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Anti-toxic Effect of Abelmoschus esculentus Leaf Diet on Monosodium Glutamate-induced Organ Dysfunctions and Genotoxicity

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ABSTRACT,

The potential of *Abelmoschus esculentus* leaf diet (AELD) in the reversal of Monosodium glutamate (MSG)-induced toxicity was assessed in this study. Eighteen (18) female Wistar rats (95±5 g) were equally divided into three (3) groups (n=6). Group A was the control (given I mL/kg distilled water) while Group B rats were exposed to MSG at 1000 mg/kg body weight for 60 days, and Group C was fed with a 20% AELD in the last 30 days of MSG exposure. On the 61st day, all rats were sacrificed with their liver, kidneys, heart, and femurs harvested for serum biochemistry assay, histology, and determination of micronuclei formation frequency. MSG caused a significant ($p<0.05$) and non-significant ($p>0.05$) increase in the body weight change compared to the control and AELD-treated groups, respectively. There was no significant ($p>0.05$) difference in the liver, relative total kidneys, and heart weights of rats in all the experimental groups. However, MSG significantly ($p<0.05$) and non-significantly ($p>0.05$) increased creatinine and BUN levels, respectively, in the MSG-treated group when compared to the control. These increases were reversed by AELD in the AELD-treated group. In addition, ALT activity was significantly ($p<0.05$) increased in the MSG-exposed group compared to other groups. AELD significantly suppressed this ALT activity, including the MSG-induced micronuclei formation and macrophage infiltration in the bone marrow. The histopathology induced by MSG in the liver, kidneys, and hearts were not completely reversed by AELD in the AELD-treated rats. In conclusion, this study shows that *Abelmoschus esculentus* is a potential anti-toxic agent in reversing MSG-induced anomalies in the liver, kidneys, heart, and bone marrow of treated rats.

Keywords: *Abelmoschus esculentus;* Genotoxicity; Histology; Monosodium glutamate

INTRODUCTION

Monosodium glutamate (MSG), also known as Vetsin or Ajinomoto, is most commonly used as a taste enhancer in food preparation worldwide. MSG is obtained from molasses through the fermentation of beet sugar, sugar cane, starch, and corn sugar (Petersen *et al*., 2024). Although classified as GRAS (Generally Recognized as Safe) by regulatory agencies, including the FDA and WHO (Lisiecka, 2025), mounting evidence suggests that its toxicity manifests particularly with long-term use or at high concentrations (Ragab, 2018). The LD_{50} of MSG in rats is 15,000-18,000 mg/kg (Abdulsalam *et al*., 2018), yet chronic exposure at sub-lethal doses has been associated with various pathological conditions (Surendra *et al*., 2021).

The deleterious effects of MSG on major organs, including the liver (Surendra *et al*., 2021) and heart (Geha *et al.,* 2001; Kesherwani *et al*., 2022), have been documented. The mechanism of MSG toxicity is multifaceted: MSG binds to glutamate receptors found in several tissues, including the kidney, liver, and heart (Abdou *et al*., 2020; Surendra *et al*., 2021), thereby altering their structural integrity and functional capacity. Additionally, MSG has been implicated in the generation of reactive oxygen species (ROS), leading to oxidative stress-mediated cellular damage (Farombi and Onyema, 2006; Kesherwani *et al*., 2022).

Research has demonstrated that MSG intake may induce the development of micronuclei (MN) and reactive oxygen species (ROS), which are acknowledged indicators of carcinogenesis and mutagenic potential (Aghaei *et al.,* 2021; Kesherwani *et al*., 2022). At a dosage of 4 mg/g body weight, MSG was reported to significantly induce the formation of micronucleated polychromatic erythrocytes (MNPCEs) in rats, indicating chromosomal damage during erythropoiesis (Farombi and Onyema, 2006). MSG was found to cause toxicity to the nuclear organization of host cells, leading to alterations in chromatin structure and DNA integrity (Umbuzeiro *et al.,* 2017; Hamdy *et al.,* 2018).

