Effect of Antiepileptic Drug Sodium Valproate on Stomach Tissue Glycoproteins: The Protective Role of Moringa Extract

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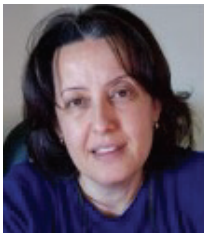

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Abstract

Objective: Sialic acid, hexoses, hexosamines and fucose are components of glycoprotein, glycolipid and/or ganglioside. These glycoconjugates are essential components of cellular membrane and receptors, which are required for normal cellular activities. The levels of these aforementioned glycans are likely to be obstructed under biological conditions (such as oxidative stress) that leads to cellular and tissue damage. Despite the efficacy of valproate as a broad-spectrum antiepileptic drug, it administration is linked to oxidative stress and multiple organ damage. *Moringa oleifera* leaves have been proven to be bioactive food with diverse biochemical benefits, that include antioxidant, wound healing and tissue protective effects.

Methods: In this study, female Sprague-Dawley rats were grouped into four. Group 1: control group given physiological saline; Group 2: animals given only 70% ethanol Moringa leaves extract (0.3 g/kg b.w./day); Group 3: animals that received only sodium valproate (0.5 g/kg b.w./day); Group 4: animals given similar dose of sodium valproate + Moringa extract. The treatments were administered orally for 15 days, and the animals were then fasted overnight and sacrificed. Stomach tissues collected were homogenized in ice-cold normal saline, using a glass homogenizer to make up 10% w/v tissue homogenate.

Results: Analysis revealed that valproate administration resulted in elevated levels of sialic acid, hexoses, hexosamine, and fucose in the stomach tissue homogenates. Conversely, the administration of Moringa extract mitigated the adverse effect of valproate on glycan levels.

Conclusion: Thus, Moringa leave extract can be a good candidate for attenuating valproate-induced toxicity on stomach tissue.

Keywords: Moringa, valproic acid, stomach, sialic acid, hexose, hexosamine, fucose

INTRODUCTION

The stomach is a vital organ of the digestive system involved in the digestion of food. Gastric juice (containing hydrochloric acid and the digestive enzymes pepsin) are secreted by gastric glands.[1] Hydrochloric acid in addition to intrinsic factors is secreted by the parietal cells of the stomach, while chief and neuroendocrine cells secrete pepsinogen and serotonin respectively.[2] The biological action of the fundic glands, cardiac glands, and pyloric glands protects the stomach against corrosion by gastric acid and proteolysis by pepsin at different junctions via secretion of a protective mucus layer. More so, bicarbonate secreted by fundic glands neutralizes excess gastric acid.[1,3]

The protective mucus layer of the stomach is chiefly composed of glycoproteins and glycolipids. In addition to protecting the stomach cells against microbial infiltration and toxins, digestive enzymes and gastric acid corrosion, these glycoproteins and glycolipids play other crucial biological roles that include transport, cell differentiation, cell regeneration, cells' recognition and cell.[4-6]

Sialic acids are a class of alpha-keto acid sugars made up of nine carbon atoms. There are primary components of glycoproteins, glycolipids and gangliosides that have diverse biological functions.[5] The most common member of the sialic acids is N-acetylneuraminic acid. Hexose on the other hand refers to monosaccharides with six carbon atoms. The most common aldohexoses of biological importance are glucose, mannose, and galactose. The biologically most important ketohexose is fructose.[7] The amino form of hexose, formed by the addition of an amino group to a hexose sugar is simply referred to as hexosamine. Essential among hexosamines are glucosamine, fructosamine, galactosamine, and mannosamine. These metabolites are essential substrates for protein glycosylation and the biosynthesis of UDP-N-acetylglucosamine. Altered levels of these metabolites in a tissue are an indication of biochemical derangement such as observed in cancer cells and nutritional stress state.[8-10] Fucose is a 6-deoxy hexose sugar found in found in several glycolipids and glycoproteins of mammalian cell origin. The sugar is unique an unusual because it exists in the L-configuration. Alterations of the fucose levels and structure are implicated in several biological derangements involving immunity and cancer.[11] Therefore, tracking fucose levels in tissue

samples can serve as a tool for the diagnosis and prognosis of tissue malfunction.

