

			TIME	
			Cold dry season	Hot humid season
Group	Parameters	Sex	Mean±SEM	Mean±SEM
NS	Central square duration	M	7.75±1.46	10.50±2.41
		F	5.67±1.69	6.67±2.79
NAC	Central square duration	M	5.00±1.46	5.00±2.42
		F	8.00±1.69	7.00±2.79
NS	Central square entry	M	15.75±5.36	15.50±3.77
		F	15.33±6.19	7.33±4.36
NAC	Central square entry	M	21.75±5.36	12.50±3.77
		F	10.33±6.19	17.67±4.36
NS	Stretch attend posture	M	1.25±0.85	1.25±0.52
		F	1.33±0.98	1.67±0.60
NAC	Stretch attend posture	M	5.25±0.85	2.75±0.52
		F	1.33±0.98	1.67±0.60
NS	Line crossing frequency	M	101.75±12.91 #	74.75±11.13
		F	83.67±14.90	90.00±12.85
NAC	Line crossing frequency	M	62.00±12.91	75.00±11.13
		F	96.00±14.90	79.00±12.85
NS	Rearing	M	43.50±5.95	28.25±6.30
		F	40.67±6.87	29.33±7.28
NAC	Rearing	M	26.75±5.95 a	30.00±6.30
		F	59.67±6.87 *	35.67±7.28
NS	Grooming	M	2.00±0.61 *	6.00±1.21
		F	2.667±0.70	4.67±1.39
NAC	Grooming	M	2.00±0.61	3.25±1.21
		F	2.00±0.70	2.33±1.39
NS	Defecation	M	6.00±0.89	3.00±0.90 a
		F	4.33±1.02	6.67±1.04 #
NAC	Defecation	M	4.00±0.86	3.50±0.90
		F	6.33±1.02*	2.33±1.04

The results of meteorological indices from our study demonstrated an increase in relative humidity and heat index in the early morning hours (6:00h) compared to the later hours of the day (18:00h) in cold dry season (Table 1). Furthermore, the significant increase observed in relative humidity and heat index in the hot-humid season at all-time intervals during the period of the study demonstrated an increase in heat stress in the hot-humid season compared to the cold dry season.

The evaluation of rectal temperature in laboratory animals remains an essential index in determination of heat stress induced by changes in environmental and physiochemical conditions (Lee *et al*., 2015). Heat stress induces many pathophysiological changes, impacting significantly on brain functions (Lee *et al*., 2015). Studies have demonstrated that exposure to heat stress has adverse effects on brain function, leading to anxiety-like behaviour, motor and memory impairment, by suppressing mRNA expression of tryptophan hydroxylase 2 (*tph2*) and increasing serum hippocampal MDA, apoptotic proteins, HSP-70 levels, oxidised glutathione (GSSG), reactive oxidative species (ROS), protein oxidation level and decrease reduced glutathione (GSH) levels in the hippocampal tissue, consequently leading to perturbation in BDNF/ERK1/2/CREB axis (Lee *et al*., 2015; Chauhan *et al*., 2021; Otsuka *et al*., 2022). Although the effects of heat stress have long been studied, the influence of heat stress associated with seasonal changes on anxiety like behaviours and the modulatory role of NAC has not been established in the tropics.

The results from our study demonstrated significant decreases in rectal temperature of male mice treated with NAC in both the cold-dry and hot-humid seasons, respectively, demonstrating the modulatory role of NAC on heat stress across the two seasons (Table 2). This agrees with previous findings by Santos *et al*. (2017), who reported anxiolytic properties of NAC in stress induced hyperthermia in mice. N-Acetyl cysteine has been demonstrated to ameliorate heat stress in different experimental models through modulation of different pathways including oxidative stress, CREB and neuroinflammation (Zhao *et al*., 2021; Pertiwi *et al*., 2024). However, our study demonstrated the season dependent influence of NAC on anxiety-like behaviour bringing new insight on the modulatory role of NAC on anxiety induced by thermal stressors.

