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Curcuma longa supplement increases anxiety-like behavior and blood glucose level in Swiss albino mice

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Abstract: *Curcuma longa* (*C. longa*), also known as curcumin, is a lipophilic polyphenol substance proven to have cholesterol-lowering, anti-diabetic, anti-inflammatory, anti-oxidant, and anti-cancer properties in both *in vitro* and *in vivo* models. Most previous studies investigated the effect of *C. longa* on diabetic mice and therefore, there is a need to investigate the effect of *C. longa* on normoglycemic mice. Depression is a common consequence of anxiety that affects 21% of the world's population. Since the prevalence of diabetes and depression is on the rise globally, it is important to search for safer and cost-effective management for these disorders. In doing so, it is therefore essential to investigate its effect in normoglycemic mice. The current study determines the effect of *C. longa* on blood glucose level and anxiety-like behavior in normoglycemic Swiss albino mice. A total of 20 mice were divided into four groups of five (n=5 per group). Group I (control) received distilled water 10 ml/kg, groups II, III, and IV received *C. longa* at 5%, 10%, and 20%, respectively, for 14 days. We found that 20% *C. longa* group showed a significant ($p<0.05$) increase in fasting blood glucose level (195.84±14.46 mg/dl) after 14 days of administration compared with the control group (134.60±4.52 mg/dl). We also found that 20% *C. longa* increased the anxiety-like behavior in normoglycemic Swiss albino mice compared with the control group. However, there was no significant ($p>0.05$) difference in both fasting blood glucose level and anxiety-like behavior between the mice treated with 5% and 10% *C. longa* and the control group. This study indicates that *C. longa* at high concentration is unsafe for consumption by normoglycemic Swiss albino mice.

Keywords: *Curcuma longa*; anxiety-like behavior; blood glucose; depression

1.0 INTRODUCTION

Diabetes mellitus (DM) affected more than 415 million people in 2015, and this figure is projected to double by 2040. A reported prevalence of 0.8% to 11% involving rural and urban dwellers, with about 2% reported in Zaria, a semi-urban community in Northern Nigeria (Dahiru et al., 2008). The management of diabetes places a great burden on both individuals and the government. In 2015, the total global expenditure on diabetes was estimated to be between USD673 billion to USD1,197 billion, and this value is projected to rise to about USD802 to USD1,452 billion (Federation., 2019). In Nigeria, about USD500 million to USD5 billion is spent annually on diabetes (Federation., 2019).

Anxiety is an unpleasant state of inner turmoil, which causes nervous behavior such as fear, apprehension, and worries. It can lead to feelings of dread over something unlikely to happen, such as a feeling of imminent death (Alzahrani et al., 2017). Moreover, anxiety disorder is an emotion that is characterized by

feelings of worrying thoughts, tension, and physical changes such as increased blood pressure (Alzahrani et al., 2017). Since anxiety disorder is common among patients with diabetes, with approximately 40% of these patients exhibiting it symptoms and a reported prevalence of about 14% (Grigsby et al., 2002), a strong relationship between diabetes mellitus, major depression, and anxiety disorder was established (Deschênes et al., 2015). Furthermore, major depression and anxiety disorders among patients with diabetes have been associated with poor glycaemic control, greater disability, and poor clinical outcome (Deschênes et al., 2015; Whitworth et al., 2016).

There are several forms of anxiety, including generalized anxiety disorder (GAD), social anxiety disorder, panic disorder, and specific phobias (Dew et al., 2004). GAD is an extravagant tension and worries about daily calamities and problems on most days. This disorder can last for at least 24 weeks, and the patient experiences difficulty in performing day-to-day tasks (Kessler & Wittchen, 2002). Additionally, some GAD symptoms include autonomic hyperactivity, increased motor tension, and increased vigilance and scanning with lacking panic attacks (Singewald et al., 2015).

