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Research Article
Pyridoxine and Zanamivir Alter Levels of Dopamine in Brain of Rats with Induced Hyperglycemia by Inhibition of Oxidative Stress

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Abstract
The aim of this study was to evaluate the effect of pyridoxine and zanamivir on dopamine (DA) levels and biomarkers of oxidative stress in brain of rats in presence of sucrose. Thirty six Wistar rats divided in six groups, comprising of 6 rats each. The rats were made to receive for 5 consecutive days intraperitoneal administrations of the following: group 1 (saline solution (NaCl 0.9%), control), group 2 (zanamivir 2.5 mg kg^{-1}), group 3 (pyridoxine 10 mg kg^{-1}), group 4 (sucrose 20%), group 5 (zanamivir+sucrose) and group 6 (pyridoxine+sucrose). The animals were sacrificed at the end of treatment and their brains were dissected in cortex, hemispheres, cerebellum/stem to measure dopamine (DA), glutathione (GSH), lipoperoxidation (TBARS) and enzymatic activities of ATPase using previously validated methods. The levels of glucose were not altered in rats treated with zanamivir and pyridoxine in presence of sucrose. In hemispheres, the levels of DA decreased in groups 3 (pyridoxine) and 4 (sucrose). Glutathione increased in the groups 5 (zanamivir+sucrose) and 6 (pyridoxine+sucrose). In cerebellum/medulla oblongata, DA increased in groups 2 (zanamivir), 3, 5 and 6. In cerebellum/medulla oblongata and cortex peroxidation decreased in group 2, while ATPase activity increased in groups 2 and 3 in cortex regions. Alterations of dopamine levels were seen in animals treated with pyridoxine and zanamivir. Reduction of oxidative stress may be involved in these effects.

Key words: Antiviral, brain, dopamine, pyridoxine, oxidative stress

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Zanamivir is an inhibitor of neuraminidase that is used in the treatment of common cold and in prophylaxis of influenza viral A and B. Wang *et al*. (2012) suggested that the use of zanamivir in prevention of intra-domiciliary flu transmission is modest and based on lesser solid test suggesting that the clinical efficacy of inhibitors of neuraminidase in sick children is still uncertain (Wang *et al*., 2012). Antiviral oseltamivir prescription has been associated with neuropsychiatric behaviors in young patients (Toovey *et al*., 2012). Zanamivir does not reduce the risk of complications of influenza, particularly pneumonia, or the risk of hospital admission or death, suggesting that this group of patients may also be at risk (Heneghan *et al*., 2014).

Deleterious effects of oxygen derived Free Radicals (FR) released during influenza virus infection have been reported. Mice infected with influenza virus type A suffered a significant decrease in pulmonary concentrations of catalase, reduced glutathione and superoxide dismutase. These findings lead the authors to conclude that during influenza virus infection there is oxidative stress (Kumar *et al*., 2005). On the other hand, evidence that oseltamivir administration produces oxidative stress has been provided by the study of El-Sayed and Al-Kahtani (2011). Till date however, there is no evidence that zanamivir treatment provokes oxidative stress.

Any alternative to combat the presence of endogenous FR should require the presence of FR scavengers (Valko *et al*., 2007). The later could be stimulated by using supplementary agents with antioxidant activity (Rani and Panneerselvam, 2001). Calderon-Guzman *et al*. (2004) have proved that vitamin B6 (pyridoxine) possesses antioxidant characteristics and could be a supplementary alternative for this (Calderon-Guzman *et al*., 2004). This hydrosoluble vitamin is eliminated through urine and could be daily replaced with diet. In other words, if this supplement forms part of children's diet, the needed FR scavengers in this age group to ameliorate oxidative stress would be a by-gone incidence. Pyridoxine participates in production of electrolytes which are converted to pyridoxamine and pyridoxal phosphate. These substances act as coenzyme for transaminase and in biosynthesis of neurotransmitters which are important for the development and function of Central Nervous System (CNS) (Rogers and Mohan 1994).

Clinical characteristics of children during pandemic influenza A/H1N1 infection in 2009 in Korea, described by Lee *et al*. (2011) showed that children were more likely to have higher level of serum glucose. In this sense Wang *et al*. (2011) reported that plasma level in fasting animals, was significantly and positively associated with H1N1 virus infection (OR = 1.377, 95% CI: 1.062-1.786, p = 0.016). Moreover, diabetes which is a major risk factor for H1N1 infection, has been frequently observed among severe cases and death due to it (Hanslik *et al*., 2010). In Latin population, obese patients with pneumonia by influenza A/H1N1 have prolonged hospitalization time with a higher morbi-mortality (Arancibia *et al*., 2011). Therefore evaluation of the effect of zanamivir administration on the glucose level seems to be a relevant issue.

