Journal of Complementary and Alternative Medical Research

Volume 25, Issue 8, Page 14-27, 2024; Article no.JOCAMR.120239
ISSN: 2456-6276

Ameliorative Effect of Bambara Nut on Cognito-motor Functions of Konzo-induced Wistar Rats Model

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Authors' contributions

This work was carried out in collaboration among all authors. Authors DLK and AAA conceptualized and designed the study. Author DVO conducted the experiment and collected the data. Author DVO performed the statistical analysis and wrote the manuscript. All authors critically reviewed and edited the manuscript, contributed to the interpretation of results and approved the final version for submission. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jocamr/2024/v25i8556

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/120239

Original Research Article

Received: 22/05/2024
Accepted: 25/07/2024
Published: 30/07/2024

ABSTRACT

Introduction: Konzo is a neurological disease characterized by a sudden onset of symmetrical and spastic paraparesis due to selective upper motoneuron damage.

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Cite as: *V. O., Dimkpa, David L. K., and Ajah A. A. 2024. "Ameliorative Effect of Bambara Nut on Cognito-Motor Functions of Konzo-Induced Wistar Rats Model". Journal of Complementary and Alternative Medical Research 25 (8):14-27. https://doi.org/10.9734/jocamr/2024/v25i8556.*

Aim: To determine the Cognito-motor modulatory functions of Bambara nut (*Vigna subterranea*) extract in improperly processed bitter cassava (*Manihot esculenta*) flour Konzo-induced male Wistar rats.
Methodology: Twenty-five (25) Adult Male Wistar rats weighing between 180–200g were randomly divided into five (5) groups of five rats per group. The rats were allowed to acclimatize for 2 weeks. Konzo was induced following unprocessed bitter cassava flour feeding for a period of three weeks and fed with Bambara nut (BN). Feeding was as follows for 28 days: Group 1: Control group - rats in this group were fed with water and rat feed; Group 2 rats were fed with improperly processed bitter cassava flour to induce Konzo: Groups 3-5 rats were fed with different doses of Bambara nut extract 100mg/kg, 200mg/kg and 300mg/kg respectively. Neurobehavioral assessments were conducted weekly using barnes maze and hand grip tasks to assess cognition, perception, motor functions and muscular strength. The data was analyzed using Graphpad Prism and Microsoft Excel 2019.
Results: In weeks 1, 2, 3 and 4 trials, konzo-induced rats (Group 2) displayed significantly increased trial times for both cognitive and motor functions compared to Group 1 rats ($p < 0.05$). Administration of graded doses of BN extract amongst Groups 3, 4, and 5 rats resulted in significant reduction in trial times compared to Group 2 rats ($p < 0.05$), suggesting a possible modulatory function of the extract of Bambara nut in bitter cassava induced neurotoxicity.
Conclusion: Bambara nut extract improved cognito-motor functions in a dose dependent manner, resulting in a possible prevention of Konzo in male Wistar rats.

Keywords: Bambara nut; konzo; bitter cassava; neurotoxicity.

1. INTRODUCTION

Konzo is a neurological disease. It is characterized by a sudden onset of symmetrical, non-progressive and spastic paraparesis that are due to selective upper motoneuron damage. Children from 2 years of age and women of childbearing age are mostly affected, leading to gait difficulties within a few hours to one week [1]. Konzo is prevalent in Africa, and involves about ten countries. As a matter of fact., the prevalence of konzo is underestimated, as about 6788 cases alone, were reported in 2009 [2]. Konzo remains a poorly known disease that cannot be properly diagnosed by most orthodox health practitioners [2]. Furthermore, affected communities are poor, have low education, live in remote areas, and hold several cultural and religious beliefs regarding konzo. As a result, patients with konzo do not resort to conventional health structures [2,3].

Globally, there is an increasing dependence on medicinal plants for the treatment and prevention of various illnesses. It is estimated that, a large chunk of the population in developing countries depend largely on medicinal plants and the services of trad medical practitioners to meet their health needs [4]. This may be attributed to its affordability, accessibility, and minimal side effects, as compared to modern medicines [5]. Bambara nut (BN), as a native crop, is widely cultivated in most sub-Sahara African countries. It is an indigenous African legume belonging to the family of *Fabaceae* and sub-family of *Faboidea*. The common names of BN vary across the continent. In Igbo-speaking parts of Nigeria it is called Okpa, while the Yoruba parts call it Epa-roro, and the Hausa-speaking parts call it Gurujia. In South Africa, it is Njugo; Malawi is Nzama; while Zambia is Katoyo [6]. Bambara nut (BN) is mainly cultivated for its seed in most rural areas in sub-Saharan Africa [7]. Mayes et al*.* [8] reported that BN has been less researched on despite its anecdotal applications in traditional medicine. It's been anecdotally reported to possess nutraceutical properties including antimicrobial, antidiabetic and antioxidant potentials [9].

There are anecdotal reports suggesting that Bambara nut is used in the treatment of neurological disorders. This is so as recent neuroscience researches encompass different neurscientific domains like neurogenomics, neuroimaging, neurophysiology, neurorehabilitation, and most importantly neuroepidemiology [10], although the African continent has less than adequate reliable data on neurological disorders, even as it is largely affected by varying neurological diseases such as Konzo [11]. The present study therefore, aims to determine the cognito-motor modulatory

functions of Bambara nut (*Vigna subterranea*) extract in Bitter Cassava (*Manihot esculenta*) flour Konzo-induced Male Wistar rats. Its significance lies in its contribution to konzo studies and neurological diseases, thereby serving as a reference for other researchers, and providing information on the benefits of Bambara nuts as a nutrient-rich plant nut, as well as its significance in the fields of neuroscience, nutrition, and dietetics. As a matter of fact, understanding the protective effects of Bambara nut extracts on the neurological damage caused by cassava toxicity can contribute in developing strategies for prevention and treatment of konzo, as well as other related neurological diseases.