Abelmoschus esculentus, commonly known as okra or lady's finger, is a flowering plant belonging to the family

Malvaceae (Vipin and Yadav, 2021). It is native to Africa and is widely cultivated in tropical, subtropical, and warm temperate regions due to its nutritional and medicinal properties (Grubben and Denton, 2004; Vipin and Yadav, 2021). The pods have been traditionally used to soothe the digestive tract and reduce cholesterol levels because of their antioxidant and anti-inflammatory properties attributed to phenolic compounds, flavonoids, and vitamin C content (Abdel-Razek *et al.,* 2023). It is also thought to have potential anti-diabetic properties due to its ability to regulate blood sugar through inhibition of α-glucosidase and α-amylase enzymes (Khan *et al*., 2022). The plant's bioactive components include quercetin, catechin, and epicatechin, which contribute to its free radical scavenging capacity (Marwa *et al*., 2023). Its leaves, however, have not been extensively explored for their medicinal benefits and remain sparsely studied for their nutritional usage compared to the pods. Therefore, this study aims to investigate the effect of *Abelmoschus esculentus* leaf diet in ameliorating MSG-induced toxicity in the liver, kidney, heart, and bone marrow.

MATERIALS AND METHODS

Plant collection and authentication

Fresh leaves of *Abelmoschus esculentus* were bought from the general market at Igbo-Ora Community of Oyo State, Nigeria. The leaves were authenticated by a herbarium curator at the Department of Biosciences and Biotechnology, University of Medical Sciences, Ondo state. Voucher specimen of the plant sample (UNIMED P.B.T.H No, 035) was thereafter deposited in the herbarium of the same Department.

Preparation of feed

The feed formulation was carried out in line with previous studies (Rouch *et al.,* 1994; Aruomaren *et al.,* 2023). The diet contains 20% powdered *Abelmoschus esculentus* leaf, 34% Maize, 23% SBM, 10% Wheat Offal, 6% PKE meal, 5.05% Limestone, 1% DCP, 0.2% Lysine, 0.2% Methionine, 0.3% Common salt, and 0.25% Vitamin Premix. The mixture was pelletized for easy consumption by the experimental rat model.

Experimental animals

Eighteen (18) female Wistar rats weighing between 90-100g were procured from the Animal House of the University of Medical Sciences, Ondo state. The animals were given unrestrained access to standard rat diet and clean water at *ad libitum*. Ethical approval number (UNIMED-AREC/Apv/2024/003) was obtained from the institutional animal ethics committee.

Experimental design

The rats were randomly distributed into three groups with each group consisting of six animals. The animals were exposed to the following treatment regimen:

- Group 1: Control group received 1 mL/kg distilled water only orally
- Group 2: 1000 mg/kg of MSG only administered by oral gavage for 60 days (Agada *et al.,* 2023)
- Group 3:1000 mg/kg of MSG administered by oral gavage for 60 days + 20% *Abelmoschus esculentus* feed formulation for the last 30 days.

The rats were weighed every week throughout the duration of the study. Twenty hours following the last treatment, the rats were anaesthetised (50 mg/kg Ketamine), bled through the retro-orbital sinus, and euthanised by cervical dislocation, following which the liver, kidneys, and heart tissues harvested.

Biochemical assays

Blood samples were collected into plain test tubes and used to determine the blood urea nitrogen (BUN) and creatinine levels, and alanine aminotransferase (ALT) activity using the RANDOX kit as described by the manufacturer.

Histology

4% neutral buffered-formalin fixed tissues (liver, kidneys, heart) were processed for routine paraffin embedding and subsequent tissue sectioning (5 µm thick), haematoxylin and eosin (H&E) staining, and histological examination as described by Chayen *et al.* (1973). The stained slides were afterwards evaluated under a bright field light microscope (Olympus Corporation, Tokyo) and photomicrographs obtained.

Genotoxicity

The *in-vivo* micronucleus assay in this study was carried out as described by Schmid (1975) and Samuel *et al.* (2024). Briefly, the femur was removed and the bone marrow extruded unto a prepared glass slide which contain a drop of foetal bovine serum. The bone marrow was smeared and air dried for 24 hours. After 24 hours, the slides were fixed in absolute methanol for 3 - 5 minutes, air dried, and stained with May-Gruenwald stain and subsequently with 5% Giemsa solution 3 - 5 minutes each. The stained slides were viewed under the light microscope and scored for the frequency of micronucleated polychromatic erythrocytes (mPCEs) in 1000 polychromatic erythrocytes (PCEs) using a tally counter.

Statistical analysis

The data collected were subjected to one-way analysis of variance with Turkey's multiple comparisons test using GraphPad prism version 8. All values were expressed as mean ± standard error of the mean (SEM) with p-value (<0.05) quoted as statistically significant.

