Experimed 2022; 12(2): 38-43

ORIGINAL ARTICLE

DOI: 10.26650/experimed.1085407

The Protective Effects of S-Methyl Methionine Sulfonium Chloride on Brain Tissue Damage in D-Galactosamine-Induced Hepatotoxicity

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Cite this article as: Bayrak BB, Mahmarzayeva D, Turkyilmaz IB, Sacan O, Yanardag R. The protective effects of s-methyl methionine sulfonium chloride on brain tissue damage in D-galactosamine-induced hepatotoxicity. Experimed 2022; 12(2): 38-43.

ABSTRACT

Objective: The objective of the current work is to examine the protective effects of S-methyl methionine sulfonium chloride (MMSC) on brain in galactosamine (GalN)-induced hepatotoxicity in rats.

Materials and Methods: A total of twenty two female Sprague-Dawley rats were randomly assigned into four groups as follows: Group I (n=5), intact control animals; Group II (n=6), animals that received 50 mg/kg/day of MMSC by gavage technique for 3 consecutive days; Group III (n=5), animals injected with a single dose of 500 mg/kg of GalN intraperitoneally (i.p.); and Group IV (n=6) are animals injected with the same dose of GalN (i.p.) 1 hour after MMSC treatment. At the end of the experiments (after 6 hours of the last GalN treatment), all animals were sacrificed under anaesthesia, and brain tissues were dissected out.

Results: A statistically remarkable increase in lipid peroxidation, hydroxyproline, and nitric oxide levels, was detected while a notable decline in the activities of sodium/potassium ATPase was observed in GalN group in comparison with control rats. In contrast, all alterations observed were reversed when MMSC was given to GalN groups.

Conclusion: Consequently, it may be considered that MMSC has a protective role on brain in GalN-induced hepatotoxicity in rats.

Keywords: Galactosamine, S-methyl methionine sulfonium chloride, brain, antioxidant effect, oxidative stress

INTRODUCTION

Galactosamine (GalN) is a derivative of six-carbon amino sugar (galactose) whose extreme accumulation, or formation in organisms give rise to liver injury. The usage of GalN, as a well-known hepatotoxin, alone [or in combination with lipopolysaccharide (LPS)] has been shown to not only triggering depletion of uracil-containing nucleotides, but also disruption of the biosynthesis of uridylate nucleotides in hepatocytes, thereby causing hepatotoxicity/liver dysfunction in animal models (1). Given that the liver is the main organ responsible for the detoxification/biotransformation of chemicals/drugs and foods, damage to the liver by chemicals such as GalN may cause unwanted effects in other major organs (2). In the hepatic and brain cells of mice, the chronic administration of LPS/GalN has been revealed to cause oxidative stress, by disrupting oxidant/antioxidant balance in favour of oxidant substances [e.g., reactive oxygen species, (ROS)], along with inducing clear DNA migration (3). On the other hand, the structure and function of biomolecules (e.g., nucleic acids, lipids, carbohydrates, and proteins) may easily change when oxidative stress conditions emerge (4). Given that the brain consumes vast amount of oxygen and is characterized by being rich in polyunsaturated fatty acids (PUFAs) content, its regions (especially the hippocampus, amygdala, and

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Submitted: 10.03.2022 **Revision Requested:** 20.05.2022 **Last Revision Received:** 24.05.2022 **Accepted:** 06.06.2022

cerebellar cells) are prone to detrimental effects of oxidative stress and ROS damage as well (5).

S-methyl methionine sulfonium chloride (MMSC), also known as vitamin U, has a sulfonium group containing derivative of the essential amino acid L-methionine, that the vegetable source is Brassica species such as white cabbage, Brussel sprouts, kohlrabi, and kale (6). It is also called a vitamin because of its vitamin-like effects. It is being studied as a source of anti-peptic ulcer dietary factors. As of its clinical importance, MMSC had several biofunctions such as cytoprotective agents against gastrointestinal disorders (6) and anti-epileptic effects (7). Besides, MMSC plays an important role by acting as a radical scavenger, as well as anti-fibrotic and anti-inflammatory agent under oxidative stress conditions (8).

The main objective of the present work designed due to limited published articles on the effect of GalN on the brain was to assess the potential protective roles of MMSC on brain injury GalN-induced hepatotoxicity in rats.