2. MATERIALS AND METHODS

2.1 Procurement of Animals

Twenty-five (25) adult male Wistar rats weighing between 80–200g were procured from the Animal House of the Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt, based on availability. The rats were housed in clean, disinfected wooden cages with sawdust as bedding. The animal house provided a controlled environment with a 12-hour light/dark cycle, 50–60% humidity, and a temperature of roughly 30°C. These conditions were maintained throughout the acclimatization and experimental periods. The rats had free access to clean water and standard animal feed.

2.2 Collection and Identification of Plant Materials

Bitter cassava root and Bambara nut were procured from the Ministry of Agriculture, Agricultural Development Programme Retail Outlet, Rumuodumaya, Rivers State, Nigeria. The plant materials were identified by Dr. Edwin Nwosu of the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria.

2.3 Preparation of Bitter Cassava Root

The cassava roots were washed in water to eliminate any strenuous materials. Peeled roots were cut into little chunks (2-5cm thickness) and minimally air dried to retain its cyanide content. It was subsequently grinded to powder for easy digestion and fed the animals.

2.4 Extraction of Barbara Nut

Procured Bambara nuts were washed to eliminate dust and other impurities. The nuts were air-dried at room temperature for a minimum of 7 days to remove moisture. Nuts were weighed and grounded into powder using an electric grinding machine [12]. Specifically, 2 kilograms of the powdered material was macerated in 80% methanol for 72 hours. After three-days, the mixture was filtered through a double-layered muslin cloth to remove debris, and the liquid portion was further filtered through Whatman filter paper. The collected filtrate was concentrated at a temperature of 45°C using a rotary evaporator. It was subsequently transferred to an evaporating dish and dried over a water bath. Once dried, the extract was safely stored in a desiccator [13,14,15].

2.5 Induction of Konzo Disease

After an initial period of acclimatization, the experimental animals were fed improperly processed bitter cassava flour for a period of three weeks. The quantity of powdered cassava flour administered was equivalent to 86 grams per kilogram of the rats' body weight as was earlier described by Enefa et al. [16].

2.6 Acute Toxicity Study

The acute toxicity of Bambara nut (*Vigna subterranean*) extract was determined and found to be consistent with Megwas et al. [17]. The study was conducted in accordance with the guidelines for the care and use of laboratory animals [18].

2.7 Experimental Design

Twenty-five (25) adult male Wistar rats weighing between 180–200g were randomly divided into five (5) groups of five (n=5) rats per group. After an initial period of acclimatization, Konzo was induced following improperly processed bitter cassava flour feeding for a period of three weeks and administered BN extract. Neurobehavioral assessments were conducted weekly (7 days interval) using the Barnes Maze Task and Hand Grip Task. Treatment was as follows for 28 days:

Group 1: Control group - rats in this group received normal distilled water and rat chow
Group 2: Konzo-Induced group – rats in this group received improperly processed bitter cassava flour
Group 3: Low Dose Extract group - rats in this group received 100mg/kg BN
Group 4: Medium Dose Extract group - rats in this group received 200mg/kg BN
Group 5: High Dose Extract group - rats in this group received 300mg/kg BN

2.8 Neurobehavioral Assessment

2.8.1 Barnes maze

This is a dryland-based rodent's behavioral paradigm, used for assessing spatial learning and memory. The rats use extra-maze visual cues to locate an escape hole that allows them to escape from open space and bright light into a dark box beneath the maze. The time it takes to locate the escape hole into the dark box beneath the maze was recorded [19,20]. The maze was assembled in a noise-free area. Before testing, the maze was cleaned with 70% ethanol to eliminate dirt. The animals were positioned in the maze's high circular center. The round surface rotated clockwise at a modest pace. After the circular surface stopped spinning, the animal's retreat from the open area and blinding light into the dark box under the maze was recorded for up to 300 seconds. Each animal was tested three times over three days.

2.8.2 Hand grip strength test

The hand grip strength test as a neurobehavioral test is used to evaluate motor function and deficit in laboratory models of central nervous system disorders. It is more convenient, and gives less stress to animals, and has been widely used to assess neuromuscular disorder and to evaluate the effect of chemicals on motor performance. This study modified the conventional hand grip strength by horizontally pulling the tail of the rat, and placing the rat on a horizontal wire grid [21]. The maximum time the rat stayed before releasing its forepaws from the wire grid was recorded with a stop watch monitored by another inspector. This procedure was carried out three times at 10 minutes intervals to allow animals rest, and the average time was recorded.

2.9 Data Analysis

Graph Pad Prism and Microsoft Excel 2019 were used to analyze the data. Values are presented as mean ± standard error of mean. One-way analysis of variance (ANOVA) was used to compare the groups. The confidence interval was set at 95%, and a p-value less than 0.05 was considered.

3. RESULTS

3.1 Results Showing the Assessment of Cognition and Perceptual Activities Using Barnes Maze Task

Konzo-induced rats consistently exhibited significant prolonged trial times compared to Group 1 (Control) rats ($p < 0.05$), indicating impaired cognitive function. Administration of BN extract at varying doses resulted in significant improvement in trial times compared to both Groups 1 and 2 (Control and Konzo-induced) ($p < 0.05$): These findings highlight the potential neuroprotective effects of BN. In weeks 1, 2, 3 and 4 trials, konzo-induced rats (Group 2) displayed significantly increased trial times compared to Group 1 rats ($p < 0.05$). Administration of BN extract at different doses amongst Groups 3, 4, and 5 rats resulted in significant reduction in trial times compared to Group 2 rats ($p < 0.05$), suggesting a possible modulatory function of the extract of Bambara nut in bitter cassava induced neurotoxicity.

3.2 Results of Assessment of Motor Function and Muscular Strength Using Hand Grip Task

Figs. 1-8 present results for the assessment of motor function and muscular strength using hand grip task from week 1 to week 4. The hand grip task, assessing motor function and muscular strength in rats, showed significant differences between Groups 1 and 2 (Control and konzo-induced) rats ($p<0.05$), indicating possible adverse effects of konzo on motor performance. Compared to Group 1, Group 2 rats (konzo induced group) exhibited lower hand grip trial times across all weeks (weeks 1-4), reflecting impaired motor function caused by Konzo. Administration of varying doses (100mg/kg, 200mg/kg, and 300mg/kg) of BN demonstrated significant improvement in hand grip times compared to both Groups 1 and 2 ($p<0.05$), highlighting the potential of BN in mitigating the adverse effects associated with konzo on motor function.