Moringa oleifera is a plant with plethora nutritional and diverse therapeutic benefits. The plant has received remarkable attention for use as nutraceuticals, food supplements and/or as herbs.[12,13] The antioxidant,[14] antiinflammatory,[15] wound healing,[16] enzyme inhibition[14,17] and protective effect on tissues such as liver,[18,19] kidney[20] and heart[21,22] make the plant a primary candidate for studies related to drug-induced toxicity.

In this study, the levels of sialic acid, hexose, hexosamine, and fucose in stomach tissue homogenates of valproic acid-administered rats were evaluated. Also, the protective effect of Moringa leaves extract against valproic-induced stomach damaged in rats was assessed.

METHODS

Extract Preparation

M. oleifera leaves obtained from farms located in Sokoto town, Sokoto State of Nigeria were identified and authenticated at the Botany Unit of Biological Sciences Department, Usmanu Danfodiyo University Sokoto. The plant samples were dried under shade, at room temperature before pulverizing. This was followed by Soxhlet extraction using analytic grade 70% ethanol (150 mL) for each 10 g of leave powder. The extraction was performed until a clear siphon of the sample was observed. Under reduced pressure, a rotary evaporator was used to remove the solvent from the extract. The residues were kept at -20 °C in an airtight container until required for use.

Experimental Protocol

Approval of the experimental protocol was obtained from the Experimental Animal Local Ethical Committee of Marmara University (MÜHDEK) (protocol number: 11.2020.mar, date: 10.02.2020). Female Sprague-Dawley rats were randomly divided into four groups. Group 1: control group (n=8); Group 2: animals given only 70% ethanol extract of Moringa leaves for 15 days (0.3 g/kg b.w./day; n=8); Group 3: animals that received only sodium valproate for 15 days (0.5 g/kg b.w./day; n=15); Group 4: animals administered with similar dose of sodium valproate + Moringa extract for 15 days (n=15). Moringa extract and sodium valproate were administered orally. Group 1 were orally given a similar doses of physiologic saline. On the 15th day of the experiment, the animals were fasted overnight, sacrificed, then stomach tissues collected and homogenized in ice-cold normal saline, using a glass homogenizer to make up 10% w/v tissue homogenate.

MAIN POINTS

- The central nervous system (CNS) is primarily affected by epileptic conditions.
- The biochemical composition of epileptic individuals can be distorted either due to loss of coordination of the CNS, antiepileptic drug toxicity or due to injuries.
- Antiepileptic drugs such as valproic acid are associated with elevated oxidative stress levels, distortion of the cellular membrane, and multiple organ damage.
- *Moringa oleifera* is a plant with plethora nutritional and diverse therapeutic benefits.
- The plant has pronounced antioxidant effects, as well as antiinflammatory and wound healing effects.
- The administration of Moringa leaves extract mitigated the adverse effect of valproate on the glycoprotein levels.
- Moringa leaves can be a good candidate for attenuating valproate-induced toxicity.

Biochemical Analysis

The sialic acid levels in stomach tissue homogenates were estimated according to the method of Warren[23] (1959). The levels of hexose and hexosamine were estimated according to the Winzler[24] (1955) method. Fucose levels were measured based on the method of Dische and Shettles[25] (1948), while protein levels in the homogenates were quantified based on the method of Lowry et al.[26] (1951).

Statistical Analysis

Graph-Pad Prism 6.0 (GraphPad Software, San Diego, CA, USA) program was used to analyse obtained data, by using one-way analysis of variance (ANOVA). Differences between groups were determined with Tukey's multiple comparisons test. The results were expressed as mean±standard deviation. The significance of differences was taken at $p<0.05$.

