The Ameliorating Effect of lactate-rich *akamu* in Reversing Depressive Symptoms in Rats

**Okoli, F.A.** 1\***, Anazodo, C.A**1**. and Chukwura, E.I.** 1

1Applied Microbiology and Brewing Department, Nnamdi Azikiwe University, PMB 5025, Awka, Nigeria

Corresponding Author: kc.agu@unizik.edu.ng

**ABSTRACT**

Depression is a debilitating mental health condition affecting millions worldwide. Traditional treatments include a range of antidepressant medications and therapies, yet not all patients respond adequately. This study was designed to evaluate the effect of lactic acid-rich *akamu* on depression and its antidepressant like quality in rodents. Eighteen rats were used in the study, 6 were fed *akamu* a local food produced from the fermentation of *Zea mays*, rich in lactate, 6 were administered a standard antidepressant (escitalopram) while the other 6 served as control. The chronic mild stress protocol was used to induce depression. After 3weeks, there was a 71.43% reduction (on the average) in sucrose consumption which was indicative of depression. On the 56th day of the study, 3 weeks after treatment commenced, there was a reversal of depressive symptoms and a rapid increase in the weight of rats that were fed *akamu* while there was a 14% decrease in the rats that were administered the standard drug. Although this reduction was statistically non-significant (p>0.05). Furthermore, after feeding the rats with *akamu,* there was a 62.5% average increase in sucrose consumption, indicative of recovery from depression. The level of lactate in the stool of rats before the feeding trial was between 66-80mg/kg. After the feeding trial, it ranged between 153-216mg/kg with the *akamu* group having the highest value.

**Keywords:** depression, *akamu*, lactate, rats

**INTRODUCTION**

##### **Background on Depression**

Depression is a widespread mental health disorder characterized by persistent sadness, lack of interest in activities, and a variety of physical and cognitive symptoms. It significantly impacts quality of life and can lead to severe outcomes, including suicide. Current treatments, including antidepressants and psychotherapy, do not work for all patients, prompting the need for alternative or complementary therapies (World Health Organization, 2024).

**Akamu and Lactate**

Nigeria has many local foods that can be fermented, and these foods can be used to create small businesses in rural areas. These businesses use simple equipment to turn crops grown locally into more valuable products, like "akamu" a fermented corn porridge (Latunde-Dada, 2020). *Akamu,* also known as *Ogi*, is a traditional fermented maize porridge commonly consumed in West Africa. One of the major cereals consumed by Africans and Nigeria in particular is Maize (Okoli *et al.,* 2023). The three main tribes in Nigeria refer to fermented maize gruel as *akamu* (Igbo), *ogi* (Yoruba) and *koko* (Hausa). *Akamu* is a common fermented semisolid food with a variety of colors depending on the maize used to prepare it. The colour could be white, cream or yellow. It is usually taken as a traditional breakfast with other foods in Nigeria. In addition, it is used as baby food or weaning food. (Akinsola *et al*., 2021). The fermentation process of *Akamu* results in the production of lactate, a compound with known neuroprotective and anti-inflammatory properties. Lactate has been shown to influence brain function positively and may play a role in mood regulation (Magistretti and Allaman, 2018).

However, recent research has uncovered its significant role as a signaling molecule in the brain. Lactate can cross the blood-brain barrier and influence neuronal function and plasticity. This has profound implications for understanding and potentially treating depression.

Beyond the popular akamu, Nigeria boasts a treasure trove of traditional foods championed by researchers for their health benefits. These include ogili (Okpalla *et al.,* 2012), kunu (Mbachu *et* *al*., 2014), burukutu (Anaukwu *et al.,* 2015), and cocoyam flour (Agu *et al*., 2023). But the bounty extends even further. Nigerian cuisine offers a range of health-promoting options like wine (Okeke *et al*., 2015), yogurt (Agu *et al*., 2015; Awah *et al.,* 2016), and refreshing vegetable and mixed fruit salads (Agu *et al*., 2014; Anaukwu *et al.,* 2015).