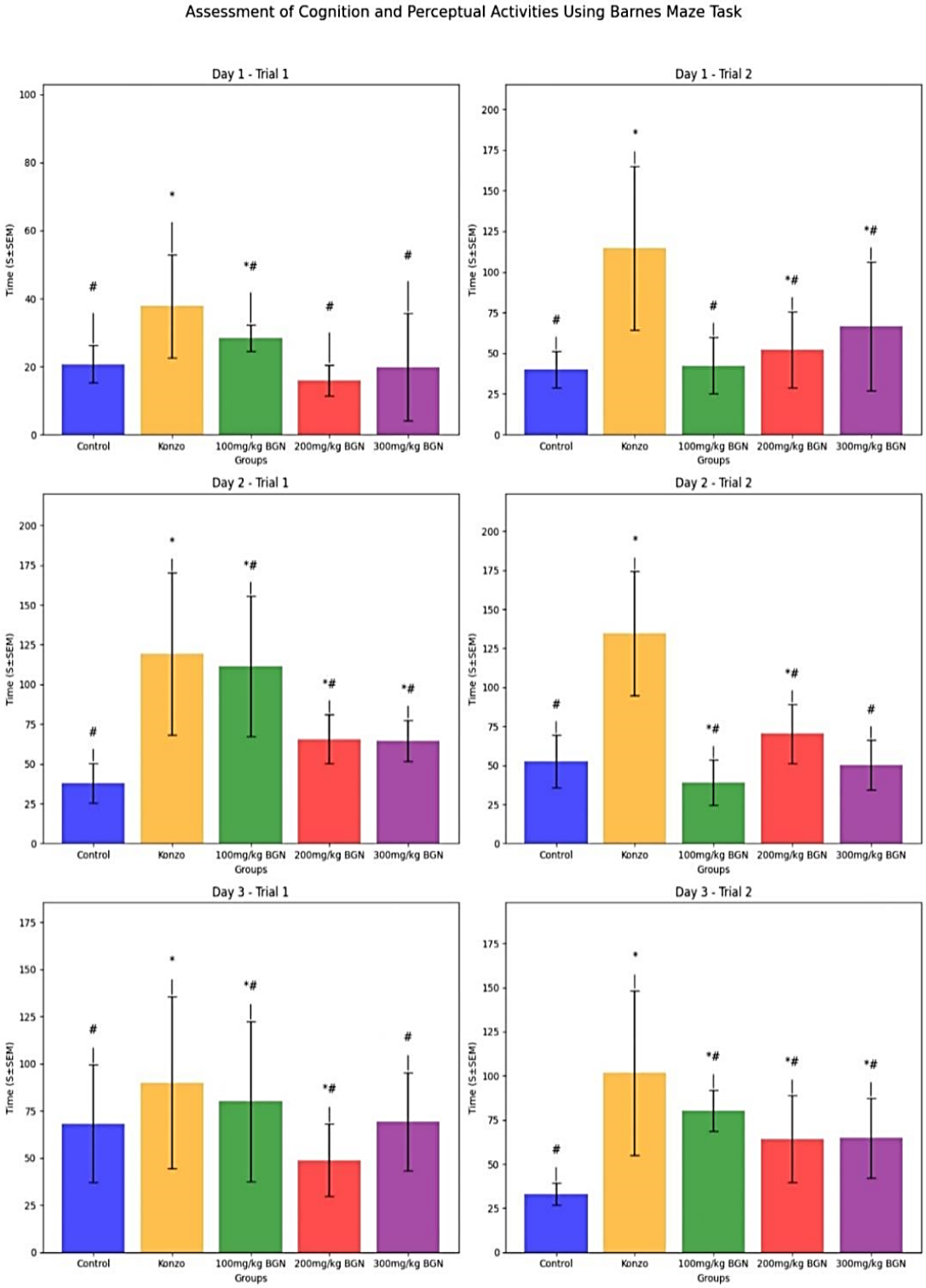

Fig. 1. Assessment of cognition and perceptual activities using barnes maze task on konzo-induced and BN fed rats in week 1

*Values are presented in Mean ± SEM; n=5, * = means values are statistically significant at p<0.05 when compared to the control values; # =means values are statistically significant at p<0.05 when compared to group 2 (konzo) values*