Depression comes because of anxiety and affects approximately 21% of the world populace (Organization, 2017). Curcumin, a major constituent of *Curcuma longa,* demonstrated anti-inflammatory, anti-cancer, and anti-oxidant (Deogade & Ghate, 2015), anti-hyperlipidemic (Pari & Murugan, 2007), and anti-anxiety activities (Lee & Lee, 2018) in both *in vitro* and *in vivo* experimental settings. Therefore, the need to search for safer and cost-effective management for these disorders cannot be overemphasized. However, there is a need to investigate its safety in normoglycemic mice. While many studies on the effect of *C. longa* (turmeric) on diabetic mice, there is a paucity of data on its effect on normoglycemic mice. Also, diabetes and anxiety-like behavior have been linked but poorly investigated. Hence, this study determined the effect *C. longa* on blood glucose level and anxiety-like behavior in normoglycemic Swiss albino mice.

2.0 MATERIALS AND METHODS

2.1 Animals and supplementation of curcumin

A total of twenty Swiss albino mice weighing 20-30 g were housed in plastic cages under standard laboratory conditions with free access to food and water for two weeks to acclimatize in the laboratory environment before starting the experiments. Ethical clearance was obtained from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC/051) and in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All drugs and reagents were obtained commercially and are of analytical grade. *C. longa* was obtained from the College of Agriculture, Bauchi State. Mr. Modibbo Sale of the Forestry Department identified the plant and gave it a voucher number of 1466. The rhizomes of the turmeric were first washed and sliced into pieces, and dried. The dried rhizomes were then ground to a fine powder. A digital glucometer (an Accu-Check Advantage, Roche Diagnostic, Germany) was used to determine the blood glucose level of the animals. The animals were divided into four groups of five mice each and fed with an appropriate combination of thoroughly mixed vital feed and *C. longa* for 14 days as described in Table 1.

Table 1: Composition of animal feed for each group

Groups	Treatments
Group I	Distilled water and 100% vital feed (9.7% moisture, 2 % ash, 9% crude fiber, 10% fat, 20 % crude protein and 49.3% carbohydrate),
Group II	*C. longa* 5% and 95% of vital feed (9.2% moisture, 1.9 % ash, 8.6% crude fiber, 9.5% fat, 19 % crude protein and 46.8% carbohydrate),
Group III	*C. longa* 10% and 90% of vital feed (8.7% moisture, 1.8% ash, 8.1% crude fiber, 9% fat, 18 % crude protein and 44.4% carbohydrate)
Group IV	*C. longa* 20% and 80% of vital feed (7.8% moisture, 1.6% ash, 7.2% crude fiber, 8% fat, 16 % crude protein and 39.4% carbohydrate)

2.2 Estimation of blood glucose level

We obtained the blood sample from sequential snipping off the tail in accordance with Fluttert et al. (2000). Animals fasted for approximately 12 h (overnight) before the determination of fasting blood glucose level (Sun et al., 2016). A digital glucometer was used to measure the blood glucose level (Beach & Turner, 1958), and results were recorded in mg/dL.

2.3 Elevated plus maze (EPM) for anxiety assessment

An elevated plus-maze (EPM) test was conducted as previously described (Komada et al., 2008). The elevated plus-maze consists of two open arms (28×5 cm) and two closed arms (30×5 cm) with a 15 cm high wall. The arms and central square were made of wooden plates elevated 51 cm above the floor. Notably, the arms of the same type are located opposite from each other. Each mouse was placed in the central square of the maze (5×5 cm), facing one of the closed arms. Then, the behavior of the mouse, including the time spent in the open and closed arm, were recorded and analyzed during a 5 min test period. The mice were subjected to EPM test 3 times, and the average measurement was used.

2.4 Statistical analysis

Data obtained were expressed as mean±standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc tests to compare the level of significance between control and experimental groups was used to analyze the data using SPSS software version 22. Statistical tests with $p<0.05$ were considered significant.

