ACTA BIOSCIENTIA
Journal of Biomedical and Biological Sciences

Acta Bioscienctia **2025**:1(3);140-152 **OPEN ACCESS**
https://doi.org/10.71181/actabioscientia12290

Feed-added Inulin mitigates ketamine-induced behaviours and hippocampal neurotoxicity via modulation of gut Lactobacilli count and brain oxidative stress

Lawal Ismail Adetayo[1], Onaolapo Olakunle James[2], Onaolapo Adejoke Yetunde[3]

1. Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Al-hikmah University, Ilorin, Nigeria, ORCID: 0000-0002-0584
2. Behavioural Neuroscience and Neuropharmacology Unit, Department of Pharmacology and Therapeutics, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria, orcid.org/0000-0003-2142-6046
3. Behavioural Neuroscience and Neurobiology Unit, Department of Anatomy, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria, orcid.org/0000-0001-7126-7050

Correspondence:
Lawal Ismail Adetayo**.**
Department of Anatomy,
Al-hikmah University ,
Ilorin, Kwara State, Nigeria.
Email: lawalismail@alhikmah.edu.ng. **Tel:** 09033563919

ABSTRACT

Schizophrenia is a complex psychiatric disorder characterised by positive and negative symptoms, with current pharmacotherapies showing limited efficacy in managing negative symptoms. Hence, the problem of treatment resistance and high relapse rates. Ketamine, an N-methyl-D-aspartate (NMDA) receptor antagonist, is frequently used to induce schizophrenia-like symptoms in rodents. Recent research has highlighted the gut-brain axis as a novel target for mitigating schizophrenia disorder, with prebiotics such as inulin demonstrating potential in modulating neurobehavior through antioxidant properties and neurotransmitter regulation. Eighty (80) male mice were randomly assigned into eight groups (n=10). Group A received normal saline (control), Groups B - D were administered inulin in feed at 2g, 4g, and 8g/kg, Group E received ketamine alone (50 mg/kg, intraperitoneally), and Groups F, G and H received ketamine followed by inulin supplemented feed. Ketamine and saline were administered intraperitoneally for 5 days, while inulin was fed to them for 30 days. Faecal samples were collected weekly for *Lactobacillus* quantification. On day 36, behavioural tests (social interaction and Y-maze) were conducted. On day 37, animals were sacrificed for histological (haematoxylin and eosin and cresyl fast violet staining, biochemical analyses including lipid peroxidation, total antioxidant capacity and nitric oxide levels. Results revealed that ketamine-treated mice exhibited social withdrawal, oxidative stress and evidence of hippocampal neurotoxicity. The administration of Inulin however reversed Ketamine induced behavioural and hippocampal alterations. In conclusion this study has demonstrated the benefits of feed-added inulin in mitigating ketamine induced schizophrenia-like behaviours and hippocampal neurotoxicity in mice, however more studies are required to ascertain its beneficials effects in humans.

KEYWORDS: Schizophrenia, Ketamine Model, Inulin, Gut-Brain Axis, Oxidative Stress

1. Introduction

The gut microbiota, which consists of microorganisms which are extensively dispersed in the gastrointestinal tract (GIT) of animals and humans, is crucial for preserving homeostasis, or the balance of the host's metabolic and physiologic processes [1, 2]. Each individual's gut microbiome is quite copious and distinct [3]. About a quarter of the entire microbiome community is made up of bacteria of *Firmicutes* and *Bacteroides* phyla [1, 4]. In the last decade or more there has been increasing evidence of the beneficial effects of the gut microbiome [5]. There is also ample evidence to suggest that

microbiome-based therapies including the use of synbiotics, probiotics and prebiotics could significantly alter current management protocols for neurodegeneration [6].

Prebiotics are fermented dietary compounds which have been shown to promote the activity and growth of beneficial strains of gut flora in biological hosts, contributing to improved health and wellbeing [7]. Research has shown that fructo-oligosaccharides [FOS] and galacto-oligosaccharides are the two most important groups of prebiotics that have health benefits in humans [8]. Fructo-oligosaccharide comprise a broad class of oligosaccharides that include short chain FOS, Levan oligosaccharides and inulin. Inulin is a naturally occurring prebiotic present in various plants including onions, chicory root and garlic [9, 10]. Reports from recent studies have revealed that prebiotics have beneficial effect on brain in health or disease. Galactooligosaccharides were reported to mitigate postoperative cognitive dysfunction and neuroinflammation through the targeting of the gut-brain axis [11]. Bimuno galactooligosaccharide also alters the gut microbiota and has benefits on cognitive function in psychosis [12]. Inulin has also been shown to exert neuroprotective effects including the mitigation of post-stroke depressive like behaviour [9] and attenuation of blood-brain barrier permeability and behavioural dysfunction [10]. While there is ample evidence of its benefits in attenuating brain neurodegeneration and depressive disorders, there is a dearth of information on its benefits in the management of schizophrenia.

Schizophrenia is a chronic and debilitating neurodevelopmental disorder characterised by deficits in cognition, mood, perception of reality and interpersonal relationships. [13-15]. Research has demonstrated that the development of schizophrenia is influenced by genes and the environment [16, 17]. The rising schizophrenia burden worldwide has increased the need for research into the development of more efficient and less toxic therapies for the management of schizophrenia [18]. Also, the search for alternative therapies has become increasingly important, as current pharmacological treatments, namely first- and second-generation antipsychotics, often fail to provide adequate symptom relief [19]. These medications have been reported to primarily target positive symptoms including hallucinations and delusions showing limited efficacy in addressing negative symptoms such as withdrawal, anhedonia and cognitive deficits [19]. Moreover, a significant proportion of persons on schizophrenia therapy develop treatment resistance, with approximately 20–30% of these individuals experiencing little to no improvement despite adherence to prescribed antipsychotic regimens. These limitations underscore the urgent need for novel therapeutic strategies, including non-pharmacological and integrative approaches, to improve outcomes and quality of life for those affected [19, 20].

In the last decade or more, increasing awareness of the possible impact of gut microbiota-brain interactions in the development and progression of schizophrenia [21, 22] has led to suggestions that prebiotics like inulin could be beneficial in the management of schizophrenia. Therefore, this study examined the effect of feed-added inulin on behavioural and morphological changes in the hippocampus in a ketamine model of schizophrenia in mice.

2. Materials and Methodology

2.1 Animals

A total of 80 male mice weighing between 25-32 g were procured from Umarahmarkeen Nigeria Global Ventures, Ilorin. They were housed in fenestrated plastic cages and allowed to acclimatise for two (2) weeks. The mice were given access to clean water at all times and were fed normal rodent chow purchased from Ogo-Oluwa feeds, Sango, Ilorin.

2.2 Experimental Methods

Eighty (80) male Swiss albino mice, were used in this study. Animals were randomly assigned to 8 groups of 10 mice each. Group A was the control (CTR), while groups B, C, and D received inulin-(IN) in feed at 2g, 4g, and 8g per kg of feed respectively. Mice in group E received intraperitoneal injection (i.p) of ketamine (KET) alone at 50 mg/kg, while three intervention groups (F, G and H) received i.p KET. Intraperitoneal injection of saline was administered to mice in group A-D while mice in groups E-H received i.p KET for the first five days. Standard diet was fed to amice in groups A and E, while those in the inulin groups were fed Inulin incorporated into standard diet daily for 30 days. Body weight and faecal samples were taken weekly. At the end of the treatment period mice were exposed to the social interaction and Y-maze paradigms. Twenty-four hours after the last behavioural test, animals were sacrificed and the hippocampus was removed and either homogenised for biochemical assays [oxidative stress markers (glutathione [GSH] & nitric oxide [NO]) and brain neurotransmitter (dopamine and serotonin) levels] or processed for histological study using Cresyl violet and haematoxylin and Eosin stains

2.3 Body weight

Body weight of the animals was recorded weekly using a sensitive precision weighing balance as previously described [23].

.

2.4 Lactobacillus evaluation

The plate count technique for determining the concentration of lactobacillus in mice faecal material was used for this research. Briefly, 180mg of stool samples were diluted in 1 ml phosphate-buffered saline solution (7.2 pH) to produce homogenous faecal solutions, which was spread over the Man Rogosa Sharpe (MRS) agar for lactobacillus and incubated for 48 hours at 37^0C. Observable lactobacillus colonies were counted and the average number of lactobacillus colonies was multiplied by the inverse of the dilution factor and presented as colony-forming units (CFU) per millilitre [24, 25].

2.5 Neurobehavioral paradigms

2.5.1 Social interaction (SI) test

Estimating the amount of time spent interacting socially or withdrawing from a "stranger" mouse is an important aspect of the social interaction (SI) test. The 3-chamber sociability box is used to evaluate social interaction and novelty preference in a two-phase test [26T. The three-chamber sociability chamber sociability test apparatus consists of a centre section and two adjacent chambers [27]. To assess sociability, a subject mouse was placed in the centre of a three-chambered sociability apparatus and allowed to explore freely for three minutes. During this session, the number of contacts and the duration of time spent interacting with a conspecific mouse in one chamber, compared to an empty chamber was recorded. In a subsequent session designed to evaluate social memory and preference for social novelty, a second unfamiliar (novel) mouse was introduced into the previously empty chamber. The subject mouse's interactions with both the familiar and novel mice were then observed for an additional three minutes. The primary parameter measured was the frequency of interactions between the subject mouse and the unfamiliar conspecifics [26-28].

2.5.2 Y-maze test

The Y-maze test is a spontaneous behavioural test that relies on the working memory or natural curiosity of the animals. Instead of going back to an arm they have already investigated, the animals frequently choose to investigate a new one [29 30]. The Y-maze paradigm is Y shaped wooden maze consisting of three identical arms arranged at 120° angles from each other [29, 30]. The experimental animal was placed at the centre of the maze and allowed to explore all three arms freely. An alternation was defined as the animal entering a different arm from the one it had just exited, which was considered a correct response. In contrast, re-entering the previously visited arm was regarded as an error. Both the total number of arm entries and the sequence of entries were recorded to calculate the percentage of spontaneous alternation.