RESULTS

In Table 1, the MSG caused a significant ($p<0.05$) and non-significant ($p>0.05$) increase in the body weight change compared to the control and AELD-treated groups, respectively. There was no significant ($p>0.05$) difference in the relative total kidneys, liver, and heart weights of rats in all the experimental groups (Table 1).

In Figure 1, MSG significantly ($p<0.05$) and non-significantly ($p>0.05$) increased creatinine and BUN levels, respectively, in the MSG-treated group when compared to the control. These increases were reversed by AELD in the AELD-treated group (Figure 1). In addition, ALT activity was significantly ($p<0.05$) increased in the MSG -exposed group compared to the control and AELD-treated groups (Figure 1).

Table 1: Body and organ weight changes in treated groups

Parameters (%)	GROUP A	GROUP B	GROUP C
Body weight change	47.60 ±7.49	67.15±12.86[a]	50.96±10.43
Relative total kidney weight	0.712±0.043	0.709±0.019	0.727±0.048
Relative liver weight	4.398±0.425	3.827±0.428	4.228±0.160
Relative heart weight	0.370±0.017	0.420±0.069	0.370±0.045

Values are expressed as mean ± standard error of the mean (SEM). [a] Significantly (p<0.05) different from the control group A. Treatment groups: Group A: 1 mL/kg distilled water (Control), Group B: 1000 mg/kg MSG only; Group C: 1000 mg/kg MSG + 20% *Abelmoschus esculentus* leaf diet. N = 6.

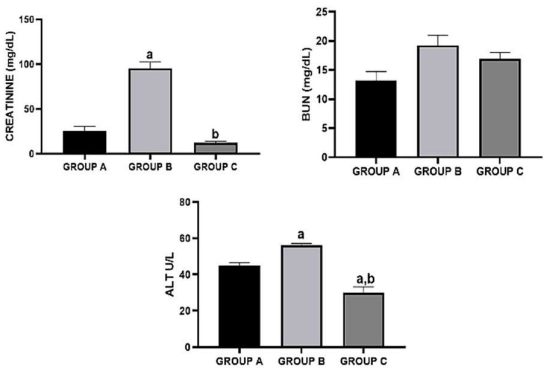

Figure 1: AELD reserved MSG-induced kidney and liver biomarkers in treated rats. [a] Significantly (p<0.05) different from the control group, [b] Significantly (p<0.05) different from the Monosodium glutamate (MSG) group. Treatment groups: Group A: 1 mL/kg distilled water (Control), Group B: 1000 mg/kg MSG only; Group C: 1000 mg/kg MSG + 20% *Abelmoschus esculentus* leaf diet. N = 6.

Figures 2 and 3 showed the formation of micronuclei and macrophage infiltration in the bone marrow cells of the treated experimental rats. The frequency of micronuclei was significantly (p< 0.05) increased in the MSG-treated group B compared to the control and AELD-treated groups. Also, MSG significantly (p<0.05) stimulated the infiltration of macrophages in the MSG-treated rats compared to other groups (Figure 3). The AELD attenuates the induced micronuclei formation and macrophage infiltration by MSG, in the bone marrow (Figures 2 and 3.

The photomicrograph of the liver tissue of treated experimental rats are shown in Figure 4. No significant visible changes in the architecture of the liver were observed in the groups. However, in the MSG alone-treated rats in group B, the liver showed moderate random hepatocellular degeneration and mild central vein congestion. Group C rats showed mild random hepatocellular degeneration with mild central vein congestion and mild oedema. In addition, MSG-exposed rats showed mild glomerular tuft hypercellularity, mild random single cell necrosis and mild mixed cell infiltration in the glomeruli, tubules and interstitial in the kidney tissues (Figure 4).