MATERIALS AND METHODS

Chemicals and Apparatus

All chemicals were of the highest purity. They were obtained commercially from Sigma-Aldrich Chemical Company (St. Louis, MO, USA) and/or Merck Chemical Company (Darmstadt, Germany). The GalN (catalog number 48250) and MMSC (catalog number 64382) were supplied by Fluka Chemie AG (Buchs, Switzerland).

Animals and Animal Grouping

Female Sprague-Dawley rats (about 200-250 gr and 26-28 weeks old) were chosen for the study. They were acclimated to standard laboratory conditions. Fresh tap water and standard pellet chow were supplied. A total of twenty two rats were randomly assigned into four groups as follows: Group I (n=5), intact control animals; Group II (n=6), animals that received 50 mg/kg/day of MMSC by gavage technique for three consecutive days; Group III (n=5), animals injected a single dose of 500 mg/kg of GalN intraperitoneally (i.p.) (9); Group IV (n=6), animals injected with the same dose of GalN (i.p.) 1 hour after MMSC treatment. At the end of the experiments (after 6 hours of last GalN treatment), all rats were sacrificed under anaesthesia, and brain tissues were dissected out. The study was carried out according to the permission of the Animal Experimentation Local Ethics Committee of Marmara University (Protocol/Approval Number: 053.2020.mar).

Biochemical Analyses

Tissue samples were homogenized in cold physiologic saline using a glass apparatus to obtain a homogenate (1/10 w/v). After centrifugation (at 10,000 xg at +4°C for 10 min), clear supernatants were obtained, which were thereafter used for biochemical analyses.

Lipid peroxidation (LPO) levels were estimated according to Ledwozyw et al. (10). Briefly, appropriate volumes of tissue homogenate and trichloroacetic acid solution were mixed and allowed to stand at room temperature for 15 min. The thiobarbituric acid solution was pipetted to the reaction medium and then boiled in a water bath at 95°C for 30 min. The resultant mixture was mixed with the appropriate volume of n-butanol solution to extract organic phase. The absorbance of the organic phase at 532 nm was then monitored using a spectrophotometer (Shimadzu UV-Mini-1240, Kyoto, Japan) in terms of brain malondialdehyde (MDA), which is undertaken as an index of LPO. Results were expressed as nmol MDA/mg protein.

The hydroxyproline levels were determined by the method of Reddy and Enwemeka (11). This assay is based on alkaline hydrolysis of tissue homogenates thereafter subsequent determination of free hydroxyproline content in hydrolysates. After the hydrolyzation process was done at 110°C for 3 h, hydrolysates were mixed with chloramine-T solution and the oxidation of free hydroxyproline contents was allowed to proceed for 25 min at room temperature. Ehrlich's reagent [*p*-dimethylaminobenzaldehyde in n-propanol/perchloric acid (2:1 ratio v/v) solution] was added to reaction media and the chromophore was developed by incubating the samples at 65°C for 20 min. The absorbance of the samples were then recorded at 550 nm using a spectrophotometer. Results were expressed as µg hydroxyproline/mg protein.

Nitric oxide (NO) levels were carried out by spectrophotometric method of Miranda et al. (12). The principle of this assay is reduction of nitrate to nitrite by VCl_3 in an acidic reaction media containing Griess reagent [mixed an equal volume of sulfanilamide (2% w/v) and *N*-(1-Naphtyl) ethylenediamine dihydrochloride (0.1% w/v)]. After the coloured diazonium complex completed, absorbance of the resultant mixture was measured at 540 nm by a spectrophotometer, and the results were expressed as nmol NO/mg protein.

The sodium/potassium adenosine triphosphatase (Na^+/K^+-ATPase) activities in the brain tissue homogenates were determined by the method developed by Ridderstap and Bonting (13). The main principle of this method was based on determination of inorganic phosphate (P_i) after the hydrolyzation of ATP when the homogenates were incubated with the appropriate amount of ATP. First, total ATPase activities were assayed thereafter Mg^{2+}-ATPase activities were determined in the presence of ouabain. The Na^+/K^+-ATPase activities were calculated by subtraction from the total ATPase of Mg^{2+}-ATPase. The obtained results were expressed as micromoles of P_i/mg protein/h.