Depression in rats is commonly induced through chronic stress or pharmacological methods, which lead to behavioral changes analogous to human depressive symptoms. Key indicators include reduced interest in pleasurable activities, increased immobility in the forced swim test, and changes in social interaction (WHO, 2024). These models provide a reliable means to study the efficacy of potential antidepressants, like *akamu*. This study investigated the potential of lactate-rich *Akamu,* a traditional fermented maize porridge, in alleviating depressive symptoms in rats

**Lactate's Antidepressant Mechanisms**

Several studies have examined how lactate administration affects depressive behaviors in rats. One significant study by Carrard *et al.* (2018) demonstrated that lactate administration led to an increase in the levels of brain-derived neurotrophic factor (BDNF) in the hippocampus of rats, a region crucial for mood regulation and cognitive function. BDNF is known for its role in neurogenesis and synaptic plasticity, and its upregulation is associated with the alleviation of depressive symptoms. Furthermore, research by Tang *et al.* (2014) showed that lactate could modulate the activity of NMDA receptors, which are critical for synaptic plasticity and memory function. Dysregulation of NMDA receptor activity has been linked to depression, and lactate's modulatory effects suggest a mechanism by which it can restore normal synaptic function and improve mood.

**MATERIALS AND METHODS**

Samplesof *Akamu* were purchased from Ake Awka market, Awka Anambra State. They were further placed on a tray and dried in an oven for 2 hours. The dried maize cakes were further crushed using a mixer blender (Silver Crest, China).

**Feed formulation protocol**

A standard traditional plate of *akamu* weighs 200g.

Thus, 200g = 2.86g/kg

 70kg

With 70kg being the average weight of man according to Ashar 2018. The foods are regularly consumed and thus can form one tenth of a daily meal. One tenth of 1000g of food consumed by an adult. An adult consumes an average of 1000g of food (FAO, 2018).

Thus, 2.86g of food/kg body weight. In a cage containing 6 animals weighing 100g on the average

each, the value of food that will be consumed: 600g ×2.86 = 1.72g of food / day.

 1000

**Proportional mixing (W/W)**

As earlier stated that the food made up one tenth of their meal, i.e 1/10 of 1000g =200g. Thus, 100g of food sample per 1000g of feed; 1g of food sample/10g of feed. Each rat consumes about 50g of feed daily, thus 6 rats were fed 30g of food sample/300g of feed daily (On the average).

**Procedure for Chronic Mild Stress (CMS) for inducing depression in rats**

The procedures involving animals were reviewed and approved by the Animal research ethics committee of Nnamdi Azikiwe University Awka, Nigeria. Witsar rats were procured from the animal house of the Biochemistry Department of Nnamdi Azikiwe University. Eighteen rats were weighed and randomized into three different groups in accordance to the different treatments for each group. The rats were allowed to acclimatize for two weeks. After acclimatization, they were subjected to daily unpredictable mild stressors to induce depression using the Pap and Willner (2023) Protocol for inducing depression. The stressors include; wetting their bedding, food and water deprivation, tilting of cage at 45˚, strobe lighting and sudden withdrawal of all stressors. This protocol has been used for decades to induce depression in rats. The chronic mild stress (CMS) procedure is widely used and most extensively validated (Willner, 2017). Every week, the level of sucrose consumption of the rats was measured because a decrease in the consumption of sucrose is indicative of a successful rat model of depression. It is important to note that sucrose consumption in rodents can return to normal levels with the administration of antidepressant drugs (Willner 2016, 2017). The depression in the rats can be reversed by administering antidepressants such as selective serotonin reuptake inhibitors (SSRIs) and monoamine oxidase (MAO) inhibitors (Pap and Willner, 2023). However, in order to test the effectiveness of lactate-rich *akamu* in reversing depressive symptoms or ameliorating depression, the food was administered to the rats after depression was induced.

A total of 18 rats were used in this research and they were divided into 3 groups. The first group were administered *akamu*, the second group was administered the standard drug (escitalopram) while the third group served as the control.

An ethical approval for the use of animals was obtained and all procedures for animal handling followed the ethical standards of the Nnamdi Azikiwe University Animal Research Ethics Committee.

**Lactate Levels**

The lactate level in the stool of rodents was analyzed using Gas Chromatography- Flame Ionization Detector (GC-FID) before and after administration for any changes. It was carried out following the manufacturer’s instruction. The Gas chromatography equipped with flame ionization detector (FID); M910 equipped with a flame ionization detector was purchased from Buckscientific, USA was used. The samples were well homogenized with the help of a spatula and an aliquot (100 mg) were weighted in a vial and acidified with 0.25 mL of sulfuric acid 50% w/v. The solution was shaken by means of a vortex device for 3 min. The acidic solution was then added with 50 μL of internal standard solution (IS1) (iC6, 19.8 × 103 μM in ethyl ether in order to achieve a final concentration in the sample of 330 μM), extracted with 1 mL of ethyl ether and centrifuged for 5 min at 2800 × g. The organic phase was collected into another vial. The extraction was repeated thrice, collecting 3 mL of organic phase. At the end, 0.5 μL of the collected organic phase were injected into the GC for analysis.