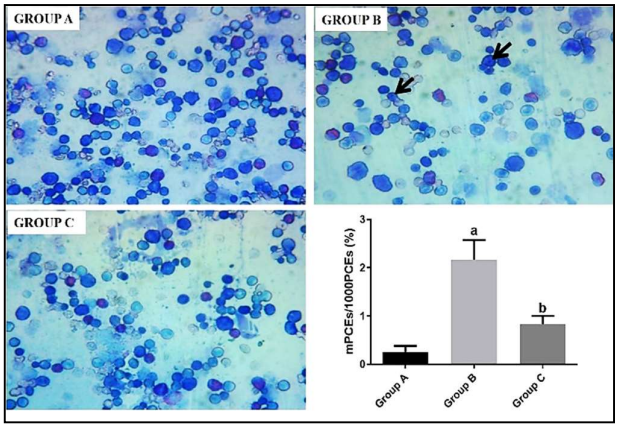

Figure 2: AELD suppressed MSG-induced micronuclei formation in treated rats bone marrow. [a] Significantly (p<0.05) different from the control group, [b] Significantly (p<0.05) different from the Monosodium glutamate (MSG) group. Treatment groups: Group A: 1 mL/kg distilled water (Control), Group B: 1000 mg/kg MSG only; Group C: 1000 mg/kg MSG + 20% *Abelmoschus esculentus* leaf diet. Magnification is x400. N = 6.

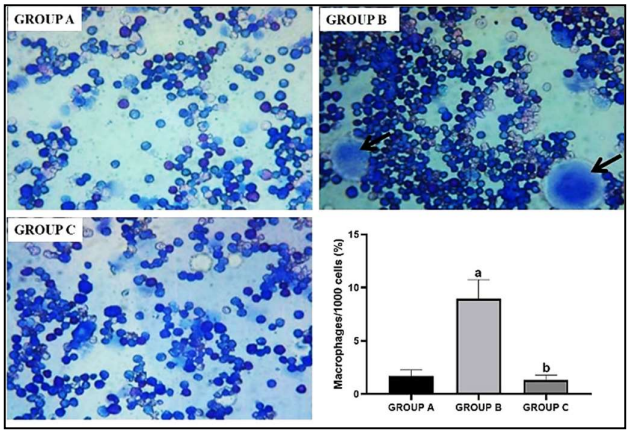

Figure 3: AELD suppressed MSG-induced macrophage infiltration in treated rats bone marrow. [a] Significantly (p<0.05) different from the control group, [b] Significantly (p<0.05) different from the Monosodium glutamate (MSG) group. Treatment groups: Group A: I mL/kg distilled water (Control), Group B: 1000 mg/kg MSG only; Group C: 1000 mg/kg MSG + 20% *Abelmoschus esculentus* leaf diet. Magnification is x400. N = 6.

The kidney tubules are apparently healthy in all the groups (Figure 4). For the cardiac tissues, there were no visible lesions observed in group A (Figure 4), except that in group B, loss of myocardial striations is seen in locally extensive areas and a mild focal disruption in myocardial architecture (Figure 4).