2.5.3 Beam Balance test

The beam apparatus consisted of a one-meter-long wooden beam with a flat surface 12 mm wide, supported by two poles and elevated 50 cm above the tabletop [31]. A black plywood box, positioned at one end of the beam and containing nesting material from the home cage, served as the goal area to motivate the mouse to complete the task. Beginning on day 15 of inulin administration, each mouse was trained to traverse the 12 mm-wide beam three times per day. After the treatment period concluded, each mouse performed a single test trial. During this trial, the number of hindlimb slips (hind slides) and the time taken to traverse the 80 cm central section of the beam were recorded. Video recording, using a mobile device-mounted camera, began when the mouse's nose entered the central 80 cm portion of the beam and ended when it reached the goal box [32].

2.6 Tissue Homogenisation

Sections of the hippocampus were weighed and homogenized in phosphate buffered saline (PBS) solution, centrifuged and the supernatant was

2.7 Biochemical tests

Tissue levels of glutathione, protein, dopamine, and nitric oxide were assayed using commercially available ELISA Assay (Biovision Incorporated Milpitas, California U.S.A) kits and by following the manufacturer's instructions for respective tests.

2.8 Tissue Histology

Rat brains were dissected, sectioned and fixed in neutral-buffered formolsaline. The hippocampus was then processed for paraffin-embedding, cut at 5 µm and stained with haematoxylin and eosin and cresyl fast violet for general histological study.

2.9 Photomicrography

Histological slides of the cerebral cortex were examined under an Olympus binocular light microscope. Images were captured using a Canon PowerShot 2500 Digital camera.

2.8 Statistical Analysis

Statistical analysis was done using the Chris Rorden's ezANOVA (V0.985) application and Microsoft Excel for the production of charts. Darta analysis was done using One or Two factor Analysis of Variance (ANOVA) followed by Tukey's post-hoc test. Results were expressed as mean ± SEM and a p-value of $p < 0.05$ was considered statistically significant

3. Results

3.1 Effects of inulin on the body weight and feed intake

Figures 1 and 2 show the effect of inulin on weekly changes in mean body weight (figure 1) and weekly changes in mean feed intake (figure 2) respectively. Two factor ANOVA of body weight data showed a significant effect of treatment [$F(7, 432) = 134$, $p < 0.001$] and duration of administration (6 weeks) [$F(5, 432) = 28.80$, $p < 0.001$] and significant interaction between treatment and duration of administration [$F(35, 432) = 13.70$, $p < 0.001$]. Pairwise comparisons revealed a significant decrease in body weight in weeks 2, 3, 4, 5 and 6 in the groups administered inulin at 4 and 8 g/kg of feed, ketamine (KET), and KET with Inulin (IN) at 2, 4 and 8 g/kg of feed compared to control. Compared to KET, body weight increased significantly in weeks 3, 4, 5 and 6 with KET/IN at 2, 4 and 8 g/kg of feed

Two factor ANOVA of feed intake data showed a significant effect of treatment [$F(7, 432) = 112$, $p < 0.001$] and duration of administration (6 weeks) [$F(5, 432) = 13.45$, $p < 0.001$] and significant interaction between treatment and duration of administration [$F(35, 432) = 9.25$, $p < 0.001$]. Pairwise comparisons revealed a significant decrease in feed intake in weeks 4, 5 and 6 with IN at 8g/kg, KET and KET/IN at 2, 4 and 8 g/kg of feed compared to control. Compared to KET, feed intake increased significantly in weeks 4, 5 and 6 with KET/IN at 2, 4 and 8 g/kg of feed respectively.

3.2 Effects of inulin on faecal lactobacillus count

Figure 3 shows the effect of inulin on lactobacillus count in mice exposed to ketamine. Lactobacillus count increased significantly ($p<0.05$) with IN at 2g and 8g, and with KET/IN at 2g, 4g and 8g groups while it reduced ($p<0.05$) with IN at 4g and in the KET-only groups compared to control. Compared with KET, lactobacillus count increased ($p<0.05$) in KET/IN at 2g, 4g, and 8g groups.

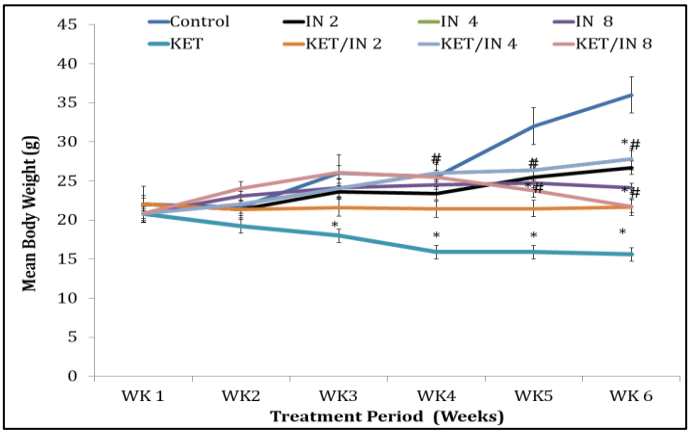

Figure 1: The effect of inulin on body weight measured as mean weekly body weight (g) in ketamine-treated mice. Lines represent Mean ± SEM, *p<0.05 compared to the control group, #p<0.05 compared to the KET group. IN – Inulin, KET - Ketamine.

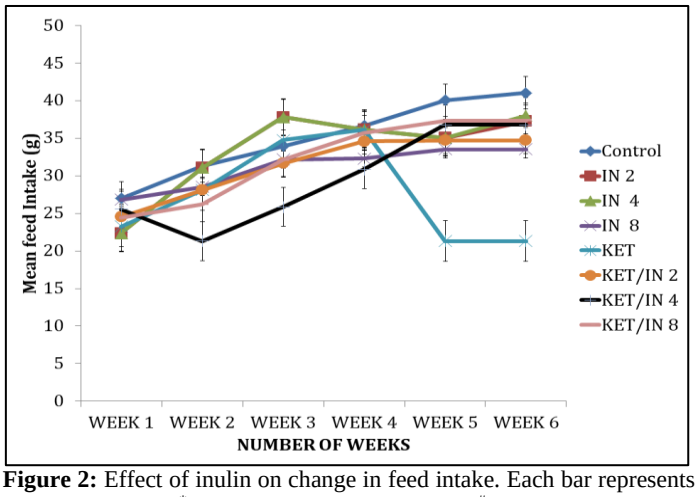

Figure 2: Effect of inulin on change in feed intake. Each bar represents Mean ± S.E.M, *p<0.05 vs. normal control, #p<0.05 vs. ketamine control, number of mice/group =10, IN: Inulin: KET: Ketamine.

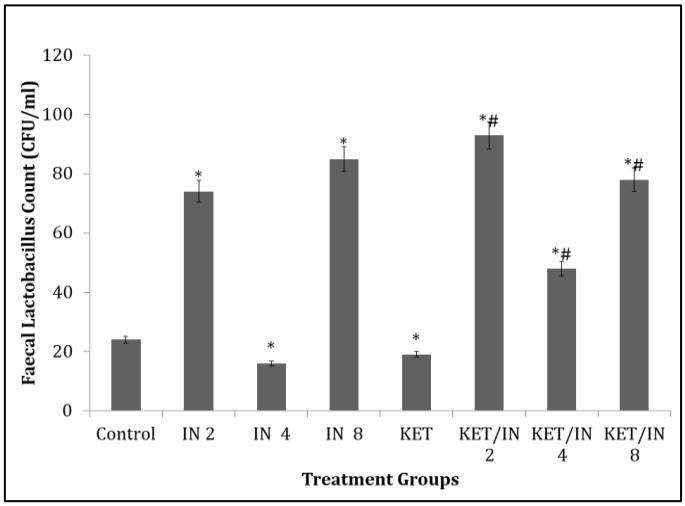

Figure 3: The effect of inulin on faecal lactobacillus. Each bar represents Mean ± SEM, *p<0.05 vs. normal control, #p<0.05 vs.

ketamine control, number of mice/group =10, IN: Inulin: KET: Ketamine.

3.3 Effect of Inulin on spatial working memory

Figure 4 shows the effect of inulin on spatial working memory changes in the Y-maze measured as % alternation/5 minutes. Pairwise comparisons of spatial working memory data revealed a significant [F (5, 72) =8.63, p < 0.001] increase in % alternation with IN at 2, 4 and 8, and KET/IN at 2, 4 and 8 g/kg of feed and a decrease with KET compared to control. Compared to KET, % alternation increased significantly with KET/IN at 2, 4 and 8 g/kg of feed.

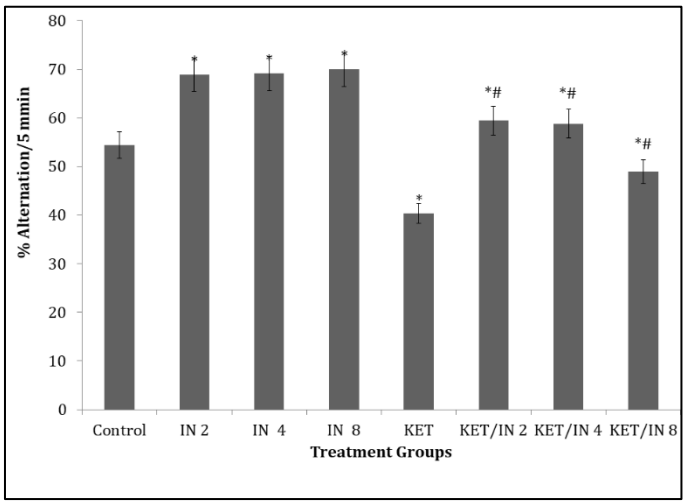

Figure 6: Effect of inulin on spatial working memory in the Y-maze. Each bar represents Mean ± SEM, *p<0.05 compared to the control group, #p<0.05 compared to the KET group. Number of mice/groups =10. IN – Inulin, KET – Ketamine

3.4 Effect of Inulin on anxiety-related behaviours

Figure 5 shows the effect of inulin on time spent in the open and closed arms of the elevated plus maze. Pairwise comparisons of data from time spent in the open arm revealed a significant [F (5, 72) =16.99, p < 0.001] increase in time spent in the open arm with IN at 2 and 4, and a decrease with KET compared to control. Compared to KET, open arm time decreased significantly with KET/IN at 2, 4 and 8 g/kg of feed. Pairwise comparisons of data from time spent in the closed arm revealed no significant difference in time spent in the closed arm in any of the groups compared to control or KET.