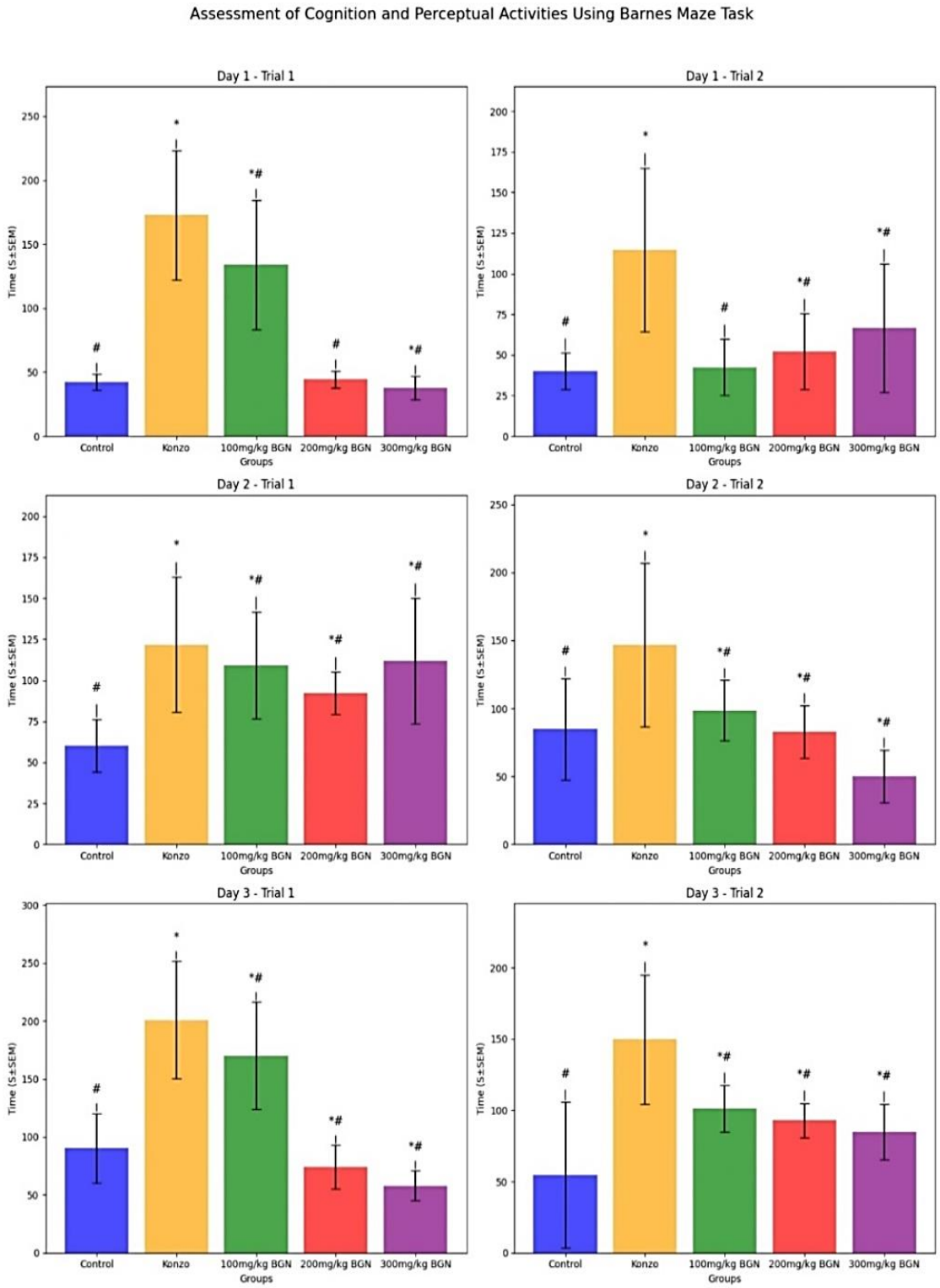

Fig. 2. Assessment of cognition and perceptual activities using barnes maze task on konzo-induced and BN fed rats in week 2

*Values are presented in Mean ± SEM; n=5, * = means values are statistically significant at p<0.05 when compared to the control values; # =means values are statistically significant at p<0.05 when compared to group 2 (konzo) values*

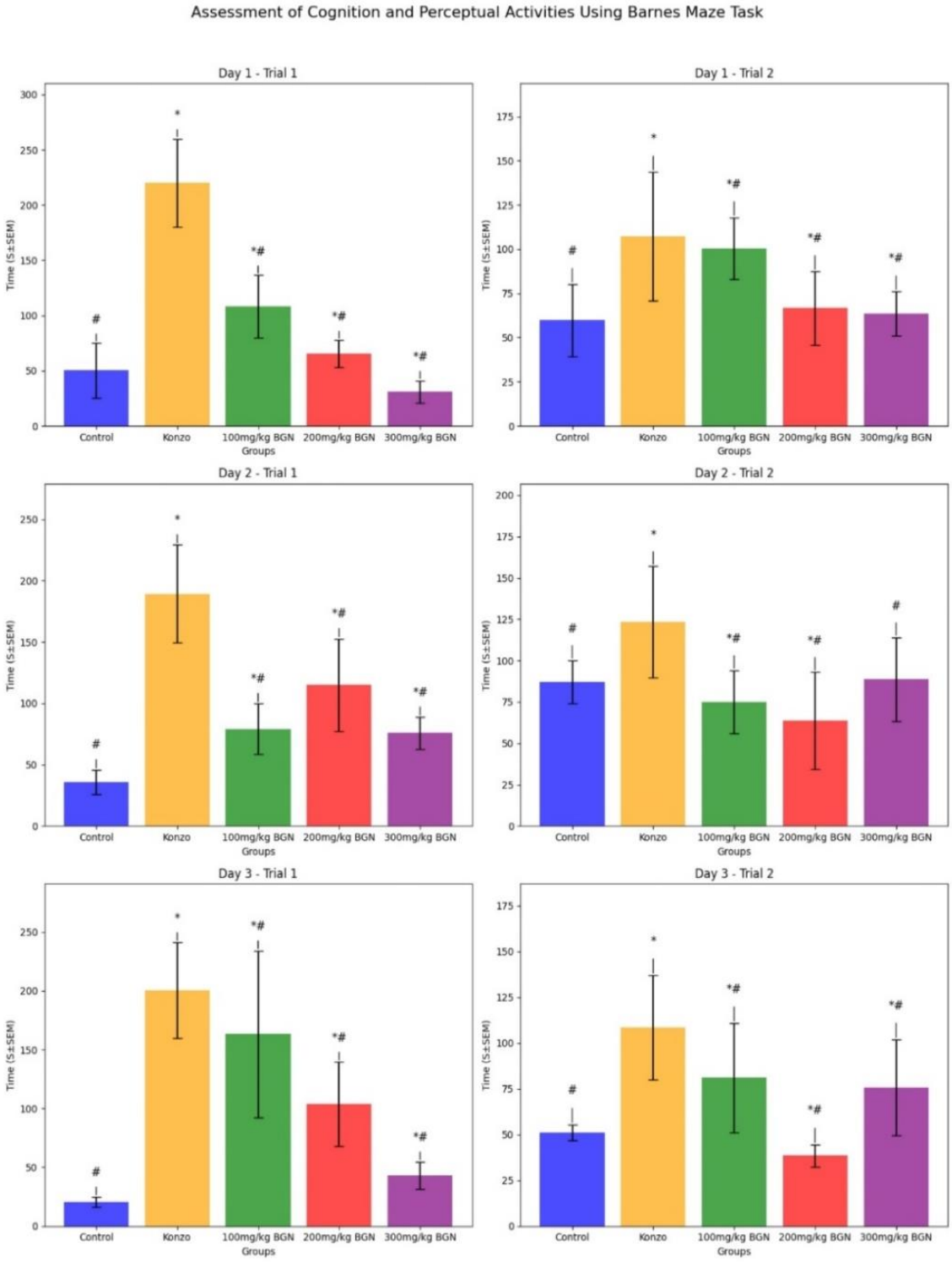

Fig. 3. Assessment of cognition and perceptual activities using barnes maze task on konzo-induced and BN fed rats in week 3