RESULTS

The sialic acid levels in the stomach tissues of all experimental groups are presented in Figure 1. According to the results obtained, a significant difference ($p_{ANOVA}=0.0013$) was observed when the sialic acid levels of all experimental groups were compared with each other. Between the control group and the Moringa administered group, no significant difference ($p>0.05$) was observed. In comparison to normal control rats, the sialic acid of valproate administered rats was significantly increased ($p<0.01$). Interestingly, Moringa extract administration to the valproate group led to a significantly lower ($p<0.01$) SA level in the Moringa + valproate group compared to rats solely given valproate.

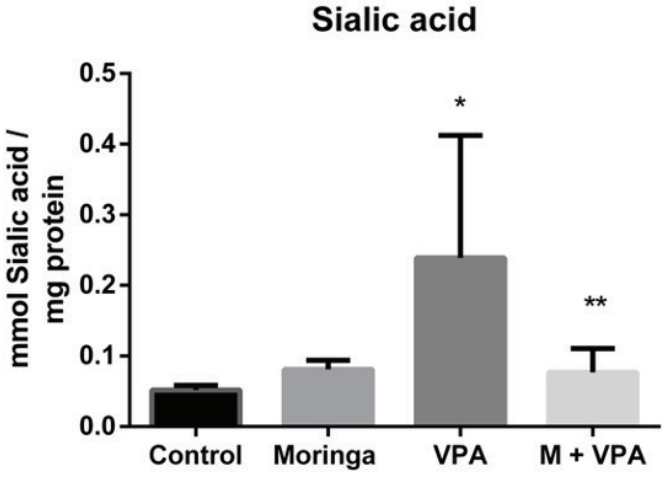

Figure 1. Sialic acid levels of the stomach tissue homogenates of experimental rats
*p<0.01 versus control group; **p<0.01 versus VPA group
VPA: Valproate, M: Moringa

As observed in Figure 2, a significant difference (p_{ANOVA}=0.0001) was observed between hexose levels of all experimental groups. The administration of Moringa leaves extracts to experimental control animals resulted in an insignificant increase (p>0.05) of hexose sugars levels in stomach tissue. In comparison to moreover, valproate administration resulted in a significant elevation (p<0.0001) of stomach tissue hexose sugar levels of control animals. In comparison to the valproate group, treatment of valproate rats with Moringa resulted in a significant decline (p<0.0001) of hexose concentration in the stomach tissue of Moringa + valproate group.

According to the results presented in Figure 3, comparison of hexosamine of all four groups indicated a significant difference (p_{ANOVA}=0.0001). The sole administration of both Moringa extract and valproate resulted in a significant rise in hexosamine levels in experimental rats (p<0.05, p<0.0001 respectively). In comparison to the solely valproate administered group, Moringa treatment to the valproate animals resulted in a significant decline (p<0.0001) of hexosamine level.

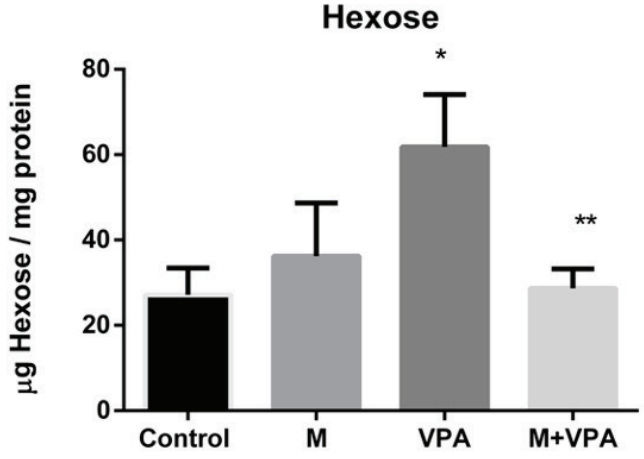

Figure 2. Hexose levels of the stomach tissue homogenates of experimental rats
*p<0.0001 versus control group; **p<0.0001 versus VPA group
VPA: Valproate, M: Moringa