3.5 Effect of Inulin on Marble burying behaviours

Figure 6 shows the effect of inulin on marble burying behaviour. Pairwise comparisons of data from number of marbles buried revealed a significant [F (5, 72) =7.00, p < 0.001] increase in number of marbles buried with IN at 4 and 8 g/kg, KET and KET/IN at 2, 4 and 8 g/kg of feed compared to control. Compared to KET, number of marbles buried decreased significantly with KET/IN at 2, 4 and 8 g/kg of feed respectively.

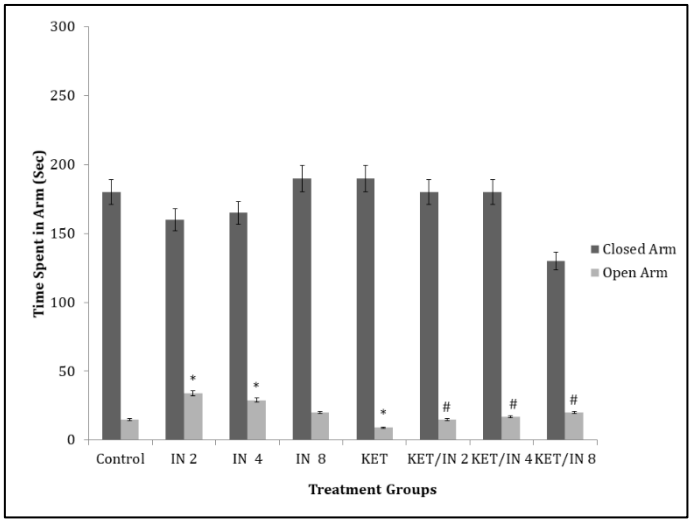

Figure 5: Effect of inulin on time spent in the arms of the elevated plus mase /5 minutes. Each bar represents Mean ± S.E.M, *p<0.05 vs. normal control, #p<0.05 vs. ketamine control, number of mice/group =10, IN: Inulin: KET: Ketamine.

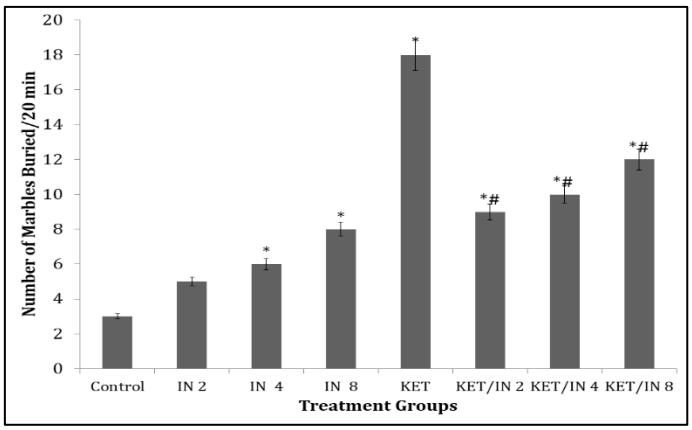

Figure 6: Effect of inulin on marble burying behaviour measured as % alternation/5 minutes. Each bar represents Mean ± S.E.M, *p<0.05 vs. normal control, #p<0.05 vs. ketamine control, number of mice/group =10, IN: Inulin: KET: Ketamine.

3.6 Effect of Inulin on social interaction phase I

Figure 7 shows the effect of inulin on social interaction behaviour measured as time spent in the stranger or empty compartment. Two factor ANOVA of social interaction data revealed no significant effect of Treatment [F (7,144) = 1.86 p<0.081], but a significant effect of chamber (Empty vs Stranger) [F (1,144) = 29.2 p<0.001] and significant interaction between treatment and chamber [F (7,144) = 8.39 p<0.001]. Pairwise comparisons revealed no difference in time spent in the empty compartment in any group compared to vehicle control or ketamine control. There was however a significant increase in time spent in the stranger compartment with IN at 2 and 8 g/kg and KET/IN at 8 g/kg of feed and a decrease with

KET compared to control. Compared to KET, time spent in the stranger compartment increased significantly with KET/IN at 2, 4 and 8 g/kg of feed.

Effect of on Inulin on social interaction Phase 1I

Figure 8 shows the effect of inulin on social interaction behaviour measured as number of contacts with "Stranger mouse. Pairwise comparisons revealed a significant [$F(7,72) = 12.4$, $p < 0.001$] increase in number of contacts with IN at 2, 4 and 8 g/kg and KET/IN at 2, 4 and 8 g/kg of feed and a decrease with KET compared to control. Compared to KET, number of contacts with stranger mouse increased significantly with KET/IN at 2, 4 and 8 g/kg of feed.

3.8 Effect of Inulin on social memory and social novelty

Figure 9 shows the effect of inulin on social memory and novelty measured as duration of time in the chamber with the unfamiliar mouse from the sociability phase ("Stranger 1") and in the opposite chamber with a new unfamiliar mouse ("Stranger 2"). Two factor ANOVA of social memory data revealed no significant effect of Treatment [$F(7,144) = 1.87$ $p<0.078$], but a significant effect of chamber (Stranger 1 vs Stranger 2) [$F(1,144) = 0.923$ $p<0.338$] and significant interaction between treatment and chamber [$F(7,144) = 5.95$ $p<0.004$]. Pairwise comparisons revealed a significant decrease in time spent in contact with the familiar mouse (stranger 1) with IN at 4, KET and KET /IN at 2 compared to vehicle control. Compared to KET, time spent in contact with stranger 1 increased significantly with KET/IN at 4 and 8 g/kg of feed. There was also a significant increase in time spent in the stranger 2 chamber with IN at 4 and 8 g/kg and KET/IN at 4 and 8 g/kg of feed and a decrease with KET compared to control. Compared to KET, time spent in the stranger 2 compartment increased significantly with KET/IN at 2, 4 and 8 g/kg of feed.

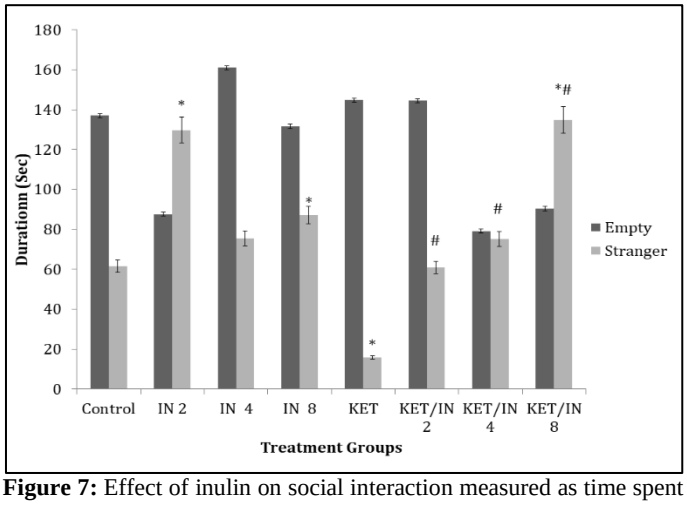

Figure 7: Effect of inulin on social interaction measured as time spent in the stranger or empty compartment. Each bar represents Mean ± S.E.M, *p<0.05 vs. normal control, #p<0.05 vs. ketamine control, number of mice/groups =10, IN: Inulin: KET: Ketamine.

3.7 Effect of Inulin on beam walking behaviours

Figures 10 and 11 show the effects of inulin on beam walking behaviour measured as latency to cross the beam (figure 10) and number of hind limb slips (Figure 11). Pairwise comparisons of data of latency to cross the beam revealed a significant [$F(5, 72) = 6.00$, $p < 0.001$] decrease in latency to cross the beam with IN at 2 g/kg of feed and an increase with IN at 8 g/kg of feed, KET and KET/IN 2, 4 and 8 g/kg of feed compared to control. Compared to KET, latency to cross the beam decreased significantly with KET/IN at 2, 4 and 8 g/kg of feed.

Pairwise comparisons of data of hind limb slips revealed a significant [$F(5, 72) = 12.00$, $p < 0.001$] increase in hind limb slips with compared to control. Compared to KET, number of hind limb slips decreased significantly with KET/IN at 2, 4 and 8 g/kg of feed.

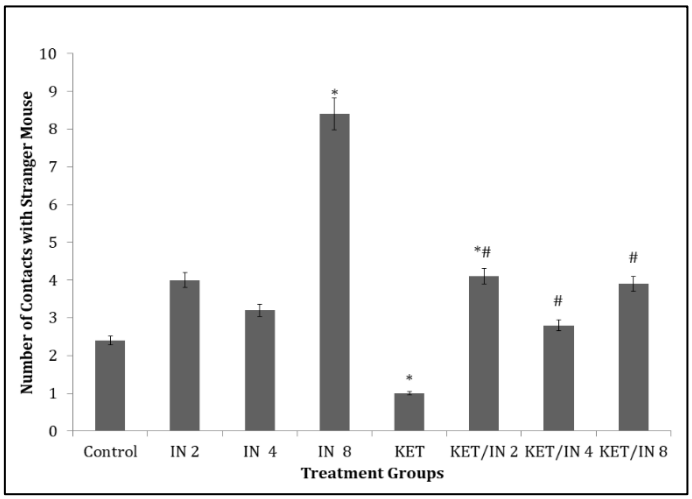

Figure 7: Effect of inulin on social interaction/sociability measured as number of contacts with "Stranger mouse". Each bar represents Mean ± S.E.M, *p<0.05 vs. normal control, #p<0.05 vs. ketamine control, number of mice/groups =10, IN: Inulin: KET: Ketamine.