The significant increase in rectal temperature in the female mice observed across the two seasons and in the treatment groups demonstrated the sex-dependent changes in rectal temperature. The rise in rectal temperature in the female mice maybe associated with hormonal changes. This however, requires further investigation to unravel the biochemical basis for the rectal temperature raise in female mice relative to the male as observed in our study.

In the normal saline group, the significant rise in rectal temperature of both male and female mice in the hot humid season demonstrated heat-stress induced rise in the rectal temperature, which was significantly reduced by NAC administration demonstrating the ameliorative potential of NAC on heat stress. This agrees with previous reports on the role of NAC in modulating heat stress-induced anxiety in mice (Santos *et al*., 2017).

Our findings shows that the measures of general anxiety-like behaviours on the basis of open arms avoidance using EPM do not differs across the two seasons. However, the significant increase in close arm duration observed in the normal saline group compared to NAC in the cold dry season (Table 3), demonstrated enhanced exploratory behaviour induced by NAC in the study. NAC is an established antioxidant that mitigate oxidative stress by replenishing glutathione (Raji *et al*., 2025). Beyond this, it produces glutathione-independent effects through the modulation of mitochondrial redox systems, ferroptosis, and the Nrf2-antioxidant response element signalling pathway (Cherneva *et al*., 2025). It also plays a significant role in neuroinflammatory processes by inhibiting the production of cytokines, the expression of iNOS, and the activation of microglia (Iciek *et al*., 2025). It was reported to elicit anxiolytic and antidepressant-like effect by modulating periterminal release of glutamate (Chakraborty *et al*., 2020). These could be the probable mechanisms via which NAC elicits its anxiolytic potential as observed in our studies.

Furthermore, within the normal saline treated group in cold dry season (Table 4), the significant statistical difference in % open arm entry and % Close arm entry was observed between male and female mice with the results showing increase in open arm avoidance in female than in male mice, which is an indication of increased anxiety level in female mice. This also correlated with the increased in rectal temperature observed in female mice in both the cold-dry and hot-humid seasons compared to male mice further demonstrating heat-stress induced anxiety in female mice.

Therefore, it could be inferred that there is more female susceptibility to anxiety in cold dry season. This is in agreement with previous investigation that female gender are more vulnerable to climate variability and predisposition to frustration and mental health challenges including depression and anxiety in cold climate (Olanrewaju *et al*., 2022).

A rodent study on anxiety has also evaluated the effects of NAC on mice models commonly used to characterize anxiolytic compounds and found that NAC has anxiolytic properties similar to diazepam (Santos *et al*., 2017). Evidence from literatures has suggested that sex hormones, particularly oestradiol and progesterone, play a significant part in the generation of sex differences on neurobehaviours. The same study further proposed that elevated level oestradiol and progesterone influence sex differences in the development of anxiety disorders (Li *et al*., 2017). Thus, this may be the reason why the female mice are more susceptible to anxiety, as observed from our study.

Earlier literature reported that the assessment of anxiety-like behaviours in rodents is described by using the ratio of time spent in the open arms to the time spent in the closed arms, and that an increase in open arm activity (duration and/or entries) reflects anxiolytic behaviour (Brolese *et al.,* 2017). Thus, this current study indicated an increase in anxiety behaviour displayed by female mice treated with normal saline during the cold dry season compared to male mice, but no difference between male and female mice administered NAC. Similarly, the significant increase in head dip in NAC treatment group, compared to the Normal Saline group in female mice in cold dry season depicts an enhanced exploratory activity of NAC in female mice, thus, further proven the anxiolytic property of NAC as explained previously.

In the same vein, the significant difference in head dip between cold dry and hot humid seasons in male mice administered Normal saline demonstrated an increase in exploratory activity by male mice in the cold dry season (Table 4). This was further supported by the significant decrease in rectal temperature in the cold-dry season compared to the hot-humid season especially in male mice. To further confirm the above argument, studies have indicated that Plasma cortisol concentration increased after heat stress on either short term or long-term exposure which could lead to anxiety-like behaviours in males (Otsuka *et al*., 2022).