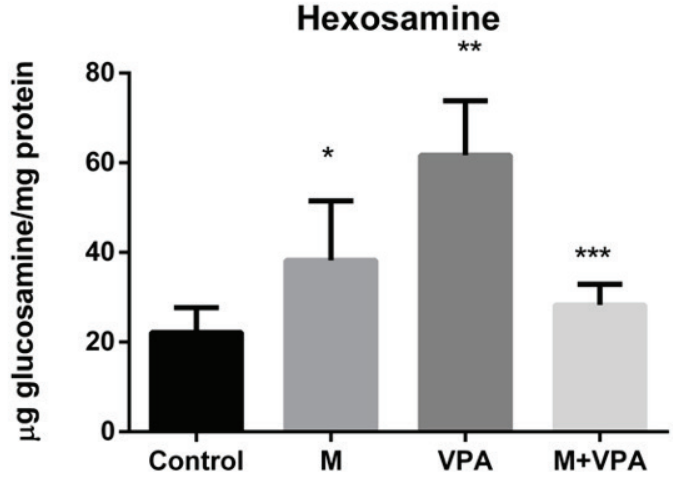

Figure 3. Hexosamine levels of the stomach tissue homogenates of experimental rats
*p<0.05 versus control group; **p<0.0001 versus control group; ***p<0.0001 versus VPA group
VPA: Valproate, M: Moringa

The fucose levels in the stomach tissue homogenates of all experimental groups are given in Figure 4. A significant difference (p_{ANOVA}=0.0001) was observed between all groups when statistically compared. In comparison to the normal control rats, the sole administration of either Moringa extract or valproate resulted in elevated fucose levels (p<0.05, p<0.0001 respectively). The fucose level of the Moringa + valproate group was significantly lower (p<0.0001) than that of the valproate group.

DISCUSSION

Generally, glycoproteins, glycolipids and gangliosides have critical biological roles essential for the normal function of cells, as well as its replication.[4-6,27] because these biomolecules are essentially made up of either proteins or lipids in combination to glycans (such as sialic acid, hexoses, fucose and/or their modified derivative), it is clear to expect abnormalities in glycan levels of tissues under any biological stress, pressure or trauma. The changes in tissue glycans levels can be a biomarker too for assessing the biological and physiological characteristics of the cell change and membrane damage.[28]

Despite the effectiveness and broad antiepileptic effect of valproate, the drug is associated with multiple organs and oxidative stress.[29-32] Moringa leaves on the other hand are rich in phytochemicals and antioxidants, which confers to it antioxidant,[17] antiinflammatory[15] and wound healing[16] effects. Thus, making it a suitable candidate for attenuating valproate-induced oxidative damage.

In this study, valproate administration to experimental animals resulted in elevated levels of all four glycans (i.e., sialic acid, hexose, hexosamine and fucose) assessed from stomach tissue homogenates of rats. The pronounced increase in sialic acid level of the valproate rats is probably linked to an increase in the activity of the sialidase enzyme. The enzyme promotes the hydrolysis of sialic acid from glycoconjugates. Hence, higher levels of sialic acid can be observed in tissue homogenates. Studies have shown that oxidative stress promotes the activity of sialidase, thereby enhancing increased desialization.[33] The increase in sialic acid (a membrane component) levels in stomach tissue homogenates of the