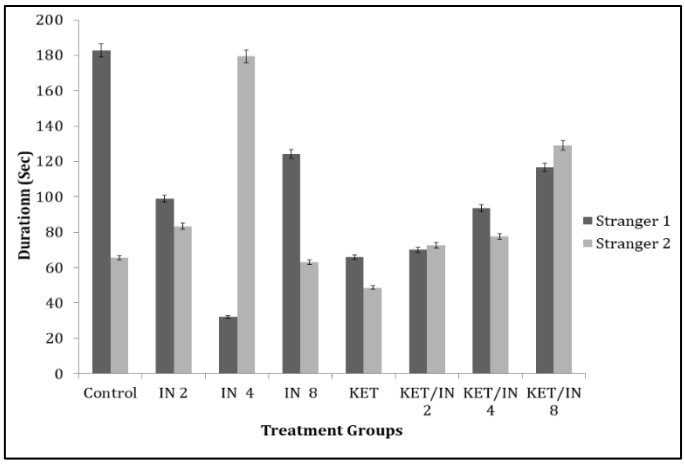

Figure 9: Effect of inulin on social memory. Each bar represents Mean ± S.E.M, *p<0.05 vs. normal control, #p<0.05 vs. ketamine control, number of mice/group =10, IN: Inulin: KET: Ketamine.

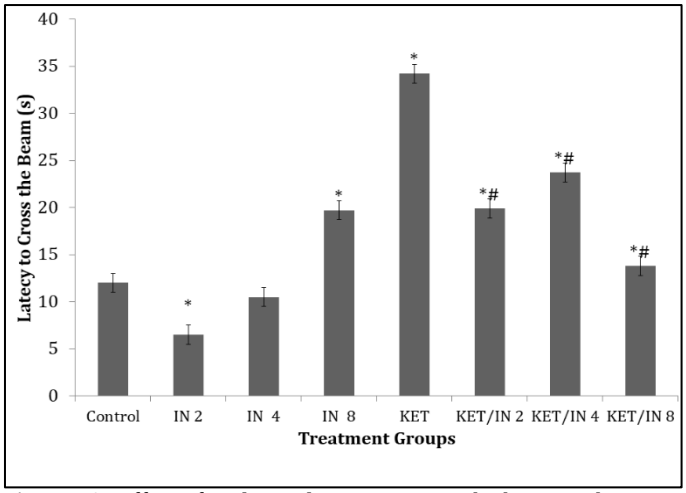

Figure 10: Effect of inulin on latency to cross the beam in the Beam balance test. Each bar represents Mean ± SEM, *p<0.05 compared to the control group, #p<0.05 compared to the KET group. Number of mice/groups =10. IN – Inulin, KET – Ketamine.

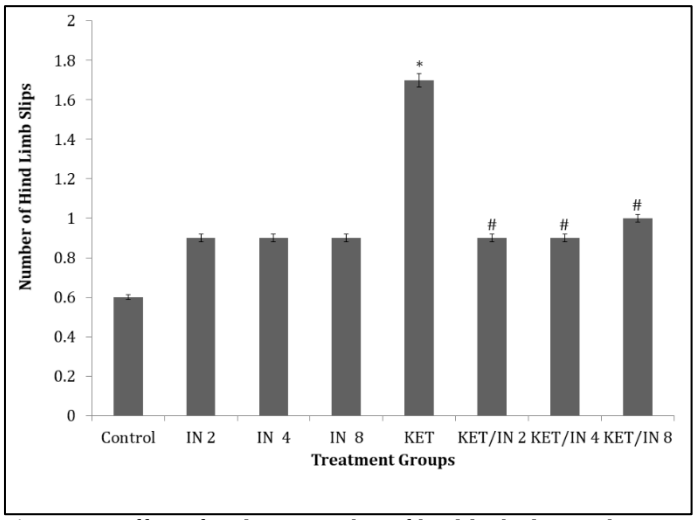

Figure 11: Effect of inulin on number of hind limb slips in the Beam balance test. Each bar represents Mean ± SEM, *p<0.05 compared to the control group, #p<0.05 compared to the KET group. Number of mice/groups =10. IN – Inulin, KET – Ketamine.

3.8 Effects of inulin on oxidative stress parameters

Table 1 shows the effect of inulin on hippocampal levels of malondialdehyde, total antioxidant capacity, glutathione, total protein and nitric oxide levels. Malondialdehyde (MDA) levels decreased significantly with IN at 2, 4 and 8 g, KET/IN at 2, 4 and 8 g respectively and increased with KET compared to control. Compared to KET, MDA levels decreased with KET/IN at 2, 4 and 8 g respectively. Total antioxidant capacity (TAC) decreased significantly with KET and increased with IN at 8 g compared to control. Compared to KET, TAC levels increased significantly with KET/IN at 2, 4 and 8 g respectively. Nitric oxide levels decreased significantly with IN at 8g, KET and KET/IN at 2 and 4 g compared to control. Compared to KET, nitric oxide levels increased significantly with KET/IN at 4 and 8 g respectively.

3.9 Effects of inulin on histomorphology of the Cornus Ammonis

Plates 1 (A - H) and Plate 2(A-H) are representative photomicrographs of haematoxylin and eosin and cresyl violet stained sections of the mouse hippocampal cornus ammonis-2 (CA2) region respectively. Examination of the photomicrographs revealed well delineated structure of the mouse cornus ammonis showing a layer of tightly bound small pyramidal neurons (black arrow) and scattered glial cells and some blood vessels in the molecular layer in the control (1A, 2A), and Inulin at 2 (1B, 2B), 4 (1C, 2C) and 8 g (1D, 2D) groups. In the groups administered ketamine (1E, 2E) loss of pyramidal neurons are observed with sparsity of pyramidal neurons in the pyramidal cell layer. Increased cellularity in the molecular layer was also observed. However, in the groups administered ketamine with increasing concentrations of inulin KET/IN at 2 (1F, 2F), 4g (IG, 2G), and 8g (1H, 2H) varying degrees of reversal of ketamine induced changes was observed.

Table 1: Effect of Inulin on hippocampal oxidative stress parameters

Groups	MDA (µM)	TAC (mM Trolox Equivalence)	Nitric oxide (µM)
Control	0.59±0.10	8.74±0.63	7.07±0.73
IN 2	0.42±0.08	7.03±0.45	6.12±0.56
IN 4	0.32 ±0.05*	7.88±0.66	5.74±0.72
IN 8	0.33±0.24*	6.31±0.47	3.73±0.13*
KET	1.88±0.05*	4.75±0.30*	3.80±0.46*
KET/IN 2	0.50±0.09#	8.87±0.74#	4.58±0.22*
KET/IN 4	0.27 ±0.03*#	6.03±0.28#	5.58±0.23*#
KET/IN 8	0.25 ±0.08*#	11.65±0.32*#	6.58±0.24#

Table 1: showing the hippocampal oxidative stress markers in Data represented as Mean ± SEM, *p<0.05 compared to the control group, #p<0.05 compared to the KET group. Number of mice/groups =10. IN – Inulin, KET – Ketamine.

3.10 Effects of inulin on the histomorphology of the dentate gyrus

Plates 3(A-H) and Plate 4(A-H) are representative photomicrographs of haematoxylin and eosin and cresyl violet stained sections of the mouse hippocampal dentate gyrus respectively. Examination of the photomicrographs of the dentate gyrus revealed well delineated granular cell layer with tightly packed granule neurons surrounded by the molecular layer within which were observed scattered glial cells in the control (3A, 4A), and Inulin at 2 (3B, 4B), 4 (3C, 4C) and 8 g (3D, 4D) groups. In the groups administered ketamine (3E, 4E) loss of granule neurons were observed with sparsity of granule

neurons in the granule cell layer of the dentate gyrus cell. In the groups administered ketamine with increasing concentrations of inulin KET/IN at 2 (3F, 4F), 4g (3G, 4G), and 8g (3H, 4H) varying degrees of reversal of ketamine induced changes was observed.

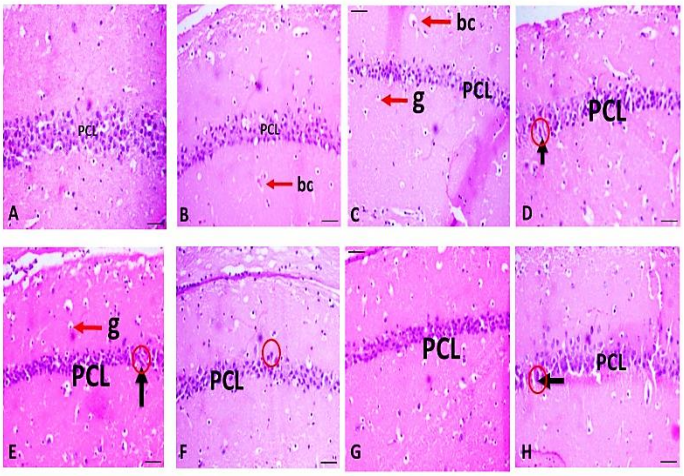

Plate 1(A-H): Representative photomicrograph of haematoxylin and Eosin-stained sections of the mouse hippocampal cornus ammonis region. Photomicrographs revealed distinct layers of the cornus ammonis with presence of small pyramidal neurons in the pyramidal cell layer with scattered glial (g) cells and some blood vessels (bc) in the molecular layer with presence Magnification: x160

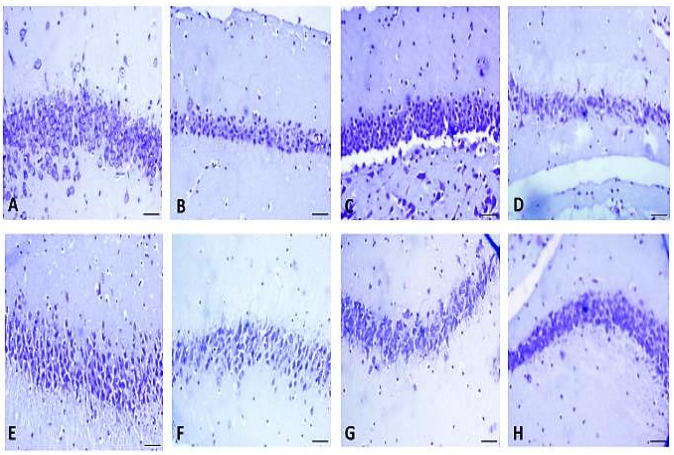

Plate 2(A-H): Representative photomicrograph of haematoxylin and Eosin-stained sections of the mouse hippocampal cornus ammonis region. Photomicrographs revealed distinct layers of the cornus ammonis with presence of small pyramidal neurons in the pyramidal cell layer Magnification: x160.