3.0 RESULTS

To determine whether *C. longa* supplementation affected fasting blood glucose level, we checked the blood glucose level weekly and observed a statistically significant means difference [$F(3)=5.284$, $p<0.001$] (Table 2) in the fasting blood glucose level between controls and the treatment groups. Moreover, result from Tukey's multiple comparison post hoc tests showed a statistically significant increase ($p<0.05$) in the fasting blood glucose level in the 20% *C. longa* supplemented group compared to the control, 5% and 10% supplemented groups, respectively (Table 2). Hence, *C. longa* at 5% and 10% did not affect fasting blood glucose level on Swiss albino mice ($p>0.05$).

Table 2: Effect of *C. longa* Supplement on Fasting Blood Glucose Level of Normoglycemic Swiss albino mice

Groups	Day 0 (mg/dl)	Day 7 (mg/dl)	Day 14 (mg/dl)
Control	87.64 ± 5.70	111.04 ± 2.69	114.60 ± 4.52
C. longa 5%	97.20 ± 10.84	126.00 ± 8.50	130.60 ± 3.78
C. longa 10%	97.80 ± 15.76	133.80 ± 15.76	115.08 ± 9.57
C. longa 20%	98.28 ± 7.46	130.68 ± 9.48	195.84 ± 14.46*

Note: Values with asterisks (*) superscript are statistically **significant F (3) = 5.284 (**$p < 0.001$)

To determine whether *C. longa* supplementation affected anxiety-like behavior, we used the elevated plus maze test and recorded the time spent by each mouse exploring both open and closed arms. In the time spent in the open arm, no statistically significant ($p>0.05$) difference between the mean of the control group (223.40±6.38 s) and the means of the treated groups (220.20±16.17 s, 200.40±2.38 s, 200.00±4.09 s for Groups II, III and IV, respectively) (Figure 1). However, a statistically significant increase [$F(3)=8.381$, $p<0.001$] (Figure 2) in the time spent in closed arms between control and treatment groups. Moreover, the result from Tukey's multiple comparison post hoc analysis revealed a significant increase ($p<0.05$) in the time spent in the closed arms in the 20% *C. longa* supplemented group compared to the control, 5% and 10% supplemented groups, respectively (Figure 2). Contrarily, *C. longa* at 5% and 10% have no effect on the time spent in the closed arm.

4.0 DISCUSSION

Diabetes and depression are highly prevalent conditions with a significant impact on health outcomes (Egede & Ellis, 2010). Recent studies have suggested a strong relationship between diabetes and depression. While some of these studies suggested bidirectional relationship (Knol et al., 2006), this relationship needs to be backed with a research-based evidence aimed at understanding the mechanism of increased anxiety and depression resulting from diabetes and vice versa. From the findings of this study, it was observed that high concentration *C. longa* treated groups (Group IV; 195.84±14.46 mg/dL)

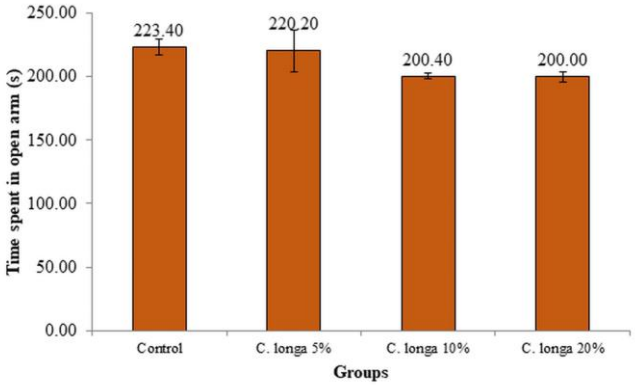

Figure 1. Effect of *C. longa* Supplement on Anxiety-like Behavior in Normoglycemic Swiss albino mice (Open Arm).