*Values are presented in Mean ± SEM; n=5, * = means values are statistically significant at p<0.05 when compared to the control values; # =means values are statistically significant at p<0.05 when compared to group 2 (konzo) values*

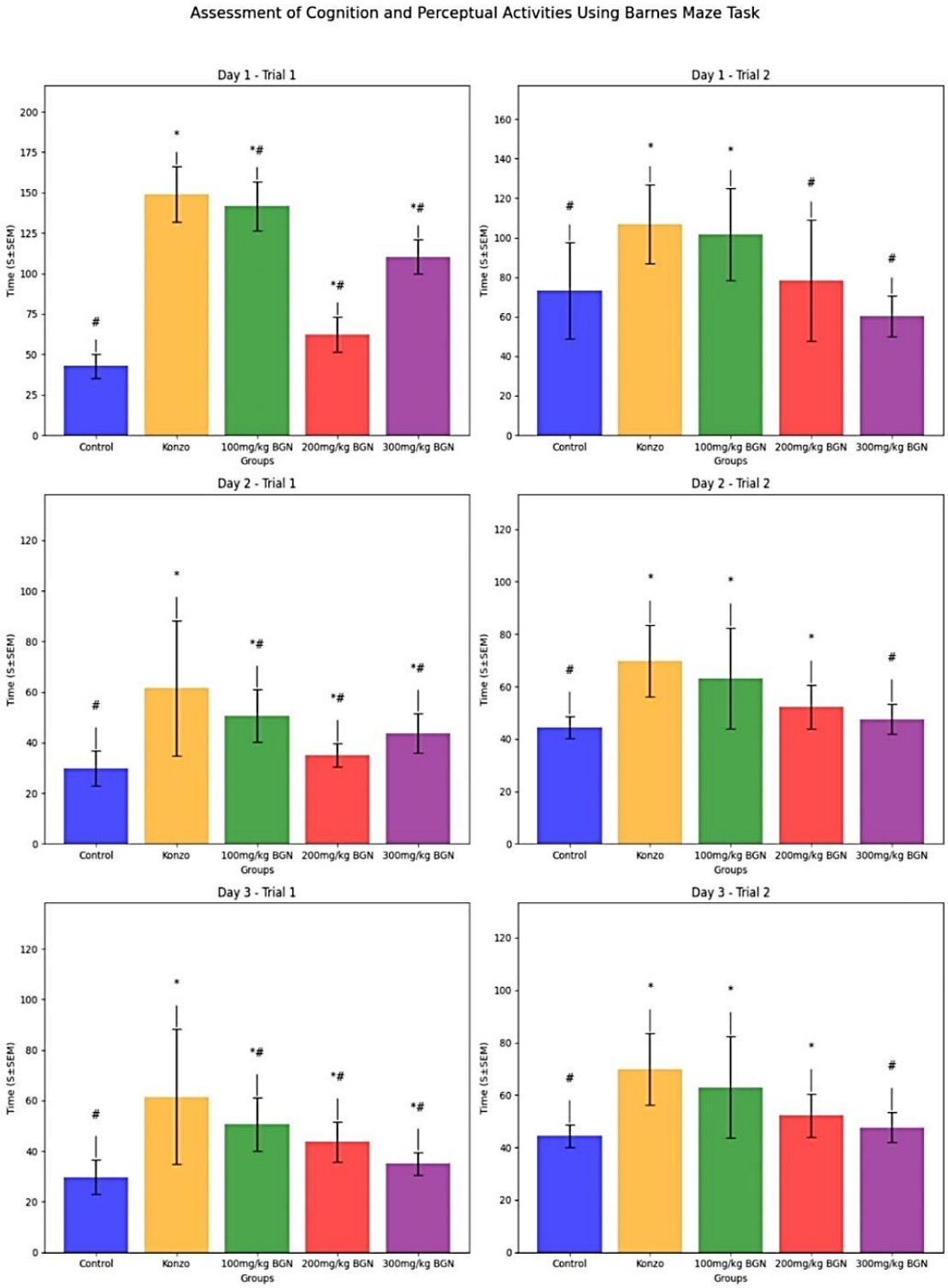

Fig. 4. Assessment of cognition and perceptual activities using barnes maze task on konzo-induced and BN fed rats in week 4

*Values are presented in Mean ± SEM; n=5, * = means values are statistically significant at p<0.05 when compared to the control values; # =means values are statistically significant at p<0.05 when compared to group 2 (konzo) values*