4. Discussion

For decades, research into the aetiopathogenesis of schizophrenia has implicated multifactorial factors including stressful life experiences, maternal infections, genetics and gene–environment interactions. In recent years, research has begun to demonstrate the ability of the gut-microbiome brain axis to modulate brain function and structure in health and disease. There have also been reports suggesting that the gut-microbiome-brain axis, plays a crucial role in the development and progression of schizophrenia [33-35].

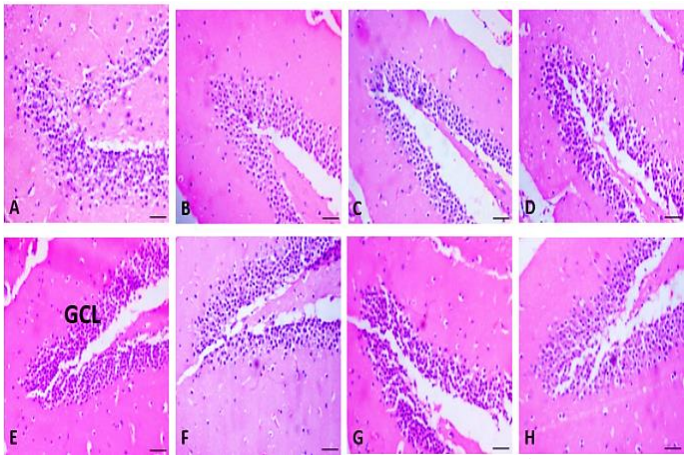

Plate 3 (A-H): Representative photomicrographs showing of the dentate gyrus of the hippocampus general structure of experimental animals. Stain – Haematoxylin and Eosin stain. Magnification – 160x. GCL – granule cell layer.

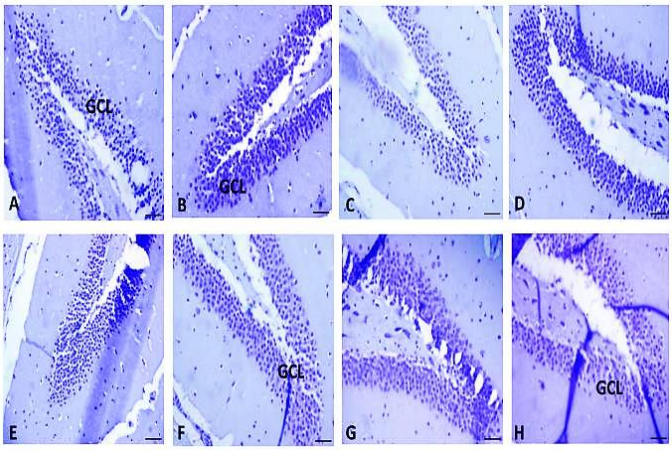

Plate 4 (A-H): Representative photomicrographs showing of the dentate gyrus of the hippocampus general structure of experimental animals. Stain – Cresyl violet. Magnification – 160x. GCL – granule cell layer.

While studies are beginning to examine the possible beneficial effects of prebiotic inulin on schizophrenia pathogenesis, there is still a dearth of scientific information on its possible benefits as a therapeutic option in schizophrenia management. In this study the possible beneficial effects of inulin on behavioural and structural changes in the hippocampus in a mouse model of ketamine induced schizophrenia was investigated. Ketamine was used to model schizophrenia in this study following previously published protocols [36-40]. Similar to the previous studies animals demonstrated phenotypes synonymous with schizophrenia in humans and rodents [37, 40, 41-43].

The results of this study showed that in healthy mice, feed-added inulin reduced feed intake and body weight compared to control, while in the groups with ketamine induced-schizophrenia, an increase in feed intake and body weight was

observed compared to ketamine control. Several studies [44-48] have examined the impact of prebiotics like inulin on food intake and body weight and reported that inulin could be beneficial in inducing weight loss and counteracting weight gain. In this study feed-added inulin was associated with reduced weight gain in health or disease. Weight loss due to inulin has been attributed to its ability to increase gut concentration of short chain fatty acids which are gut microbiota induced fermentation end-products of inulin [45]. The effect of inulin on weight could also be linked to its ability to reduce feed intake, increase energy expenditure and decrease feed conversion ratio and adiposity index [49]. In this study a decrease in feed intake was observed across all groups fed inulin compared to control. Also observed was a lag in the time a reduction in fed intake was observed (weeks 4, 5 and 6) compared to the period when a decrease in body weight was observed suggesting that inulin first caused weight loss which could be attributed to a possible increase in energy expenditure and a reduction in feed conversion ratio resulting in weight loss before a decrease in feed intake ensued. The administration of ketamine in this study was associated with an initial steady non-significant increase in feed intake which could be attributed to the acute effects of N-methyl-D-aspartate (NMDA) receptor antagonism [50-52], However a significant reduction in feed intake was observed towards the end of the study. Compared to ketamine's effect on feed intake, a progressive decline in body weight was observed throughout the experimental period. These findings are in keeping with the results of previous studies [39, 41, 53] which had reported that ketamine administration was associated with decreased feed intake and weight loss. In the ketamine groups treated with feed-added inulin a normalization of these changes in weight and feed intake was observed, buttressing the reports that prebiotics like inulin had the ability to reverse schizophrenia related changes in weight and food intake [54].

Behavioural phenotypes observed in this ketamine model of schizophrenia in mice included working memory deficits, anxiety related behaviours, social isolation and social interaction deficits. Also observed were increased repetitive behaviours and altered gait. Studies have associated the repeated administration of subanaesthetic doses of ketamine in rodents with the development of positive symptoms like working-memory deficits [55], and negative symptoms like social-interaction deficits [37] corroborating the results of this study. The ability of ketamine to accentuate repetitive behaviours particularly self-grooming has been reported severally [37-40]. In this study using the marble burying test we observed increased repetitive behaviours. The marble burying test has been suggested to be valuable for the quantification of repetitive/compulsive behaviours and anxiety [56], although it still requires proper validation [57]. In this study, compared to vehicle control an increase in marble burying behaviour was observed in the group of mice administered ketamine alone. Repetitive behaviours have been described as one of the behavioural phenotypes in schizophrenia [58, 59]. Although initially considered to be rare behavioural phenotype in schizophrenia; recent reports are beginning to demonstrate its increased prevalence and association with poor treatment outcomes [60, 61]. There have been suggestions that compulsive behaviours and psychosis possibly share common neurotransmitter pathways or loci [61]. In this study the administration of inulin was observed to reverse the effects due to ketamine on marble burying behaviour. Also, the response of inulin when administered alone or in a background of ketamine was similar to the response observed with grooming (in previous reports from our laboratory) suggesting also the possibility that similar neurotransmitter circuits modulate repetitive behavioural response observed in both tests

Repeated ketamine administration has also been associated with the development of anxiety related behaviours [38, 39], Likewise in this study we observed a decrease in time spent in the open arm of the elevated plus maze in mice in the group administered ketamine. The anxiolytic effects of prebiotic inulin have been reported severally [62-64]. However, in this study, inulin administered at 2 and 4 g/kg of feed in healthy was associated with increased anxiety. In the groups administered inulin with ketamine a reversal of ketamine-induced anxiety response was observed. Several studies have examined inulin's effect on anxiety and reported an anxiolytic response contrary to the anxiogenic response observed in this study. In the majority of these studies' inulin effect was assessed in a background of ill health [62-66], the anxiolytic response which is corroborated in this study with inulin reversing ketamine induced anxiogenic response. This would suggest that in health the administration of inulin could cause mild anxiety with a more beneficial effect in reversing anxiety. This is further supported by reports that attribute inulin's ability to reverse anxiety to the reduction of oxidative stress which was also observed in this study.

Historical characterization of behavioural phenotypes in schizophrenia had strongly associated the presence of motor dysfunctions as an integral part of schizophrenia diagnosis however, the advent of treatment relegated this symptom to a common side effect of antipsychotics, eventually becoming absent from the modern diagnostic criteria [67, 68]. In the last few decades, motor dysfunction such as gait and balance deficits are emerging as important features in schizophrenia diagnosis [69, 70]. In this study, ketamine administration was associated with functional immobility measured as an increase in latency to cross the balance beam, corroborating the result of

a few studies that had examined ketamine's ability to impair functional mobility in rodents [71]. In this study, ketamine administration increased functional immobility, Inulin on the other hand showed a dose related response with lower doses showing reduction in time spent crossing the beam and the highest does associated with increased latency to cross the beam when compared to control. In groups administered ketamine with inulin a reversal of ketamine-induced latency to cross the beam. Inulin's positive effects on motor coordination observed in this study corroborates the reports of a few studies that have also reported inulin's ability to mitigate repetitive behaviours in mice [70].