The analysis was accomplished using a gas chromatograph Buckscientific M910 GC (USA) equipped with a splitless injector and FID. The capillary chromatographic column used was a nitroterephthalic acid modified polyethyleneglycol (PEG) column (DBFFAP, 25 m, 0.25 mm i.d., 0.25 μm film thickness, purchased from Buckscientific, USA). The GC injector was maintained at 280 °C. The injection was performed in splitless mode . The oven temperature was initially set at 40 °C for 3 min, programmed at a rate of 20 °C/min to 160 °C and then at 40 °C/min to 245 °C that was held for 1.87 min, resulting in a total run time of 13 min. The carrier gas was hydrogen at a flow rate of 3.70 mL/ min. The FID temperature was maintained at 250 °C. The level of lactate detected in the stool samples was confirmed by comparison of their retention times with those of authentic standards. (Scortichini *et al*., 2020).

**RESULTS**

The table 1 below shows the average level of lactate in stool samples of rats before the administration of a standard anti-depressant and lactate-rich akamu; to test for anti- depressant like quality. The lactate level of the *akamu* group was 65.23mg/kg before the administration and increased significantly to 216mg/kg after 3 weeks of administering *akamu*. The Standard drug group (escitalopram) was 80mg/kg before administration and increased to 153mg/kg after administration while the control group slightly increased from 67.22mg/kg to 83.52mg/kg.

****

**Figure 1: The Mean lactate levels in the faeces of rats before and after administrations**

**Table 1: Average sucrose consumption (ml/g/hour) during the experimental period**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Grp/****Day** | **0** | **7** | **14** | **21** | **28** | **35** | **42** | **49** | **56** |
| **Akamu group** | 0.12069 | 0.091575 | 0.117211 | 0.103712 | 0.139623 | 0.11655 | 0.147945 | 0.189125 | 0.148649 |
| **Stand. drug group** | 0.110706 | 0.095847 | 0.102761 | 0.036232 | 0.131124 | 0.022472 | 0.235294 | 0.180723 | 0.168539 |
|  |  |  |  |  |  |  |  |  |  |

**Average sucrose consumption (ml/g/hour) during the experimental period**

From table 1 above, on the 35th day of the experiment, there was a 71.43% reduction (on the average) in sucrose consumption when compared with the initial. In rodents, a significant decrease in the sucrose consumption as seen in table 1 is usually an indication of a successful model of depression. Furthermore, it indicated the success of the chronic mild stress (CMS) protocol and the onset of depression. Although this reduction was statistically non-significant (p>0.05). After feeding with the experimental diet, there was a 62.5% average increase in sucrose consumption on the 56th day. This suggested a steady recovery in the groups with the *Akamu* group having the highest rate of increase.

**Table 2: Average weight (grams) of rat in various groups**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Days/** **Groups**  | **0**  | **7**  | **14**  | **21**  | **28**  | **35**  | **42**  | **49**  | **56**  |
|  |  |  |  |  |  |  |  |  |  |
| *Akamu*  | 72.83±3.26  | 68.25±4.77  | 84.25±5.19  | 114.50±7.46  | 112.75±7.14  | 107.25±7.14  | 91.25±6.04  | 105.75±4.48  | 111.00±4.82  |
|  |  |  |  |  |  |  |  |  |  |
| Standard drug (Escitalopram)  | 104.50±8.21  | 79.33±6.20  | 86.00±7.22  | 103.50±12.03  | 86.75±7.02  | 89.00±19.00  | 76.50±16.50  | 83.00±17.00  | 89.00±0.00  |
| Control  | 90.83±3.27  | 106.17±4.64  | 116.17±5.46  | 103.67±4.95  | 107.33±5.55  | 96.50±4.81  | 98.50±4.54  | 100.33±4.22  | 105.67±4  |

**Change in weight of rats during the experimental period**

Table 2 describe the percentage change in weight of the rats during the entire test; from day 1 to day 56. The 3 groups of rats showed varying weight changes throughout the duration of the experiment.