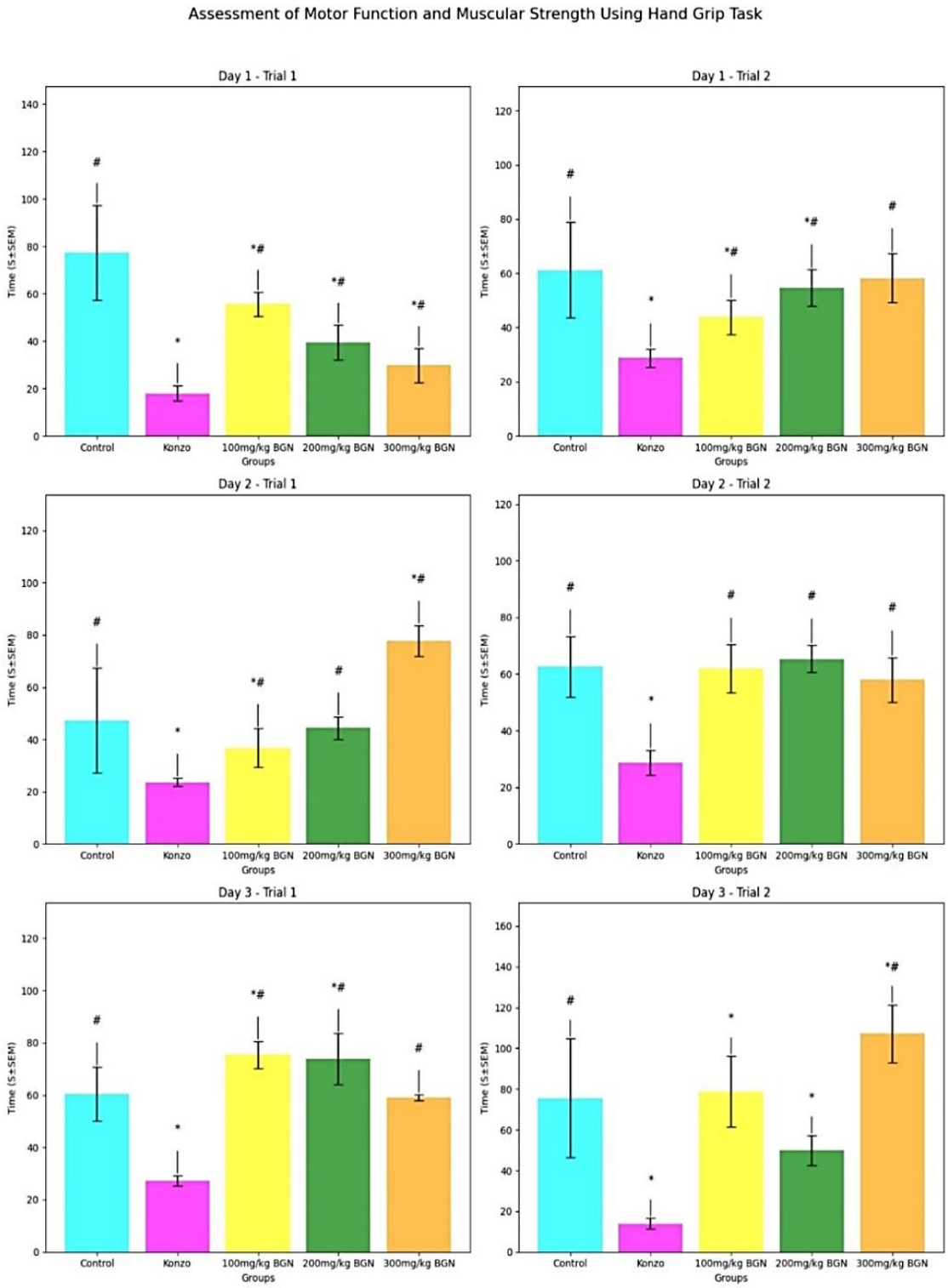

Fig. 5. Assessment of motor function and muscular strength using hand grip task on konzo-induced and BN fed rats in week 1

*Values are presented in Mean ± SEM; n=5, * = means values are statistically significant at p<0.05 when compared to the control values; # =means values are statistically significant at p<0.05 when compared to group 2 (konzo) values*