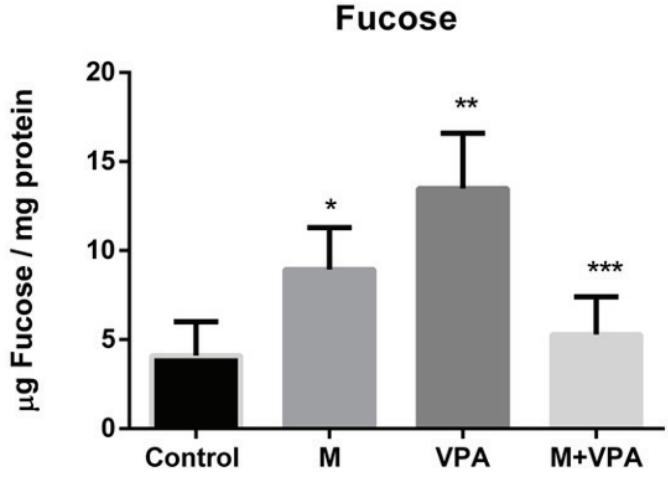

Figure 4. Fucose levels of the stomach tissue homogenates of experimental rats
*p<0.05 versus control group; **p<0.0001 versus control group; ***p<0.0001 versus VPA group
VPA: Valproate, M: Moringa

valproate group may be a direct consequence of cell membrane and tissue damage, which might have arisen due to valproate-induced oxidative damage. The administration of Moringa leaves extract in this study attenuated the negative effect of valproate on sialic acid level. This positive effect is likely because of the antioxidant, antiinflammatory, enzyme inhibition and wound healing potentials of Moringa extracts as earlier reported.[14-17]

Similarly, the concentrations of hexose, hexosamine, and fucose, which are glycoconjugate components of glycoprotein and glycolipids that makes up cell membrane and cellular receptors, were seen to have significantly increased upon administration of valproate to experimental rats. This is most likely a consequence of valproate-induced oxidative stress which damage cellular integrity and distort normal metabolic processes. Conversely, Moringa treatment to the valproate-treated animals mitigated the derangements in levels of hexose, hexosamine, and fucose of stomach tissue homogenates. Previous studies have indicated that antioxidants such naringin combined with vitamin C could prevent elevation of free glycan levels of both plasma, liver and kidney tissues streptozotocin-induced diabetes.[34] Similarly, vitamin U mitigate either amiodarone or D-galactosamine-induced hepatotoxicity, oxidative damage, as well as distortion of glycan levels.[35] Sacan et al.[36] (2021) and Turkyilmaz et al.[37] (2021) have demonstrated that antioxidant and antiinflammatory elements such as zinc could protect against cellular damage induced by streptozotocin. It also prevented oxidative stress and maintained normal levels of glycoconjugates. Just like Moringa extract, herbal formulations such as muthu marunthu[38] and convincing db[39] could protect tissue integrity and prevent the alteration of glycocomponents of glycoprotein levels in experimental animals. The ameliorative and positive biological action of Moringa against stomach tissue damage, as well as on glycoprotein glycan components is linked to it ample phytochemical components and antioxidant minerals. More so, the antioxidant potential of the plant extract must have played a vital role in preventing valproate-induced oxidative stress/damage to the stomach tissue of the experimental animals.

Study Limitations

The study limitation of this study is to fully lighten the beneficial effects of Moringa alcoholic extract on stomach biochemical parameter; thus, thus further stomach disease or toxicity models must be developed and protection of Moringa alcoholic extract must be examined on these models.

CONCLUSION

The administration of valproate to experimental rats distorted the levels of sialic acid, hexose, hexosamine, and fucose in the stomach tissue homogenate. Nevertheless, it coadministration with the Moringa extract offsets changes in tissue levels of the aforementioned glycans in the valproate group. Therefore, Moringa leaves can be a suitable candidate for mitigating valproate-induced toxicity/damage to stomach tissue.

Ethics

Ethics Committee Approval: Approval of the experimental protocol was obtained from the Experimental Animal Local Ethical Committee of Marmara University (MÜHDEK) (protocol number: 11.2020.mar, date: 10.02.2020).

Informed Consent: Animal experiment.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: U.F.M., S.M., Ö.S., R.Y., Design: S.M., Ö.S., R.Y., Data Collection or Processing: U.F.M., S.M., Ö.S., R.Y., Analysis or Interpretation: S.M., Ö.S., R.Y., Literature Search: U.F.M., Ö.S., R.Y., Writing: U.F.M., Ö.S., R.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

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