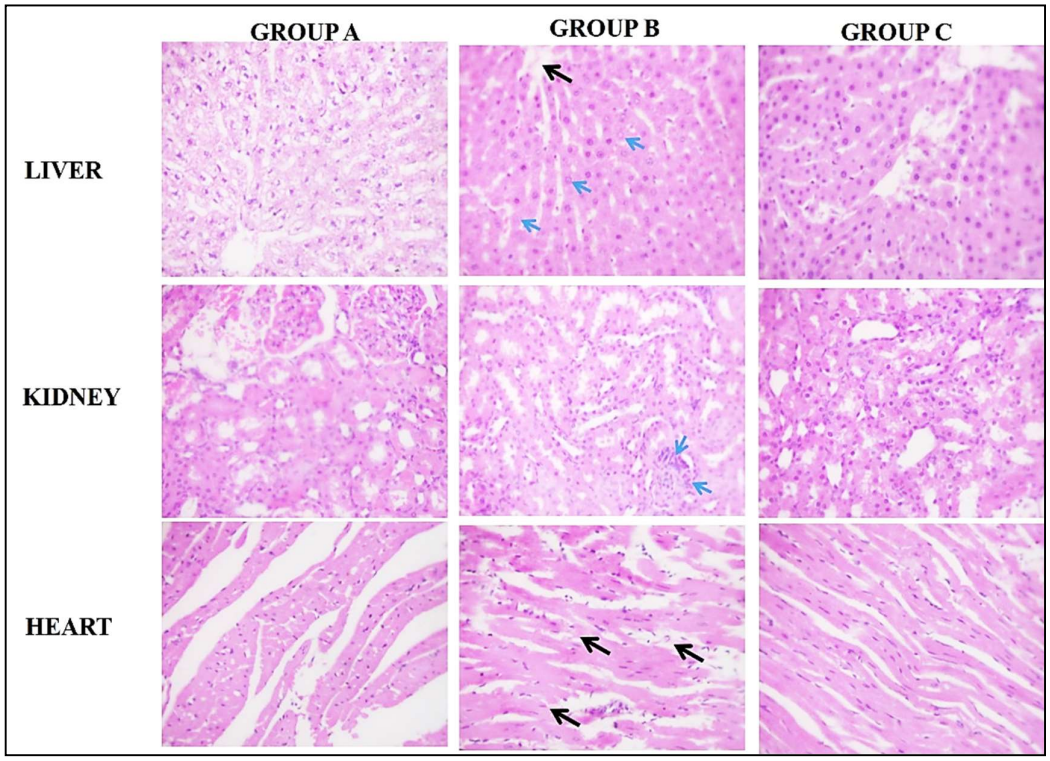

Figure 4: Photomicrographs of the liver, kidney, and heart tissues of treated rats. There are no significant visible changes in the architecture of the liver in group A. However, in Group B, the liver showed moderate random hepatocellular degeneration (blue arrow) and mild central vein congestion (black arrow). Group C rats showed mild random hepatocellular degeneration with mild central vein congestion and mild oedema. There was mild glomerular tuft hypercellularity, mild random single cell necrosis and mild mixed cell infiltration in the glomeruli, tubules and interstitial in the kidney tissues of Group B rats (blue arrow). Group B rats showed loss of myocardial striations in locally extensive areas and a mild focal disruption in myocardial architecture (black arrow). Treatment groups: Group A: 1 mL/kg distilled water (Control), Group B: 1000 mg/kg MSG only; Group C: 1000 mg/kg MSG + 20% *Abelmoschus esculentus* leaf diet. Magnification is x100, N = 6.

DISCUSSION

The deleterious effects of Monosodium glutamate (MSG) on major organs, including the liver (Surendra *et al*., 2021), and heart (Geha *et al.,* 2001; Kesherwani *et al*., 2022), have been documented.

In this study, MSG did significantly affect the body weight changes, confirming earlier findings by Mohamed *et al.* (2021), but contradicting the report of Shi *et al.* (2010), who reported no association between MSG consumption and obesity in a five-year longitudinal study. In addition, a series of five carefully controlled experiments on adult rats and mice, reported by Tordoff *et al*. (2012), found that chronic consumption of MSG caused no effect on body weight or body composition. The relative kidney weight of the MSG-exposed rats, which was not significantly different from that of the control animals, contradicts the findings of Mohamed *et al.* (2021) and Slima and Ragab (2023), who reported organ hypertrophy in MSG-exposed animals. Differences in dosage of MSG in this study, duration of administration, strain susceptibility, and metabolic efficiency may be responsible for these variations.

The notable increase in creatinine and blood urea nitrogen (BUN) levels in the MSG-exposed rats compared to the control and AELD-treated rats suggests the nephrotoxic potential of MSG, which aligns with previous reports. Al hamed *et al.* (2022) reported a significant increase in creatinine and urea levels in rats exposed to MSG, attributing these changes to oxidative stress-mediated glomerular and tubular damage. Muritala *et al.* (2024) similarly reported significant elevations in urea and creatinine levels in rats fed MSG, further corroborating the nephrotoxic effects of MSG.

This finding of elevated ALT activity in MSG-exposed rats is consistent with Tawfik and Al-Badr (2012), who demonstrated that MSG administration resulted in elevated liver enzymes. Mohamed *et al.* (2021) stated that the increase activity of ALT, as well as urea and creatinine levels in MSG-exposed rats, could signify the liver and kidney-damaging potential of MSG, respectively. Although, there was a limitation of not assessing other liver damage biomarkers, such as albumin, AST, ALP, and GGT, in this study, the ameliorative effect of AELD on these biochemical parameters suggests its hepatoprotective and nephroprotective properties, possibly mediated through its antioxidant potential (Wahyuningsih *et al.,* 2021).