Total protein levels in the homogenates were estimated according to the method of Lowry et al. (14). Briefly, proteins were reacted with Cu^{2+} ions in alkaline medium (2% Na_2CO_3 in 0.1N NaOH) and reduced by the Folin-Ciocalteu reagent. The absorbance of the blue-coloured product that colour intensity is proportional to the amount of protein in the sample was evaluated at 500 nm. Bovine serum albumin was used as a standard for determination of protein levels.

Statistical Analyses

To analyse the obtained data, an unpaired t-test and one-way analysis of variance (ANOVA) were carried out, followed by Tukey's test as a post hoc test for multiple comparisons with the aid of the GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA) statistical program. The values were illustrated as the mean±standard deviation (SD). Differences were regarded as significant when the p value is less than 0.05.

RESULTS

The LPO levels of brain tissues are depicted in Figure 1. LPO levels in the GalN group were remarkably elevated by 3.02-fold in comparison with the control group (p<0.0001). Pretreatment with MMSC in the GalN group resulted in an approximately 2.5-fold reduction in LPO levels (p<0.0001; Figure 1).

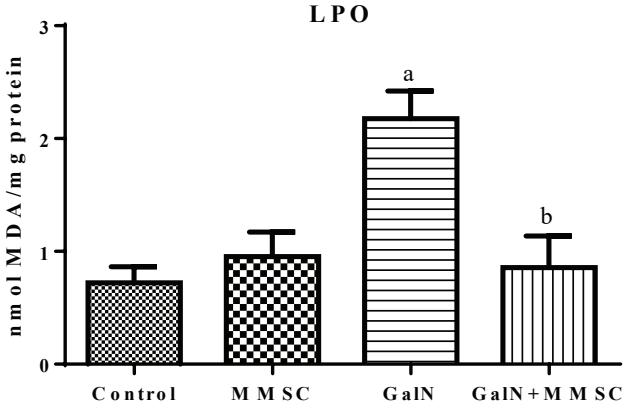

Figure 1. The effect of MMSC on brain tissues lipid peroxidation levels of all rats. Data were presented as mean±SD. Group I (n=5), intact control animals; Group II (n=6), MMSC given group; Group III (n=5), GalN given group; Group IV (n=6), GalN+MMSC given group.

Abbreviations: SD, standard deviation; ANOVA, analysis of variance; LPO, lipid peroxidation; MDA, malondialdehyde; MMSC, S-methyl methionine sulfonium chloride; GalN, galactosamine. To analyze data, an unpaired t-test and one-way ANOVA was carried out, followed by Tukey's as a post hoc test for multiple comparisons. [a]p<0.0001 vs control; [b]p<0.0001 vs GalN group.

Alteration of hydroxyproline levels of all groups is shown in Figure 2. More than 2.5-fold increase in hydroxyproline levels were detected in the GalN group as compared to the intact rats (p<0.0001). Treatment with MMSC to the GalN group led to 8.7-fold decline of hydroxyproline levels in the brain (p<0.0001; Figure 2).

Levels of NO of all groups are presented in Figure 3. NO levels were considerably higher (about 3.5-fold) in the MMSC pretreated group and the GalN group (3.6-fold) (p<0.0001) in comparison with intact rats (Figure 3). By contrast, a statistically significant (p<0.0001) diminishment (approximately 1.5-fold)

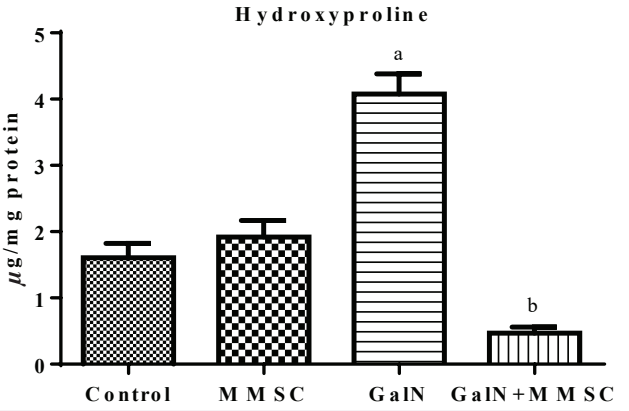

Figure 2. The effect of MMSC on brain tissues hydroxyproline levels of all rats. Data were presented as mean±SD. Group I (n=5), intact control animals; Group II (n=6), MMSC given group; Group III (n=5), GalN given group; Group IV (n=6), GalN+MMSC given group.