**DISCUSSION**

on the 35th day of the experiment, there was a 71.43% reduction (on the average) in sucrose consumption when compared with the initial values. This indicated the success of the chronic mild stress protocol and the onset of depression, though this reduction was statistically non- significant (p>0.05). The reduction in the sucrose consumption obtained in this study corroborates the findings of Papp *et al* (2016); Papp and Willner (2023).

However, after feeding with the experimental diet, there was a 62.5% average increase in sucrose consumption on the 56th day. This suggested a steady recovery in the groups with the akamu group having the highest rate of recovery. The results are in line with the report of Papp *at al.* (2016) and Papp and Willner (2023). Also, the *akamu* group had the highest reversal rate i.e they recovered from depression faster than the standard anti-depressant group. This result is in line with that of Yang *et al.* (2014) who conducted a study where lactate was given to rats and led to a reduction in depressive symptoms. In addition, Papp and Willner (2023) reported that the administration of antidepressant drugs reverses the decreased sucrose intake and other behavioral changes in the rats. Due to the anti-depressant effects of lactate-rich *akamu* which was administered to the depressed rats, within 3 weeks, there was a reversal of the depressive symptoms the standard drug group as well as in the a*kamu* group but the *akamu* group recorded a faster reversal when compared to the standard drug group. This was in line with the findings of Scavuzzo *et al*. (2020) who administered lactate to rats and observed a remarkable improvement in mood and reversal of anhedonia

From the results, there was a decrease in weight in the treatment groups during the chronic mild stress induction. This could entail a slight correlation between weight loss and borderline depression. After the 35th day (i.e. commencement of treatment), there was a rapid increase in weight in all the treatment groups except in the standard drug group where there was a 14% decrease in weight. This could be one of the side effects of the standard drug serving as a pointer to its possible detrimental effects, thus the need for consumption of the lactate-rich akamu that has no detrimental/side effects. The results support the findings of Safer and Zito, 2019 who reported that there were severe side effects of standard antidepressants.

**CONCLUSION**

The antidepressant effects of lactate observed in rat models open up exciting possibilities for human treatment. If similar effects can be confirmed in humans, lactate or its analogs could become a novel class of antidepressants, offering benefits especially for those who do not respond to current treatments. Lactate's role in reversing depressive symptoms in rats highlights its potential as a novel antidepressant. Through mechanisms involving BDNF upregulation and NMDA receptor modulation, lactate exerts significant behavioral and neurochemical effects that alleviate depression-like symptoms in animal models. While promising, further research is essential to determine its efficacy and safety in humans. The future of depression treatment could well include lactate as a key component, providing hope for more effective and comprehensive management of this pervasive condition

 **REFERENCES**

Agu, K.C., Archibong, E. J., Anekwe, D.C, Ago, C.A., Okafor, A.C. and Awah, N.S. (2014). Assessment of Bacteria Present in Yoghurt Sold on Awka Metropolis. *Scholars Journal of Applied Medical Sciences*, **2** (6D): 3071-3075.

Agu, K.C.,Ikwuka, O.I., Umeoduagu, N.D., Victor-Aduloju A.T., Uwanta L.I., Ikenwa, B.O., Nwiyi, I.U., Chidubem-Nwachinemere, N.O., Nwosu, J.C. and Okafor, I.J. (2023). Fermentation and Production of Cocoyam (*Colocasia esculenta*) Flour Fortified with Soybean Powder. Cognizance Journal of Multidisciplinary Studies, 3 (6): 449-470. **DOI: 10.47760/cognizance.2023.v03i06.031**

Akinsola, O.T, Alamu, E.O, Otegbayo, B.O, Menkir, A. and Maziya-Dixon, B. (2021). Nutritional properties of ogi powder and sensory perception of ogi porridge made from synthetic provitamin: A maize genotype. *Frontiers in Nutrition*. 8:685004.

Anaukwu, C.G., Nwangwu, F.C., Okafor, I.O., Ezemba, C.C. Orji, C.C., Agu, K.C. and Archibong, J.E. (2015). Microbiological Analysis of Burukutu beverage produced in southern part of Nigeria. *European Journal of Experimental Biology*, 5(8):18-22

Anaukwu, C.G., Ugwuoke, G.O., Ekwealor, I.A., Okafor, O.C. and Agu, K.C. (2015). Preliminary Study of Bacterial Isolates from Indigenous Ready – To – Eat Salad Vegetables. *American Journal of Life Science Researches*, (3) 4: 282-286

Ashar, B.H. (2018) In Search of the 70-kg Man, *Medical Clinics of North America*,102(1) :15-16,

Awah, N.S., Agu, K.C., Muokwe, J., Irondi, C., Okeke, C.B., Anaukwu, C.G., Archibong, E.J., Iloanusi, C.A., Ngenegbo, U. and Umeoduagu, N. (2016). Microbial Assessment of Yoghurts Sold in Amawbia, Nigeria. *Universal Journal of Microbiology Research*, **4** (2): 55-58.