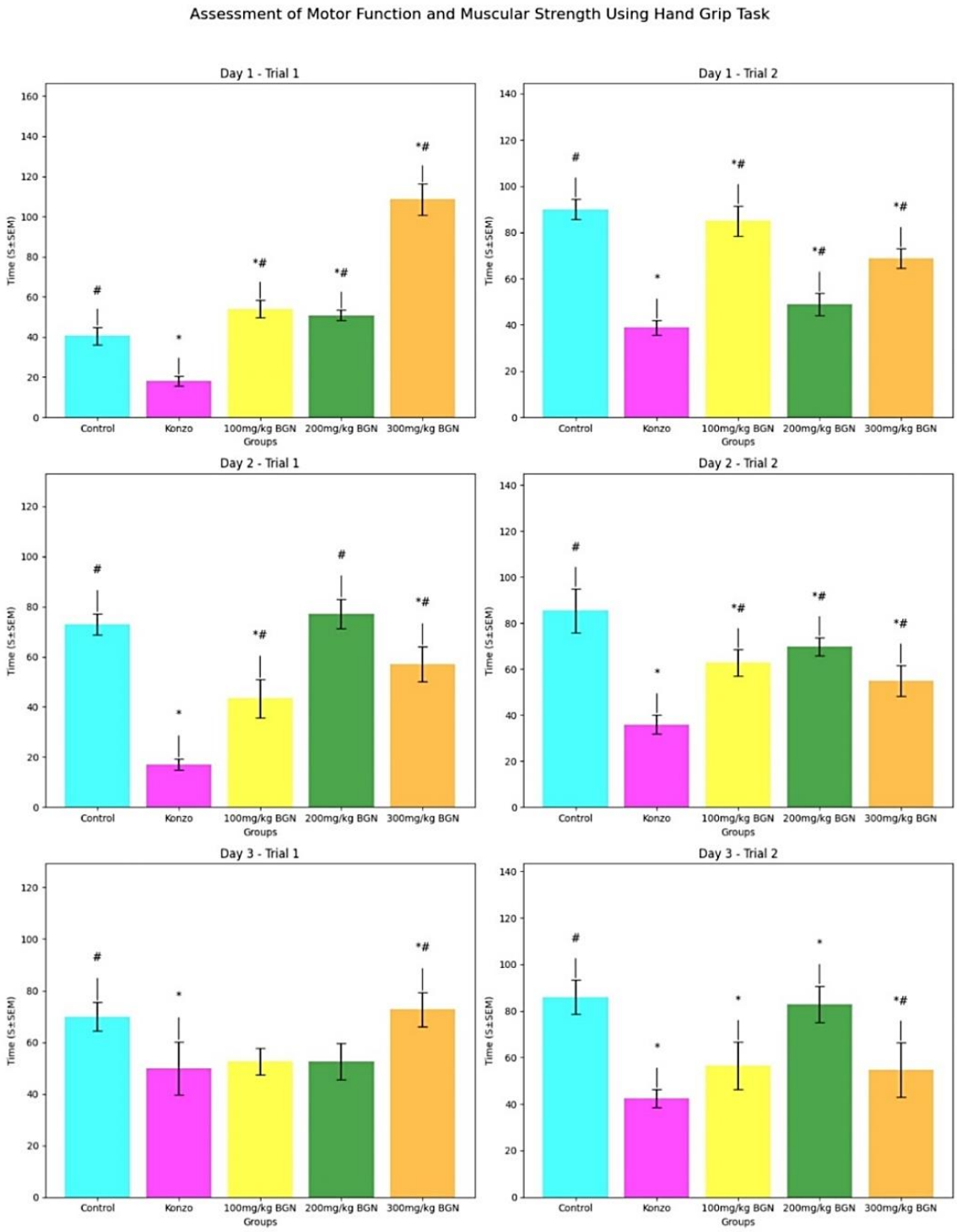

Fig. 6. Assessment of motor function and muscular strength using hand grip task on konzo-induced and BN fed rats in week 2

*Values are presented in Mean ± SEM; n=5, * = means values are statistically significant at p<0.05 when compared to the control values; # =means values are statistically significant at p<0.05 when compared to group 2 (konzo) values*

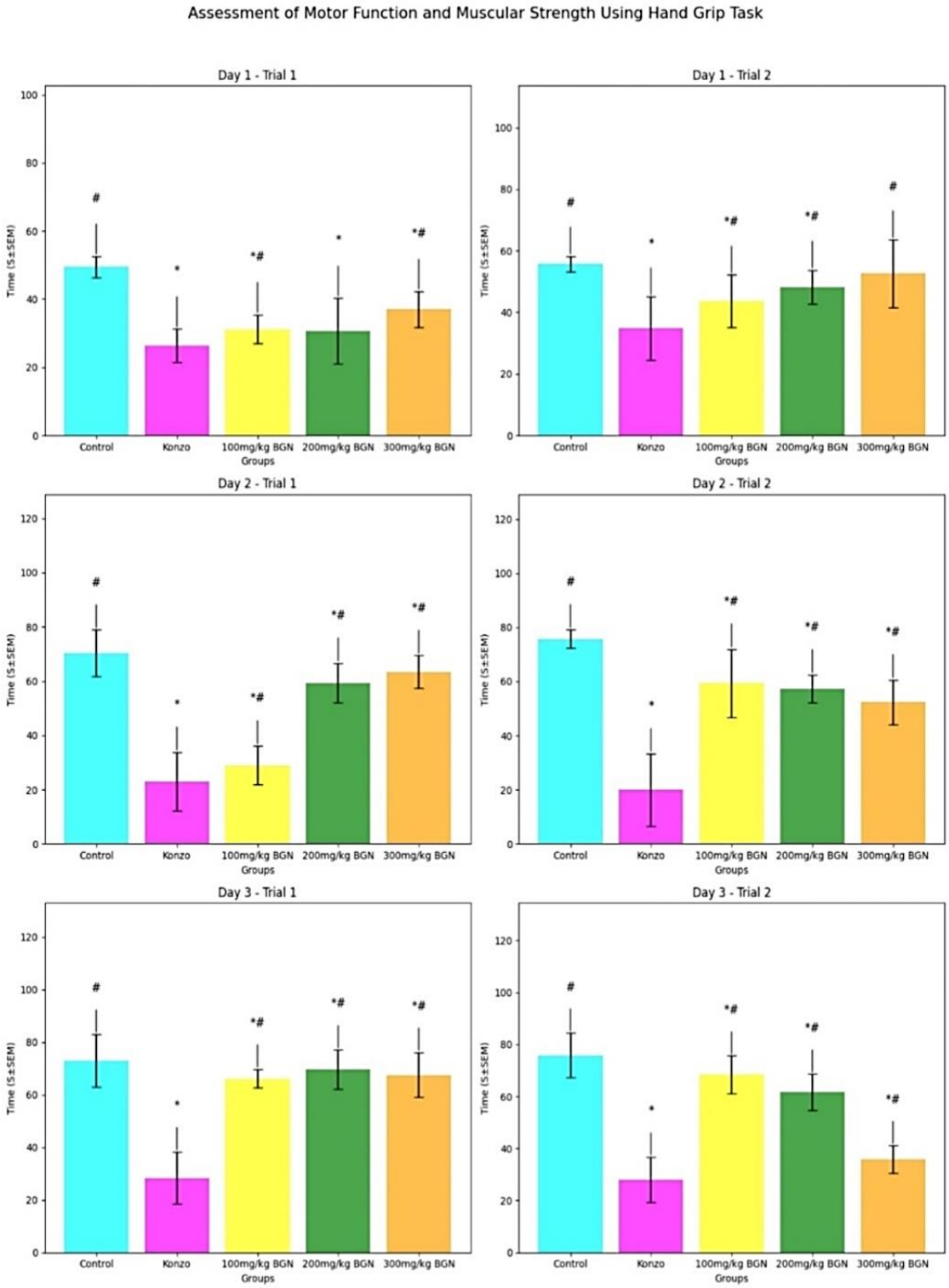

Fig. 7. Assessment of motor function and muscular strength using hand grip task on konzo-induced and BN fed rats in week 3

*Values are presented in Mean ± SEM; n=5, * = means values are statistically significant at p<0.05 when compared to the control values; # =means values are statistically significant at p<0.05 when compared to group 2 (konzo) values*