Loss of social interaction and social isolation are core negative symptoms of schizophrenia [72-74]. In this study, ketamine administration was associated with loss of social interaction and preference for empty chamber (social isolation). This behavioural response corroborates the result of previous studies that had demonstrated that deficits in social interaction were consistent features in both preclinical and clinical schizophrenia studies [38, 39, 73, 74]. Feed added inulin on the other hand was associated with increased social interaction either in groups administered inulin alone or in the ketamine-inulin groups, corroborating the results of studies that had associated inulin use with a reversal of social interaction deficits [75, 76]. Inulin, 's ability to influence social interaction behaviours have been linked to its ability to mitigate stressful conditions like anxiety (also observed in this study) and modulate the gut microbiome [77]. Deficits in social interaction have also been linked to deficits in social cognition In this study ketamine was associated with working memory deficits [40], however in groups treated with inulin a reversal of working memory loss was observed. Inulin's ability to reverse memory deficits have been linked to its ability to restore gut microbial dysbiosis, reduce oxidative stress, neuroinflammation, and also increase the levels of brain-derived neurotrophic factor [78].

The result of this study also showed that ketamine negatively altered lactobacillus count and cause gut microbial dysbiosis. While in the groups administered inulin-alone or in combination with ketamine increased lactobacillus composition was observed. Inulin's ability to positively impact lactobacillus count and by extension modulate gut microbiota composition of beneficial bacteria has been reported severally [79. 80]. Studies have associated inulin's effect with the ability to increase the growth and survival of lactobacillus [81]. The observed changes in the microbiota composition also corresponds to the observed effect on weight and feed intake. There have been reports that inulin mitigates weight gain through its ability to increase lactobacillus count [76].

Histopathological examination of hippocampus revealed loss of pyramidal neurons in the CA2 region, and loss of granule neurons in the dentate gyrus regions of the hippocampus. The effects observed with ketamine corroborate the result of an earlier study that had reported evidence of neurodegenerative changes in the hippocampus following ketamine administration [41]. The administration of inulin was associated with mitigation of these ketamine induced morphological changes in the hippocampus.

Conclusion

This study highlights the potential therapeutic benefits of inulin in mitigating ketamine-induced schizophrenia-like symptoms. The significant improvements noted in gut microbiota composition, and hippocampal structural integrity suggest that inulin may serve as a viable prebiotic intervention in schizophrenia. However, further studies are necessary to determine its benefits in humans.

Funding.
None

Availability of data and materials

Data generated during and analysed during the course of this study are available from the corresponding author on request.

Declarations Ethics approval

The ethical approval for this research was given by the Ethical Committee of the Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology (**ERCFBMS:048/06/2024**)

Competing interests

All authors of this paper declare that there is no conflict of interest related to the content of this manuscript.

Received 3rd May, 2024, Revised 10th May 2025; Accepted 15th May, 2025,

Published online:25/05/25

References

1. Carabotti, M., Scirocco, A., Maselli, M.A. and Severi, C. The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. Ann Gastroenterol, 2015;28(2):203-209.
2. Onaolapo OJ, Onaolapo AY, Olowe AO. The neurobehavioral implications of the brain and microbiota interaction. Front Biosci (Landmark Ed). 2020 Jan 1;25(2):363-397. doi: 10.2741/4810.
3. Rinninella, E., Raoul, P., Cintoni, M., Franceschi, F., Miggiano, G.A.D., Gasbarrini, A., Mele, M.C. What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. Microorganisms, 2019;7(1):14. Doi: 10.3390/microorganisms7010014.
4. Eckburg, P.B., Bik, E.M., Charles, N.B., Elizabeth, P., Les, D., Michael, S., Steven, R.G., Karen, E.N., and David, A.R. Diversity of the Human Intestinal Microbial Flora. Science, 2005;308,1635-1638(2005). DOI:10.1126/science.1110591.
5. Onaolapo AY, Onaolapo OJ. Glutamate and depression: Reflecting a deepening knowledge of the gut and brain effects of a ubiquitous molecule. World J Psychiatry. 2021 Jul 19;11(7):297-315. doi: 10.5498/wjp.v11.i7.297.

6. Onaolapo AY, Ojo FO, Olofinnade AT, Falade J, Lawal IA, Onaolapo OJ. Microbiome-Based Therapies in Parkinson's Disease: Can Tuning the Microbiota Become a Viable Therapeutic Strategy? CNS Neurol Disord Drug Targets. 2022 Sep 3. doi: 10.2174/1871527321666220903114559
7. You, S., Ma, Y., Yan, B., Pei, W., Wu, Q., Ding, C., and Huang, C. The promotion mechanism of prebiotics for probiotics: A review. Front Nutr., 2022;9:1000517. doi: 10.3389/fnut.2022.1000517.
8. Davani-Davari D, Negahdaripour M, Karimzadeh I, Seifan M, Mohkam M, Masoumi SJ, Berenjian A, Ghasemi Y. Prebiotics: Definition, Types, Sources, Mechanisms, and Clinical Applications. Foods. 2019 Mar 9;8(3):92. doi: 10.3390/foods8030092.
9. Shao R, Tan X, Pan M, Huang J, Huang L, Bi B, Huang X, Wang J, Li X. Inulin alters gut microbiota to alleviate post-stroke depressive-like behavior associated with the IGF-1-mediated MAPK signaling pathway. Brain Behav. 2024 Jan;14(1):e3387. doi: 10.1002/brb3.3387.
10. Wang L, Wang Z, Lan Y, Tuo Y, Ma S, Liu X. Inulin Attenuates Blood-Brain Barrier Permeability and Alleviates Behavioral Disorders by Modulating the TLR4/MyD88/NF-κB Pathway in Mice with Chronic Stress. J Agric Food Chem. 2023 Sep 13;71(36):13325-13337. doi: 10.1021/acs.jafc.3c03568
11. Hoffman, J.D., Yanckello, L.M., Chlipala, G., Hammond, T.C., McCulloch, S.D., Parikh, I., Sun, S., Morganti, J.M., Green, S.J., Lin, A.L. Dietary inulin alters the gut microbiome, enhances systemic metabolism and reduces neuroinflammation in an APOE4 mouse model. PLoS One, 2019;14(8):e0221828. Doi: 10.1371/journal.pone.0221828.
12. Guo, L., Xiao, P., Zhang, X., Yang, Y., Yang, M., Wang, T., Lu, H., Tian, H., Wang, H., and Liu, J. Inulin ameliorates schizophrenia: Via modulation of the gut microbiota and anti-inflammation in mice. Food and Function, 2021;12(3), 1156–1175. https://doi.org/10.1039/d0fo02778b.
13. Kizilirmak, J.M., Schott, B.H., Thuerich, H., Sweeney-Reed, C.M., Richter, A., Folta-Schoofs, K., and Richardson-Klavehn, A. Learning of novel semantic relationships via sudden comprehension is associated with a hippocampus-independent network. Conscious Cogn., 2019;69:113-132.
14. Insel, T.R. Rethinking schizophrenia. Nature, 2010;468, 187–193.
15. Onaolapo A Y, Onaolapo O J Schizophrenia Aetiology and Drug Therapy: A Tale of Progressive Demystification and Strides in Management. Advances in Pharmacology and Pharmacy, 2018, 6(2), 19 - 42. DOI: 10.13189/app.2018.060201
16. Gardner, K and Nasrallah, H.A. Managing First-Episode Psychosis. An early stage of schizophrenia with distinct treatment needs to minimize the duration of untreated psychosis; and aim for remission. Current Psychiatry, 2015;14(5).
17. Howes, O.D. and Murray, R.M. Schizophrenia: an integrated socio-developmental cognitive model. Lancet, 2014;383, 1677–1687.
18. Onaolapo OJ, Onaolapo AY. Nutrition, nutritional deficiencies, and schizophrenia: An association worthy of constant reassessment. World J Clin Cases. 2021 Oct 6;9(28):8295-8311. doi: 10.12998/wjcc.v9.i28.8295.
19. Ceraso, A, Lin, J.J., Schneider-Thoma, J., Siafis, S., Tardy, M., Komossa, K., Heres, S., Kissling, W., Davis, J.M., and Leucht, S. Maintenance treatment with antipsychotic drugs for schizophrenia. Cochrane Database Syst Rev. 2020;8(8):CD008016. doi: 10.1002/14651858.CD008016
20. El Abdellati, K., De Picker, L., Morrens, M. Antipsychotic Treatment Failure: A Systematic Review on Risk Factors and Interventions for Treatment Adherence in Psychosis. Front Neurosci. 2020;14:531763. doi: 10.3389/fnins.2020.531763.
21. Xiong RG, Li J, Cheng J, Zhou DD, Wu SX, Huang SY, Saimaiti A, Yang ZJ, Gan RY, Li HB. The Role of Gut Microbiota in Anxiety, Depression, and Other Mental Disorders as Well as the Protective Effects of Dietary Components. Nutrients. 2023 Jul 23;15(14):3258. doi: 10.3390/nu15143258.
22. Szeligowski T, Yun AL, Lennox BR, Burnet PWJ. The Gut Microbiome and Schizophrenia: The Current State of the Field and Clinical Applications. Front Psychiatry. 2020 Mar 12;11:156. doi: 10.3389/fpsyt.2020.00156.
23. Olofinnade AT, Onaolapo AY, Stefanucci A, Mollica A, Olowe OA, Onaolapo OJ. Cucumeropsis mannii reverses high-fat diet induced metabolic derangement and oxidative stress. Front Biosci (Elite Ed). 2021 Jan 1;13(1):54-76. doi: 10.2741/872.
24. Yang, X.Y., Yaoping W., Xingxing C., Hua W., Nagendra P.S., and Feng X. Enhancing flora balance in the gastrointestinal tract of mice by lactic acid bacteria from Chinese sourdough and enzyme activities indicative of metabolism of protein, fat, and carbohydrate by the flora. Journal of Dairy Science. 2016;99(10). Pages 7809-7820. https://doi.org/10.3168/jds.2016-11467.
25. Celiberto, L.S., Pinto, R.A., Rossi, E.A., Vallance, B.A., and Cavallini, D.C.U. Isolation and Characterization of Potentially Probiotic Bacterial Strains from Mice: Proof of Concept for Personalized Probiotics. Nutrients. 2018;10(11):1684. doi: 10.3390/nu10111684. PMID: 30400640; PMCID: PMC6266017.
26. Onaderu TA, Onaolapo OJ, Onaolapo AY Post-conceptional melatonin administration mitigates changes in neurobehaviour and cerebral cortex histomorphology in prenatal sodium valproate-exposed rats. Acta Bioscientia **2025**:1(1);38-45 https://doi.org/10.71181/actabioscientia12160
27. Kim, D.G., Gonzales, E.L., Kim, S., Kim, Y., Adil, K.J., Jeon, S.J., Cho, K.S., Kwon, K.J., and Shin, C.Y. Social Interaction Test in Home Cage as a Novel and Ethological Measure of Social Behavior in Mice. Exp Neurobiol. 2019;28(2):247-260. doi: 10.5607/en.2019.28.2.247. Epub 2019 Apr 30. PMID: 31138992; PMCID: PMC6526108.
28. Kaidanovich-Beilin, O., Lipina, T., Vukobradovic, I., Roder, J., and Woodgett, J.R. Assessment of social interaction behaviours. J Vis Exp. 2011;(48):2473. doi: 10.3791/2473. PMID: 21403628; PMCID: PMC3197404.
29. Onaolapo AY, Adebayo AN, Onaolapo OJ. Exogenous daytime melatonin modulates response of adolescent mice in a repeated unpredictable stress paradigm. Naunyn Schmiedebergs Arch Pharmacol. 2017 Feb;390(2):149-161. doi: 10.1007/s00210-016-1314-7.
30. Onaolapo OJ, Onaolapo AY. Subchronic Oral Bromocriptine Methanesulfonate Enhances Open Field Novelty-Induced Behavior and Spatial Memory in Male Swiss Albino Mice. Neurosci J. 2013;2013:948241. doi: 10.1155/2013/948241.
31. Onaolapo AY, Onaolapo OJ, Nwoha PU. Methyl aspartylphenylalanine, the pons and cerebellum in mice: An evaluation of motor, morphological, biochemical, immunohistochemical and apoptotic effects. J Chem Neuroanat. 2017 Dec;86:67-77. doi: 10.1016/j.jchemneu.2017.09.001.
32. Luong, T.N., Carlisle, H.J., Southwell, A., and Patterson, P.H. Assessment of motor balance and coordination in mice using the balance beam. J Vis Exp, 2011;49:2376. Doi: 10.3791/2376.
33. Szeligowski T, Yun AL, Lennox BR, Burnet PWJ. The Gut Microbiome and Schizophrenia: The Current State of the Field and Clinical Applications. Front Psychiatry. 2020 Mar 12;11:156. doi: 10.3389/fpsyt.2020.00156.
34. Socała K, Doboszewska U, Szopa A, Serefko A, Włodarczyk M, Zielińska A, Poleszak E, Fichna J, Wlaź P. The role of microbiota-gut-brain axis in neuropsychiatric and neurological disorders. Pharmacol Res. 2021 Oct;172:105840. doi: 10.1016/j.phrs.2021.105840.
35. Tsamakis K, Galinaki S, Alevyzakis E, Hortis I, Tsiptsios D, Kollintza E, Kympouropoulos S, Triantafyllou K, Smyrnis N, Rizos E. Gut Microbiome: A Brief Review on Its Role in Schizophrenia and First Episode of Psychosis. Microorganisms. 2022 May 29;10(6):1121. doi: 10.3390/microorganisms10061121.
36. Canever L, Oliveira L, D'Altoé de Luca R, Correa PT, de B Fraga D, Matos MP, Scaini G, Quevedo J, Streck EL, Zugno AI. A rodent model of schizophrenia reveals increase in creatine kinase activity with associated behavior changes. Oxid Med Cell Longev. 2010 Nov-Dec;3(6):421-7. doi: 10.4161/oxim.3.6.13446.
37. Onaolapo OJ, Paul TB, Onaolapo AY. Comparative effects of sertraline, haloperidol or olanzapine treatments on ketamine-induced changes in mouse behaviours. Metab Brain Dis. 2017a;32(5):1475-1489. doi: 10.1007/s11011-017-0031-3.
38. Onaolapo AY, Aina OA, Onaolapo OJ. Melatonin attenuates behavioural deficits and reduces brain oxidative stress in a rodent model of schizophrenia. Biomed Pharmacother. 2017b;92:373-383. doi: 10.1016/j.biopha.2017.05.094.