Abbreviations: SD, standard deviation; ANOVA, analysis of variance; MMSC, S-methyl methionine sulfonium chloride; GalN, galactosamine. To analyze data, an unpaired t-test and one-way ANOVA was carried out, followed by Tukey's as a post hoc test for multiple comparisons. [a]p<0.0001 vs control; [b]p<0.0001 vs GalN group.

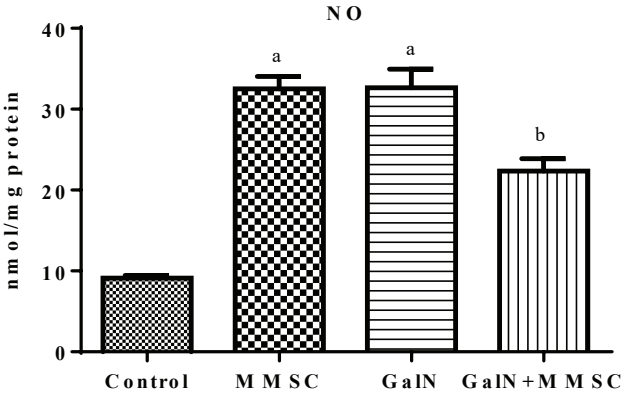

Figure 3. The effect of MMSC on brain tissues nitric oxide levels of all rats. Data were presented as mean±SD. Group I (n=5), intact control animals; Group II (n=6), MMSC given group; Group III (n=5), GalN given group; Group IV (n=6), GalN+MMSC given group.

Abbreviations: SD, standard deviation; ANOVA, analysis of variance; NO, nitric oxide; MMSC, S-methyl methionine sulfonium chloride; GalN, galactosamine. To analyze data, an unpaired t-test and one-way ANOVA was carried out, followed by Tukey's as a post hoc test for multiple comparisons. [a]p<0.0001 vs control; [b]p<0.0001 vs GalN group.

in the levels of NO was detected in brain tissues in the GalN+MMSC group compared to that of the GalN group (Figure 3).

Activities of Na^+/K^+-ATPase of experimental groups are given in Figure 4. A remarkable (more than 2.4-fold) decline in Na^+/K^+-ATPase activities was observed in the GalN group ($p<0.0001$) in comparison with the control group (Figure 4). On the other hand, a considerable elevation (more than 2.1-fold) of Na^+/K^+-ATPase activities in brain tissues in the GalN+MMSC group was detected when MMSC was pretreated with the GalN group ($p<0.0001$; Figure 4).

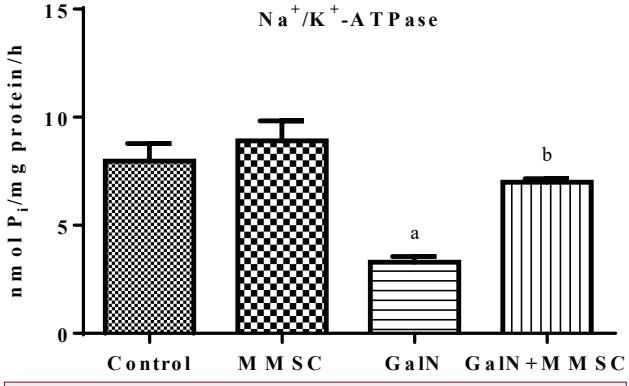

Figure 4. The effect of MMSC on brain tissues sodium/potassium ATPase activities of all rats. Data were presented as mean±SD. Group I (n=5), intact control animals; Group II (n=6), MMSC given group; Group III (n=5), GalN given group; Group IV (n=6), GalN+MMSC given group.

Abbreviations: SD, standard deviation; ANOVA, analysis of variance; Na^+/K^+-ATPase, sodium/potassium ATPase; MMSC, S-methyl methionine sulfonium chloride; GalN, galactosamine. To analyze data, an unpaired t-test and one-way ANOVA was carried out, followed by Tukey's as a post hoc test for multiple comparisons. [a]$p<0.0001$ vs control; [b]$p<0.0001$ vs GalN group.

DISCUSSION

The current outcomes revealed that GalN-induced injury resulted in alterations not only in the levels of LPO, hydroxyproline, and NO, but also in the activity of Na^+/K^+-ATPase enzyme in the rat's brain. These defects are associated with an increase in ROS formation.