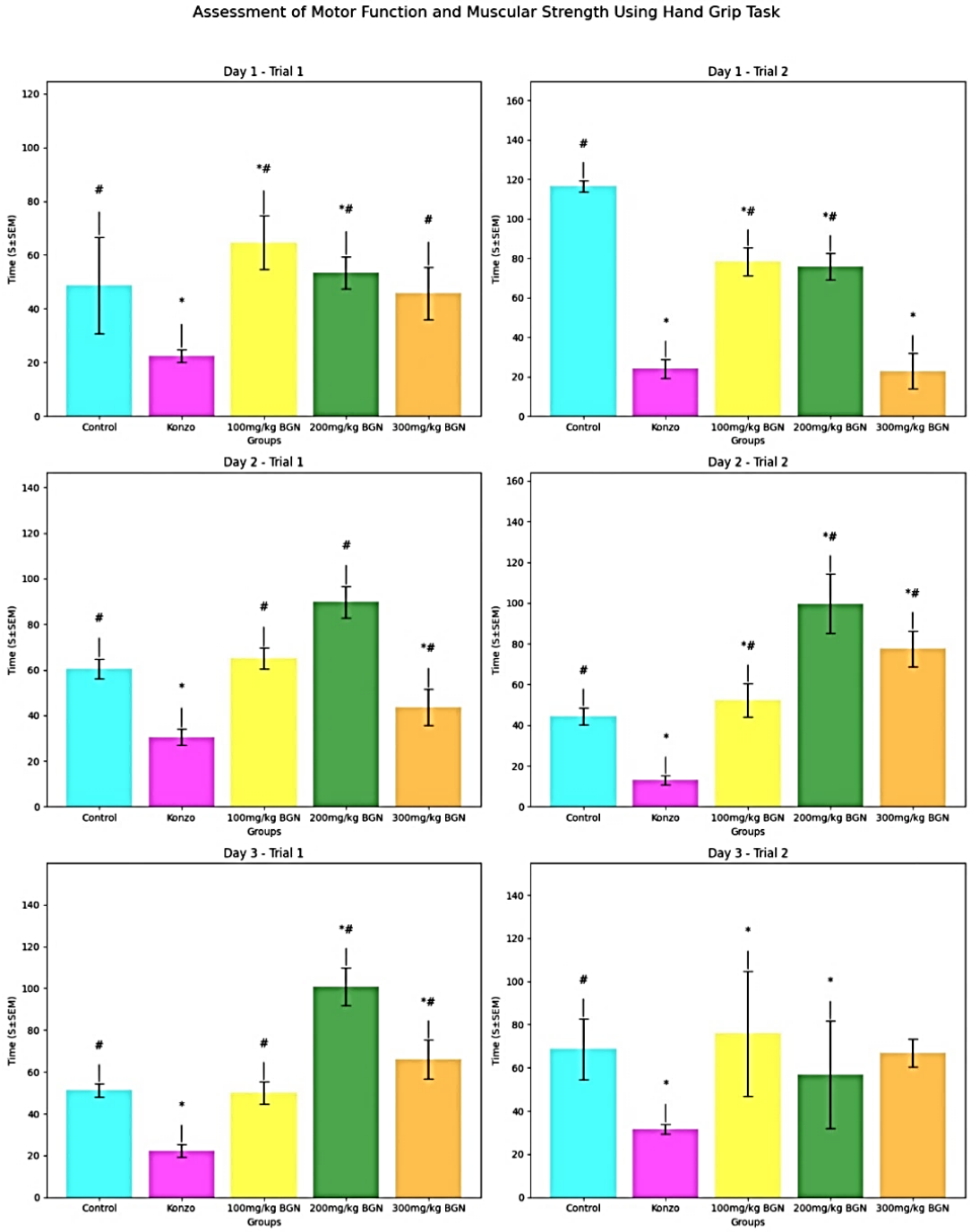

Fig. 8. Assessment of motor function and muscular strength using hand grip task on konzo-induced and BN fed rats in week 4

*Values are presented in Mean ± SEM; n=5, * = means values are statistically significant at p<0.05 when compared to the control values; # =means values are statistically significant at p<0.05 when compared to group 2 (konzo) values*

4. DISCUSSION

The modulatory influence of natural plant products on neurological and behavioral parameters has gained significant attention due to its potential therapeutic applications. Bambara nut, also called African groundnut, is a widely consumed and nutritionally rich legume in Africa.

It is highly valued for its high protein content, essential amino acids, and various bioactive compounds. Previous studies have reported its potential neuroprotective properties, but its specific effects on bitter cassava konzo-induced neurotoxicity remain largely unexplored [22]. Understanding the modulatory effects of Bambara nut extracts on neurobehavioral parameters is crucial as it may provide valuable insights into its potential application for the prevention or treatment of konzo. Hence this study investigated the modulatory influence of Bambara nut (*Vigna subterranea*) (BN) extract on neurobehavior in bitter cassava konzo-induced Wistar rats.

Assessment of neurobehavior of konzo-induced Wistar rats and its subsequent treatment with BN revealed that administration of BN extract improved cognitive and motor functions impairments induced by Konzo in rats, particularly at higher doses. BN showed significant positive effects in the Barnes Maze Task, with improvements in trial times compared to Konzo-induced rats. In the Hand Grip Task, BN, especially at higher doses, effectively ameliorated motor impairments induced by Konzo. The potential neuroprotective properties of Bambara nut may be attributed to its phytochemicals such as Flavonoids and Tannins, which have been linked to various health benefits, including anti-inflammatory, antioxidant, and neuroprotective properties [22,8]. The study's findings are consistent with previous studies which reported the neuroprotective properties of Bambara nut and its potential therapeutic applications in the treatment of various diseases, including neurodegenerative disorders [9].

5. CONCLUSION

Bambara nut extract improved cognito-motor functions in a dose dependent manner, resulting in a possible prevention of Konzo in male Wistar rats. This finding may be attributed to the rich phytochemicals identified in Bambara, such as Flavonoids and Tannins, which have been linked to various health benefits, including anti-inflammatory, antioxidant, and neuroprotective properties.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was obtained from the University of Port Harcourt Ethical Committee through a communication referenced: UPH/CEREMAD/REC/MM90/051.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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