39. Onaolapo OJ, Ademakinwa OQ, Olalekan TO, Onaolapo AY. Ketamine-induced behavioural and brain oxidative changes in mice: an assessment of possible beneficial effects of zinc as mono- or adjunct therapy. Psychopharmacology (Berl). 2017c;234(18):2707-2725. doi: 10.1007/s00213-017-4666-x.
40. Onaolapo OJ, Jegede OR, Adegoke O, Ayinde MO, Akeredolu OM, Onaolapo AY. Dietary zinc supplement militates against ketamine-induced behaviours by age-dependent modulation of oxidative stress and acetylcholinesterase activity in mice. Pharmacol Rep. 2020 Feb;72(1):55-66. doi: 10.1007/s43440-019-00003-2.
41. Onaolapo AY, Ayeni OJ, Ogundeji MO, Ajao A, Onaolapo OJ, Owolabi AR. Subchronic ketamine alters behaviour, metabolic indices and brain morphology in adolescent rats: Involvement of oxidative stress, glutamate toxicity and caspase-3-mediated apoptosis. J Chem Neuroanat. 2019 Mar;96:22-33. doi: 10.1016/j.jchemneu.2018.12.002.
42. Acevedo J, Siegel JA. Neurobiological, behavioral, and cognitive effects of ketamine in adolescents: A review of human and pre-clinical research. Behav Brain Res. 2022 Oct 28;435:114049. doi: 10.1016/j.bbr.2022.114049 Behrooz et al., 2024
43. Guess ND, Dornhorst A, Oliver N, Bell JD, Thomas EL, Frost GS. A randomized controlled trial: the effect of inulin on weight management and ectopic fat in subjects with prediabetes. Nutr Metab (Lond). 2015 Oct 24;12:36. doi: 10.1186/s12986-015-0033-2.
44. Igarashi M, Morimoto M, Suto A, Nakatani A, Hayakawa T, Hara K, Kimura I. Synthetic dietary inulin, Fuji FF, delays development of diet-induced obesity by improving gut microbiota profiles and increasing short-chain fatty acid production. PeerJ. 2020 Apr 8;8:e8893. doi: 10.7717/peerj.8893.
45. Guo L, Xiao P, Zhang X, Yang Y, Yang M, Wang T, Lu H, Tian H, Wang H, Liu J. Inulin ameliorates schizophrenia *via* modulation of the gut microbiota and anti-inflammation in mice. Food Funct. 2021 Feb 15;12(3):1156-1175. doi: 10.1039/d0fo02778b.
46. Huang Y, Ying N, Zhao Q, Chen J, Teow SY, Dong W, Lin M, Jiang L, Zheng H. Amelioration of Obesity-Related Disorders in High-Fat Diet-Fed Mice following Fecal Microbiota Transplantation from Inulin-Dosed Mice. Molecules. 2023 May 10;28(10):3997. doi: 10.3390/molecules28103997.
47. Wu H, Van Der Pol WJ, Dubois LG, Morrow CD, Tollefsbol TO. Dietary Supplementation of Inulin Contributes to the Prevention of Estrogen Receptor-Negative Mammary Cancer by Alteration of Gut Microbial Communities and Epigenetic Regulations. Int J Mol Sci. 2023 May 19;24(10):9015. doi: 10.3390/ijms24109015.
48. Koch F, Derno M, Langhammer M, Tuchscherer A, Hammon HM, Mielenz M, Metges CC, Kuhla B. A high-protein diet containing inulin/oligofructose supports body weight gain associated with lower energy expenditure and carbohydrate oxidation, and alters faecal microbiota in C57BL/6 mice. J Nutr Sci. 2021 Jul 13;10:e50. doi: 10.1017/jns.2021.42.
49. Li L, Zhang L, Zhou L, Jin M, Xu L. Chain length-dependent inulin alleviates diet-induced obesity and metabolic disorders in mice. Food Sci Nutr. 2021 May 7;9(7):3470-3482. doi: 10.1002/fsn3.2283.
50. Ouyang X, Wang Z, Luo M, Wang M, Liu X, Chen J, Feng J, Jia J, Wang X. Ketamine ameliorates depressive-like behaviors in mice through increasing glucose uptake regulated by the ERK/GLUT3 signaling pathway. Sci Rep. 2021 Sep 13;11(1):18181. doi: 10.1038/s41598-021-97758-7.
51. Dunham KE, Khaled KH, Weizman L, Venton BJ. Microdosing ketamine in Drosophila does not block serotonin reuptake, but causes complex behavioral changes mediated by glutamate and serotonin receptors. J Neurochem. 2024 Jun;168(6):1097-1112. doi: 10.1111/jnc.16070.
52. Parise EM, Alcantara LF, Warren BL, Wright KN, Hadad R, Sial OK, Kroeck KG, Iñiguez SD, Bolaños-Guzmán CA. Repeated ketamine exposure induces an enduring resilient phenotype in adolescent and adult rats. Biol Psychiatry. 2013 Nov 15;74(10):750-9. doi: 10.1016/j.biopsych.2013.04.027.
53. (Kao AC, Burnet PWJ, Lennox BR. Can prebiotics assist in the management of cognition and weight gain in schizophrenia? Psychoneuroendocrinology. 2018 Sep;95:179-185. doi: 10.1016/j.psyneuen.2018.05.027.
54. da Silva Araújo T, Maia Chaves Filho AJ, Monte AS, Isabelle de Góis Queiroz A, Cordeiro RC, de Jesus Souza Machado M, de Freitas Lima R, Freitas de Lucena D, Maes M, Macêdo D. Reversal of schizophrenia-like symptoms and immune alterations in mice by immunomodulatory drugs. J Psychiatr Res. 2017 Jan;84:49-58. doi: 10.1016/j.jpsychires.2016.09.017.
55. Taylor GT, Lerch S, Chourbaji S. Marble burying as compulsive behaviors in male and female mice. Acta Neurobiol Exp (Wars). 2017;77(3):254-260. Dixit PV, Sahu R, Mishra DK. Marble-burying behavior test as a murine model of compulsive-like behavior. J Pharmacol Toxicol Methods. 2020 Mar-Apr;102:106676. doi: 10.1016/j.vascn.2020.106676.
56. Luchins DJ, Goldman MB, Lieb M, Hanrahan P. Repetitive behaviors in chronically institutionalized schizophrenic patients. Schizophr Res. 1992 Dec;8(2):119-23. doi: 10.1016/0920-9964(92)90027-3.
57. Tracy JI, de Leon J, Qureshi G, McCann EM, McGrory A, Josiassen RC. Repetitive behaviors in schizophrenia: a single disturbance or discrete symptoms? Schizophr Res. 1996 May;20(1-2):221-9. doi: 10.1016/0920-9964(95)00104-2.
58. Vellucci L, Ciccarelli M, Buonaguro EF, Fornaro M, D'Urso G, De Simone G, Iasevoli F, Barone A, de Bartolomeis A. The Neurobiological Underpinnings of Obsessive-Compulsive Symptoms in Psychosis, Translational Issues for Treatment-Resistant Schizophrenia. Biomolecules. 2023 Aug 5;13(8):1220. doi: 10.3390/biom13081220
59. Pardossi S, Cuomo A, Fagiolini A. Unraveling the Boundaries, Overlaps, and Connections between Schizophrenia and Obsessive-Compulsive Disorder (OCD). J Clin Med. 2024 Aug 12;13(16):4739. doi: 10.3390/jcm13164739.
60. Burokas A, Arboleya S, Moloney RD, Peterson VL, Murphy K, Clarke G, Stanton C, Dinan TG, Cryan JF. Targeting the Microbiota-Gut-Brain Axis: Prebiotics Have Anxiolytic and Antidepressant-like Effects and Reverse the Impact of Chronic Stress in Mice. Biol Psychiatry. 2017 Oct 1;82(7):472-487. doi: 10.1016/j.biopsych.2016.12.031.
61. Davis GL, Minerva AR, Lario A, Simmler LD, Rodriguez CI, Gunaydin LA. Ketamine increases activity of a fronto-striatal projection that regulates compulsive behavior in SAPAP3 knockout mice. Nat Commun. 2021 Oct 15;12(1):6040. doi: 10.1038/s41467-021-26247-2. PMID: 34654803; PMCID: PMC8519915.
62. Johnstone N, Milesi C, Burn O, van den Bogert B, Nauta A, Hart K, Sowden P, Burnet PWJ, Cohen Kadosh K. Anxiolytic effects of a galacto-oligosaccharides prebiotic in healthy females (18-25 years) with corresponding changes in gut bacterial composition. Sci Rep. 2021 Apr 15;11(1):8302. doi: 10.1038/s41598-021-87865-w.
63. Chen WC, Wang TS, Chang FY, Chen PA, Chen YC. Age, Dose, and Locomotion: Decoding Vulnerability to Ketamine in C57BL/6J and BALB/c Mice. Biomedicines. 2023 Jun 25;11(7):1821. doi: 10.3390/biomedicines11071821.
64. Li K, Wei W, Xu C, Lian X, Bao J, Yang S, Wang S, Zhang X, Zheng X, Wang Y, Zhong S. Prebiotic inulin alleviates anxiety and depression-like behavior in alcohol withdrawal mice by modulating the gut microbiota and 5-HT metabolism. Phytomedicine. 2024 Dec;135:156181. doi: 10.1016/j.phymed.2024.156181.)
65. Kendler KS. Phenomenology of Schizophrenia and the Representativeness of Modern Diagnostic Criteria. JAMA Psychiatry. 2016 Oct 1;73(10):1082-1092. doi: 10.1001/jamapsychiatry.2016.1976.
66. Hirjak D, Meyer-Lindenberg A, Kubera KM, Thomann PA, Wolf RC. Motor dysfunction as research domain in the period preceding manifest schizophrenia: A systematic review. Neurosci Biobehav Rev. 2018 Apr;87:87-105. doi: 10.1016/j.neubiorev.2018.01.011.