The hippocampus, amygdala, and brain cells are highly sensitive to the harmful effects of oxidative stress-mediated tissue injury. This is because the brain is an organ containing high amounts of lipids and their derivatives and utilizes high total basal oxygen levels and consequently forms ROS (5). When ROS attack the PUFAs of membrane lipids, the abstraction of hydrogen from PUFA gives rise to the formation of carbon-centered lipid radicals, thus rapidly interacting with the O_2 to form lipid peroxyl radicals. This in turn, leads to the initiation of a chain reaction known as LPO. The lipid peroxides formed in this way are converted into highly reactive biochemical products such as acrolein, 4-hydroxynonenal, and MDA in organisms. MDA is an important biochemical marker that is frequently determined in the assessment of LPO (15). In the present study, LPO level in the GalN given group was found to be statistically higher than those of intact rats. This might be associated with a potential effect of ROS-mediated brain injury. An increase in LPO levels may be owing to the consequences of GalN-induced oxidative stress via an upsurge of ROS in the brain tissues of rats. In several studies related to central nervous system (CNS) diseases, rise in the levels of LPO has been accepted to play a crucial role in ROS-mediated brain damage (16). The reversed LPO levels may be linked to not only ROS mopping effect, but also antioxidant potential of the MMSC (8).

In general, hydroxyproline (an imino acid) is synthesized by post-translational modification of collagen (viz., hydroxylation of proline), and is one of the main components of the collagen protein. Amino acid sequences of collagen are regularly ordered as Gly-Pro-X or Gly-X-Hyp. In possible two motifs, the sequence of the Gly–Pro–Hyp occurs usually. Collagen contains approximately 99.8% of the hydroxyproline stored in the body and is therefore used as an important biomarker in the diagnosis of collagen and hydroxyproline-related diseases (17). The elevation of oxidative stress levels as well as neuroinflammation was shown to cause modification of extracellular matrix components which may cause Parkinson's disease (18). In the current study, hydroxyproline levels in brain homogenates were determined because it is an important diagnostic indicator of the severity of liver hepatitis caused by GalN (19). According to the findings, hydroxyproline levels in the GalN given group were statistically higher than that of control rats. This alteration might be associated with GalN-mediated toxicity, which causes oxidative stress as well as disruption of membrane integrity via high LPO levels. On the other hand, Ganai and his colleagues (20) reported that 250 mg/kg GalN injection to rats for 12 weeks (twice a week) resulted in the formation of fibrosis/cirrhosis in the liver, with hydroxyproline levels increasing more than 7 times compared to the control group.

NO, a short-lived, small, and freely diffusible gas molecule, is a highly reactive inorganic free radical. The high reactivity of this molecule is due to its small size, high diffusion rate and lipophilic character rather than the unpaired electrons in its structure (21). In the biological system, it is formed as a by-product of the gradual conversion of L-arginine to citrulline via hydroxylation reaction catalysed by NO synthase NOS. NO molecule is formed by the action of the NOS enzyme, which has three isoforms: neural, inducible, and endothelial. Moreover, this enzyme has very important functions in the cardiovascular and CNS (22). Apart from that, NO reacts with other molecules (e.g., superoxide, free sulfhydryl groups, and oxygen) to form reactive products known as nitrites, peroxynitrite, and nitrosothiols. Furthermore, NO has diverse functions depending on its physiologic concentration. The formation of this molecule at a low concentration may protect cells, while at higher concentrations it can act as a cytotoxin that plays a role in tumor an-

giogenesis and progression (23). More so, keeping NO levels in balance, which can have both neuroprotective and neurotoxic effects, is important in the CNS. This is because NO acts as an intracellular signal molecule by stimulating the cyclic guanosine monophosphate cascade (24). On the other hand, a rise in the production of NO has been reported to be responsible for the development of several neurodegenerative disorders (i.e., multiple sclerosis, Parkinson's and Alzheimer's diseases, and ischemia) (25). It thus can be called a "double-edged sword" that has key roles in both physiological and pathological processes (21). In the current study, NO levels in the brain tissue of rats were determined by using Griess reagent. NO levels in the MMSC group were unexpectedly found to be statistically higher than that of control animals. This might be associated with the duration of administration of MMSC (three consecutive days). Oktay et al., stated that the administration of MMSC (50 mg/kg/day) to intact animals for 7 days caused an insignificant elevation of NO levels (7). In GalN injected groups, the levels of NO were remarkably elevated as compared to intact rats. Like GalN, LPS (or in combination with GalN) is an agent that is widely used for the induction of multiple organ damage (2). Abdel-Salam et al., revealed that nitrite levels increased in LPS-induced liver and brain injury (26). On the other hand, pretreatment with MMSC gave rise to a notable decrease in NO levels in the GalN given group, which may be because of the ameliorative effect of MMSC, since NO has been shown to exert neurotoxic effects (27).