The histopathological features observed in the liver, kidney, and heart tissues of the MSG-exposed rats support the biochemistry results and confirm the pathologic effect

of MSG on these organs, which suggests an ongoing organ injury (Mohamed *et al.,* 2021). These findings are consistent with the histological lesions typically associated with chemical-induced hepatotoxicity (Han *et al.,* 2020; Abd El Hady Mousa *et al.,* 2021; Samuel *et al.,* 2024). For example, glomerular hypercellularity, random single-cell necrosis, and mixed-cell inflammation in the glomeruli and tubules have been reported in MSG toxicity (Zou *et al*., 2021; Ramos-Tovar and Muriel, 2023). The glomerular hypercellularity may represent compensatory proliferation in response to nephron loss or early signs of glomerulosclerosis. Tawfik and Al-Badr (2012) reported similar renal histopathological changes, including tubular degeneration, interstitial inflammation, and vascular congestion in MSG-treated rats, attributing these changes to oxidative stress and direct glutamate receptor-mediated toxicity in renal tissues. The MSG used in this study consistently produced toxicity in the cardiac tissues, which aligns with the report of Geha *et al.* (2001). The antioxidant and anti-inflammatory properties of *Abelmoschus esculentus*, attributed to their phytocompounds, such as flavonoids, have been reported to be responsible for their organ-protective effects (Abdel-Razek *et al.,* 2023). The plant's parts have been used to reverse histological lesions in the heart, lungs, and kidneys in chemical-induced pathologies previously (Tawfik and Al-Badr, 2012; Tirink *et al.,* 2023). The findings in these different studies share similar histological features with our observations in the present study.

The significantly increased frequency of micronuclei formation in the bone marrow smear of the MSG-administered group indicates the genotoxic capacity and effect of MSG, similar to what has been reported (Ataseven *et al.,* 2016). The toxicity of MSG in humans and experimental animals has been reported with DNA disruption and oxidative stress identified as the underlying causes of the derangement (Tawfik and Al-Badr, 2012; Aghaei *et al*., 2021). The significant reduction in the micronucleated polychromatic erythrocytes frequency in AELD-treated rats was evidence of the antigenotoxic activity of AELD. In addition, the mechanisms underlying the antigenotoxic effects of AELD may be linked to its anti-inflammatory properties, similar to what was reported on another potent medicinal plant (Samuel *et al.,* 2024; Samuel *et al.,* 2025).

Knowing that most body tissues contain resident macrophages and their induction is more prominent during inflammation, the significant macrophage infiltration in the bone marrow of MSG-treated rats suggests the proinflammatory properties of MSG (Abd El Hady Mousa *et al.,* 2021). MSG has been reported to induce macrophage infiltration and proinflammatory markers in experimental animal models (Liguori *et al.,* 2018; Sayed *et al.,* 2020; Abd El Hady Mousa *et al.,* 2021). MSG stimulates pro-inflammatory cytokines, such as TNF-α and IL-6 (Abd El Hady Mousa *et al.,* 2021). However, the immune modulatory activity of *Abelmoschus esculentus* has been documented, including its anti-inflammatory properties (Suhailah *et al*., 2021). The observed anti-inflammatory effect of AELD in this study can be attributed to its rich phytochemical composition, such as quercetin (Abdel-Razek *et al.,* 2023; Marwa *et al*., 2023). In addition, AELD has comparative anti-inflammatory effects against MSG-induced tissue injury like other notable natural products, such as Ginger and Zingerone (Abd El Hady Mousa *et al.,* 2021). One of the mechanisms of action of these natural products against MSG is via the reduction of macrophage infiltration in body tissue (Sayed *et al.,* 2020; Abd El Hady Mousa *et al.,* 2021).

Conclusion

This study investigated the effect of *Abelmoschus esculentus* leaf diet in reversing monosodium glutamate-induced toxicity in the liver, kidney, heart, and bone marrow. The results from this study suggests that *Abelmoschus esculentus* leaf diet is a potential therapeutic agent in reversing the monosodium glutamate-induced liver, kidneys, heart tissues toxicity and micronuclei formation. In addition, the suppression of macrophages infiltration in the bone marrow supports the anti-inflammatory potential of *Abelmoschus esculentus*. However, the authors would recommend the establishment of a dose-response relationship in future studies.

Conflict of Interest

The authors have no conflict of interest to declare.

Authors Contribution

ATE conceptualized, provide resources, investigate, and supervised the research, curate the data, performed the analysis, and write the original manuscript; SES provide resources, curate data and performed analysis, provide software, visualized the data, and write the original manuscript; OOS performed analysis, and contribute to the manuscript writing; AAY contribute to the manuscript writing; NRI contribute to the manuscript writing. All the authors approved the final manuscript before publication.

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