Carrard, A., Elsayed, M., Margineanu, M., Boury-Jamot, B., Fragnière, L., Meylan, E.M, Petit, J.M., Fiumelli, H., Magistretti, P.J. and Martin, J.L. (2018) Peripheral administration of lactate produces antidepressant-like effects. Molecular Psychiatry. 23(2):392-399.

Latunde-Dada, (2020). Fermented foods and cottage industries in Nigeria. *Journal Food Science,* 20:1-33

Magistretti, P. and Allaman, I. (2018) Lactate in the brain: from metabolic end-product to signalling molecule. *Nature Reviews Neuroscience;* 19, 235–249.

Mbachu A.E., Etok , C.A., Agu, K.C., Okafor, O.I., Awah, N.S., Chidi-Onuorah, L.C., Ekwueme, V.C., Okpala, J., Ogbue, M.O. and Ikele, M.O. (2014). Microbial Quality of Kunu Drink sold in Calabar, Cross River State, Nigeria. *Journal of Global* *Biosciences*, **3** (2): 511-515.

Okeke, B.C., Agu, K.C., Uba, P.O., Awah, N.S., Anaukwu, C.G., Archibong, E.J., Uwanta, L.I., Ezeneche, J.N Ezenwa, C.U. and Orji, M.U. (2015). Wine Production from Mixed Fruits (Pineapple and Watermelon) Using High Alcohol Tolerant Yeast Isolated from Palm Wine. *Universal Journal of Microbiology Research*, **3** (4): 41-45.

Okoli, F.A., Okonkwo, N.N., Agu, K.C., Nwobu, W.C., Uwanta, L.I., Ifediegwu, M.C., Umeoduagu, N.D. (2023). Identification of Potential Microbial Contaminants from Stored Pap. *International Journal of Progressive Research in Engineering Management and Science,*3 (9): 409-415

Okpalla J., Ubajekwe, C.C., Agu, K.C. and Iheukwumere, I. (2012). Biochemical Changes of Melon Seeds (*Citrillus* *vulgaris*) Fermented by pure cultures of *Bacillus* *licheniformis. International Journal of Agriculture and Bioscience.***1** (1): 42- 45.3

Papp, M. and Willner, P. (2023). Models of affective illness: Chronic mild stress in the rat. *Current Protocols*; 3: 1-13.

Papp, M., Gruca, P., Lason-Tyburkiewicz. M. and Willner, P. (2016) Antidepressant, anxiolytic and procognitive effects of rivastigmine and donepezil in the chronic mild stress model in rats.

*Psychopharmacology*. 233:1235–1243

Safer, D.J, Zito, J.M. (2019) Short- and Long-Term Antidepressant Clinical Trials for Major Depressive Disorder in Youth: Findings and Concerns. *Frontiers in Psychiatry*. 10:705

Scavuzzo, C. J., Jakowec, M. W., Petzinger, G. M., & Thomas, D. M. (2020). Systemic administration of lactate produces antidepressant-like effects in male and female mice. *Behavioural Brain Research,* 393; 112794.

Scortichini, S., Chiar- Boarelli, M., Silvi, S. and Fiorini, D. (2020). Development and Validation of a GC-FID Method for the Analysis of Short Chain Fatty Acids in Rat and Human Faeces and in Fermentation Fluids. *Journal of Chromatography B, Biomedical applications*: 121972.

Tang, J., Jiang, W., Liao, J., Wang, W., and Cao, Y. (2014). Lactate modulates NMDA receptor activity and improves cognitive functions in chronic cerebral hypoperfusion rats. *Neuroscience,* 256, 196-207.

World Health Organization. (2024). Depressive disorder (Depression). https://www.who.int/news-room/fact-sheets/detail/depression. Assessed 4 January 2024.

Yang, J., Ruchti, E., Petit, J. M., Jourdain, P., Grenningloh, G., Allaman, I., and Magistretti, P. J. (2014). Lactate promotes plasticity gene expression by potentiating NMDA signaling in neurons. *Proceedings of the National Academy of Sciences,* 111(33): 12228-12233.