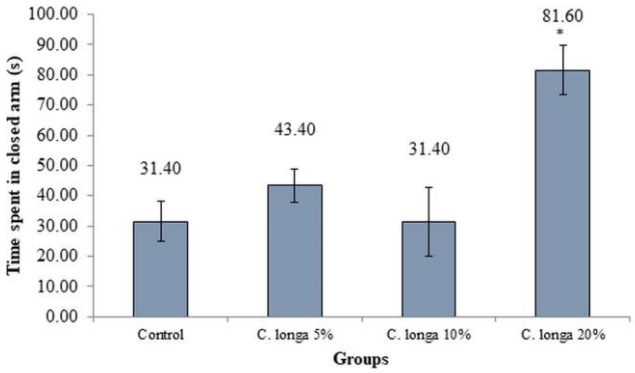

Figure 2. Effect of *C. longa* Supplement on Anxiety-like Behavior in Normoglycemic Swiss albino mice (Closed Arm). Values having asterisks (*) are statistically significant (F (3) = 8.381, (p < 0.001)

showed a significant (p<0.05) increase in fasting blood glucose levels after 14 days of administration compared with the control group (group I; 134.60±4.52 mg/dL) (Table 1). The physiological bases for this increase remain unclear, as previous studies using *C. longa* on diabetic mice showed that *C. longa* is anti-hyperglycemic (Den Hartogh et al., 2020; Hodaei et al., 2019). The result of this study shows that *C. longa* at high concentration could be harmful to normal mice.

The elevated plus-maze test is an open field test that assesses anxiety-like behaviors in experimental animals (Lee & Lee, 2018). We observed no difference in the time spent in the open arm (Figure 1). The increased proportion of the time spent in the open arm indicates a reduction or decrease in anxiety (Carobrez & Bertoglio, 2005). However, in the time spent in the closed arm, there was a statistically significant difference in the group that received *C. longa* 20% (p<0.05) compared with the control and *C. longa* 5% and 10% groups (Figure 2). This result indicated that the *C. longa* at this percentage might not be healthy for the experimental animals.

An increase in the proportion of time spent in closed arms indicates an increase in anxiety (Carobrez & Bertoglio, 2005). Therefore, our study further justifies this assertion, as shown in Table 2, where *C. longa* supplementation at 20% significantly (p<0.05) increases the blood glucose level. Hence, the rise in the blood glucose level might have affected the experimental animals, which stimulate the amygdaloid nucleus in the limbic system or by direct stimulation of the fear center in the periventricular nucleus of the hypothalamus leading to appropriate autonomic response such as sweating, pupil dilatation and side seeking escape (Carobrez & Bertoglio, 2005; Holt et al., 2014). Ultimately, a low dose of C. longa may be associated with improved anxiety and blood glucose levels (Comin et al., 2010).

The findings of this study might have been affected by the duration of the study, the doses used, or the paradigm used for assessing anxiety-like behavior. Previous studies showed that EPM is inconsistent in anxiety-like behavior assessment, hence raising issues concerning its validity (Carobrez & Bertoglio, 2005). EPM had been reported to produce mixed results concerning agents such as valium (benzodiazepine) (Carobrez & Bertoglio, 2005). Therefore, it is recommended that this same work using other models for the screening of anxiety-like behavior and depression should be employed to test further the effect of this substance on experimental animals. Passive avoidance tests and other parameters such as the number of arm entries need to be taken and analyzed. Also, the mechanism through which *C. longa* at 20% increased fasting blood glucose level is recommended for further investigation.

5.0 CONCLUSIONS

From the experimental observation, it has been shown that *C. longa* supplement at higher concentration is not suitable for normoglycemic Swiss albino mice with the report of an increase in anxiety-like behavior. It also suggested the need for further studies on the mechanism underlying the increased anxiety-like behavior in *C. longa* supplemented animals at high concentration.

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Conflicts of Interest: The authors declare no conflict of interest.

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