67. Walther S, van Harten PN, Waddington JL, Cuesta MJ, Peralta V, Dupin L, Foucher JR, Sambataro F, Morrens M, Kubera KM, Pieters LE, Stegmayer K, Strik W, Wolf RC, Hirjak D. Movement disorder and sensorimotor abnormalities in schizophrenia and other psychoses - European consensus on assessment and perspectives. Eur Neuropsychopharmacol. 2020 Sep;38:25-39. doi: 10.1016/j.euroneuro.2020.07.003.
68. Presta V, Paraboschi F, Marsella F, Lucarini V, Galli D, Mirandola P, Banchini A, Marchesi C, Galuppo L, Vitale M, Tonna M, Gobbi G. Posture and gait in the early course of schizophrenia. PLoS One. 2021 Jan 19;16(1):e0245661. doi: 10.1371/journal.pone.0245661.
69. Diaz-Gil, D.; Mueller, N.; Moreno-Duarte, I.; Lin, H.; Ayata, C.; Cusin, C.; Cotten, J.F.; Eikermann, M. Etomidate and Ketamine: Residual Motor and Adrenal Dysfunction that Persist beyond Recovery from Loss of Righting Reflex in Rats. *Pharmaceuticals* **2015**, *8*, 21-37. https://doi.org/10.3390/ph8010021
70. Wang C, Chen W, Jiang Y, Xiao X, Zou Q, Liang J, Zhao Y, Wang Q, Yuan T, Guo R, Liu X, Liu Z. A synbiotic formulation of *Lactobacillus reuteri* and inulin alleviates ASD-like behaviors in a mouse model: the mediating role of the gut-brain axis. Food Funct. 2024 Jan 2;15(1):387-400. doi: 10.1039/d3fo02663a.
71. Lalonde, R., and Strazielle, C. Probiotic Influences on Motor Skills: A Review. Curr Neuropharmacol., 2023;21(12):2481-2486. doi: 10.2174/1570159X21666230807150523
72. Weittenhiller LP, Mikhail ME, Mote J, Campellone TR, Kring AM. What gets in the way of social engagement in schizophrenia? World J Psychiatry. 2021 Jan 19;11(1):13-26. doi: 10.5498/wjp.v11.i1.13.
73. Dodell-Feder D, Tully LM, Hooker CI. Social impairment in schizophrenia: new approaches for treating a persistent problem. Curr Opin Psychiatry. 2015 May;28(3):236-42. doi: 10.1097/YCO.0000000000000154.
74. Waworuntu, R., Hain, H., Chang Q., Thiede, L., Taleen H., Brian B. Dietary prebiotics improve memory and social interactions while reducing anxiety when provided early in life to normally developing rodents (637.5). FASEB Journal, 2014; https://doi.org/10.1096/fasebj.28.1_supplement.637.5.
75. Ansari, F., Neshat, M., Pourjafar, H., Jafari, S.M., Samakkhah, S.A., Mirzakhani, E. The role of probiotics and prebiotics in modulating of the gut-brain axis. Front Nutr., 2023;10:1173660. doi: 10.3389/fnut.2023.1173660.
76. Amadieu C, Coste V, Neyrinck AM, Thijssen V, Leyrolle Q, Bindels LB, Piessevaux H, Stärkel P, de Timary P, Delzenne NM, Leclercq S. Restoring an adequate dietary fiber intake by inulin supplementation: a pilot study showing an impact on gut microbiota and sociability in alcohol use disorder patients. Gut Microbes. 2022 Jan-Dec;14(1):2007042. doi: 10.1080/19490976.2021.2007042.
77. Xu Z, Lu H, Hu C, Wen Y, Shang D, Gan T, Guo Z, Dai L, Luo Y. Inulin alleviates chronic ketamine-induced impairments in memory and prepulse inhibition by regulating the gut microbiota, inflammation, and kynurenine pathway. Int J Biol Macromol. 2025 Mar;294:139503. doi: 10.1016/j.ijbiomac.2025.139503.
78. Hussien H, Abd-Rabou HS, Saad MA. The impact of incorporating Lactobacillus acidophilus bacteriocin with inulin and FOS on yogurt quality. Sci Rep. 2022 Aug 4;12(1):13401. doi: 10.1038/s41598-022-17633-x.
79. De Giani, A., Sandionigi, A., Zampolli, J., Michelotti, A., Tursi, F., Labra, M., Di Gennaro, P. Effects of Inulin-Based Prebiotics Alone or in Combination with Probiotics on Human Gut Microbiota and Markers of Immune System: A Randomized, Double-Blind, Placebo-Controlled Study in Healthy Subjects. Microorganisms, 2022;10(6):1256. doi: 10.3390/microorganisms10061256.
80. Cerdó, T., García-Santos, J.A., Bermúdez, M., Campoy, C. The Role of Probiotics and Prebiotics in the Prevention and Treatment of Obesity. Nutrients, 2019;11(3):635. doi: 10.3390/nu11030635.
81. Xia Y, Miao J, Zhang Y, Zhang H, Kong L, Seviour R, Kong Y. Dietary inulin supplementation modulates the composition and activities of carbohydrate-metabolizing organisms in the cecal microbiota of broiler chickens. PLoS One. 2021;16(10):e0258663. doi: 10.1371/journal.pone.0258663.

Cite as: Lawal IA, Onaolapo OJ, Onaolapo AY Feed-added Inulin mitigates ketamine-induced behaviours and hippocampal neurotoxicity via modulation of gut Lactobacilli count and brain oxidative stress Acta Bioscienctia 2025:1(3);140-152 10.71181/actabioscientia12290