Na^+/K^+-ATPase, also known as Na^+/K^+-ion pump, is a cation transport protein that is localized in the plasma membrane of various eukaryotic cells. It has an indispensable role in balancing osmotic equilibrium and electrochemical membrane potential, by exporting three Na^+ ions concomitant to importing two K^+ ions across the membranes of neurons as well as other cells [by use free the energy released during the hydrolysis of one ATP molecule to ADP and P_i]. Furthermore, this enzyme is responsible for maintenance of osmoregulation of both Na^+ and K^+ in hyper- and hypotonic environment, regulating cytosolic pH and Ca^{2+} levels, transmission of nerve impulses through neuron and triggering of intracellular signalling (28). Oligomeric proteins of Na^+/K^+-ATPase are vital for its overall activity, which consist of the alpha (ten transmembrane helix subunits), beta (single transmembrane subunit), and family of small membrane proteins (FXYD) subunits (29). The alpha subunit is mainly responsible for its catalytic activity, while the glycosylated beta subunit regulates the activity and conformational stability of the alpha subunit. Also, interaction between the beta subunit and the alpha subunit is required to complete ion transport. On the other hand, the FXYD subunit is divided into two subgroups namely FXYD1 (mainly found in the heart, skeletal muscle, and brain) and FXYD2 (highly expressed in the kidney), respectively (29). In the current study, injection of GalN caused a remarkable decline in Na^+/K^+-ATPase activities. The deterioration of membrane integrity owing to the increase in LPO levels in the GalN given group may be considered as the reason for the decline in Na^+/K^+-ATPase activities. Additionally, it has been put forward that activity of this enzyme in the brain tissue decreases in conditions such as ischemia, epilepsy attacks and hypoglycemia, as well as in oxidative damage caused by the administration of toxins such as GalN (30). In parallel, decreased Na^+/K^+-ATPase activities have been reported in GalN-mediated oxidative stress in lung tissue of rats (9). According to the present findings, administration of MMSC to the GalN given group led to a notable recovery of Na^+/K^+-ATPase activities. The present research apparently revealed for the first time that treatment with MMSC before GalN injection sharply restored Na^+/K^+-ATPase activities. More so, MMSC had protective effects on the brains of GalN-injected rats. Similar outcome was previously reported by Gezginci-Oktayoglu et al. (8).

The limitation of the study is that in order to fully understand the protective effects of MMSC on brain biochemical parameters, different brain disease models need to develop and the effect of MMSC examined on these models.

CONCLUSION

A limited number of published articles on the harmful effects of GalN against brain damage encouraged the design and execution of the present estudy. The findings obtained from the current work demonstrate that GalN-mediated oxidative stress leads to an increase in LPO, hydroxyproline, and NO levels, and a decrease in Na^+/K^+-ATPase activities. In contrast, all alterations observed were reverted when MMSC was administered to the GalN groups. In the light of the current findings, it may be concluded that MMSC has therapeutic effects against GalN-induced brain toxicity in rats.

Ethics Committee Approval: The study was carried out according to the permission of the Animal Experimentation Local Ethics Committee of Marmara University (Protocol/Approval Number: 053.2020.mar).

Peer-review: Externally peer-reviewed.

Author Contributions: Conception - B.B.B., I.B.T.; Formal analysis- B.B.B., D.M., I.B.T., O.S., R.Y.; Methodology - B.B.B., O.S., R.Y.; Investigation - B.B.B., D.M., I.B.T., O.S., R.Y.; Writing - original draft - B.B.B., O.S., R.Y.; Writing - review & editing - B.B.B., O.S., R.Y.; Performing experiments- B.B.B., D.M.; Supervision - O.S., R.Y.; Final Approval and Accountability- O.S., R.Y.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declare that this study has received no financial support.

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