Conversely, there was an increase in exploratory behaviours by female mice in hot humid season than in cold dry season which suggests an increase in anxiety-like behaviours in cold dry season among female mice, compared to hot humid season. Previous studies have reported more prevalence of seasonal behavioural disorders especially seasonal affective disorders among females and concluded that such could be related to photoperiodic dysregulation of neurotransmitters like norepinephrine and dopamine in relation to unstable female hormonal cycles (Munir *et al*., 2022)

The significant increase in head dipping between male and female mice administered Normal saline in cold dry season with male mice displaying more exploratory behaviours than female mice further buttressed an increased level of anxiety in female mice compared to male mice in cold dry season.

The significant increase in defecation frequency in Normal saline, compared to NAC treated group in female mice in the cold dry season further supports the anxiolytic role of NAC in the current study. Studies have shown that stress and anxiety increase hormones, such as cortisol, adrenaline and serotonin and that serotonin in particular has been link to increased peristaltic movement in the gut leading to spasms in the entire colon which consequently produces unexpected bowel movements (Shine *et al.,* 2022).

The study also observed a significant statistical difference in Index of Open Arm Avoidance (IOAA) between male and female mice administered with normal saline in cold dry season (Table 6). An increase was recorded among female mice compared to male mice. Increase in IOAA has been associated with increase level of anxiety (Madeira *et al*., 2021). This is thus in concert with earlier discussion that anxiety is more prevalent among female than male due to hormonal factors (Munir *et al*., 2022).

The current study also evaluated thigmotaxis. Previous literatures reported that thigmotaxis is a measure of the percentage of the test time that was spent by the animal in movement adjacent to the outer wall of the maze and that its increase is considered as an index of anxiety-like behaviour (Seibenhener *et al*., 2015). Thigmotaxis can be determined from a decrease record of Central Square Entry/Duration. Thus, from the results of the analysis, it was found that there was a general decrease in the frequency of central square entry (CSE) and central square duration (CSD) across the two seasons and between the two groups, but no it was not statistically significant.

Some studies also indicated that increased rearing is in concordance with increased anxiety levels in mice (Seibenhener *et al*., 2015; Blackburme *et al.,* 2021). The results revealed an increase in rearing frequency in cold dry season compared to hot humid season (Table 7), which occurred in female mice (Table 8), thus, consolidating the argument that female mice tend to be more anxious in cold dry season.

The current study also indicated a significant increase in grooming frequency in hot-humid season compared to cold-dry season in normal saline treated group, which occurred in male mice. This suggests an increase level of anxiety in male mice in hot humid season which is agreement with previous research findings (Bouguiyoud *et al.*, 2022).

The significant increase observed in line crossing frequency (LCF) between NS and NAC group in cold dry among male mice in the Normal saline demonstrated an improved exploratory and locomotive activity and decreased anxiety level among rodents. An increase in defecation frequency has been linked to anxiety level among rodents. The current study indicated a significant decrease in defecation frequency in female mice treated with NAC in the hot-humid season when compared to the cold-dry season, further suggesting the anxiolytic effect of NAC in the current study (Table 8). This further agrees with the findings of the EPM test above. In the control group, male mice showed less defecation frequency compared to the female in the hot-humid season, which also agrees with the EPM results.

In conclusion, the study demonstrates the effects of seasonal variations on anxiety, and also the roles of the NAC. Our results demonstrated that the hot-humid season is thermally stressful to the experimental animals. There is an increase in anxiety-like behaviours in hot humid season among male mice, which could be due to increase in heat stress as a result of increased heat index, relative humidity and rectal temperature of mice observed from our study. NAC treatment significantly ameliorated the raise in rectal temperature. However, there is an increase locomotive activity in male mice in cold dry season demonstrating increase exploratory activity and decrease in level of anxiety. Female mice are more susceptibility to anxiety-like behaviours in cold dry season, which was ameliorated by NAC treatment.

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