Based on the above considerations, the objective of the present study is to evaluate the effect of zanamivir and pyridoxine in the presence or absence of sucrose on the levels of glucose, dopamine, GSH, lipoperoxidation and ATPase in brain regions of rats.

MATERIALS AND METHODS

Thirty six Wistar rats with a weight of 150 g each were deployed for the study and were procured from certified bioterium of Metropolitan University of Mexico City and housed in clean plastic cages and allowed to acclimatize in the room environment from 1 day. Animals were maintained in a mass air displacement room with a 12 h light and 12 h dark cycle at 22±2°C with a relative humidity of 50±10%. Balanced food (Rodent diet 5001) and drinking water were given to the animals *ad libitum*. Animal experiments were carried out under strict compliance with the guidelines for ethical control and supervision in the care and use of animals and all experimental procedure was done following national and international rules.

The rats were distributed in 6 groups of 6 animals each and were daily treated for 5 consecutive days as follows: group 1 (control saline solution NaCl 0.9%), group 2 (zanamivir 2.5 mg kg^{-1}), group 3 (pyridoxine 10 mg kg^{-1}), group 4 (sucrose 20%), group 5 (zanamivir 2.5 mg kg^{-1}+sucrose 20%) and group 6 (pyridoxine 10 mg kg^{-1}+sucrose 20%). All treatments were administered intraperitoneally except sucrose which was given in drinking water during the study, being the only source of water. At the end of the treatment, 20 µL of blood was drawn from the rats and used to measure levels of glucose. The animals were sacrificed by decapitation and their brains were dissected in cortex, hemispheres (includes corpus callosum, anterior and posterior commissures and hippocampal zone) and cerebellum/medulla oblongata regions. The samples were placed in a solution of NaCl 0.9% at 4°C, homogenized in 10 volumes of tris-HCl 0.05 M, pH 7.2, samples immediately frozen under liquid nitrogen and maintained at 20°C until analyzed. Determination of

thiobarbituric acid reactive substances (TBA-RS measurement and index of lipid peroxidation), GSH, dopamine levels and ATPase activity were carried out.

Method to measure blood glucose: The measurement of glucose was carried out at the end of treatment. Twenty microlitters of blood was taken from tail-end without anticoagulant and placed on a reactive paper of Accu-Chek (Roche Mannheim, Germany) and the concentration was reported as mg dL^{-1}.

Method for the measurement of dopamine (DA): The DA levels were measured in the supernatant of tissue homogenized in $HClO_4$ after centrifugation at 9,000 rpm for 10 min in a microcentrifuge (Hettich Zentrifugen, model Mikro 12-42, Germany), with a version of the method reported by Calderon-Guzman *et al*. (2008). An aliquot of the $HClO_4$ supernatant and 1.9 mL of buffer (0.003 M octyl-sulphate, 0.035 M KH_2PO_4, 0.03 M citric acid, 0.001 M ascorbic acid) were placed in a test tube. The mixture was incubated for 5 min at room temperature in total darkness and subsequently, the samples were read in a spectrofluorometer (Perkin Elmer LS 55, England) with 282 nm excitation and 315 nm emission lengths. The FL Win Lab version 4.00.02 software was used. Values were inferred in a previously standardized curve and reported as nmol g^{-1} of wet tissue.

Method for the measurement of glutathione (GSH): The levels of GSH were measured from a sample of the supernatant tissue homogenized in $HClO_4$, which was got after being centrifuged at 9000 rpm for 5 min (in a microcentrifuge Mikro 12-42, Germany), according to the method by Hissin and Hilf (1976). Briefly, 1.8 mL of phosphate buffer pH 8.0 with 0.2% of EDTA were added to an aliquot of 20 µL of the supernatant tissue in $HClO_4$ and 100 µL of ortho-phtaldialdehyde (concentration of 1 mg mL^{-1} in methanol) and incubated for 15 min at ambient temperature in total darkness. At the end of incubation, the samples were read in a spectrofluorometer (Perkin Elmer LS 55) with excitation longitude of 350 nm and emission of 420 nm. The values were inferred in a previously standardized standard curve and reported as nmol g^{-1} of wet tissue.

Method for measuring lipid peroxidation (TBARS): Determination of TBARS was carried out using the modified method of Gutteridge and Halliwell (1990), as described below: From the homogenized brain in tris-HCl 0.05 M pH 7.4, 1 mL was taken and 2 mL of thiobarbaturic acid (TBA) which contains 1.25 g of TBA, 40 g of trichloroacetic acid and 6.25 mL of concentrated chlorhydric acid (diluted in 250 mL of deionized H_2O) was added. Samples were heated to boiling point for 30 min (Thermomix 1420) after which they were immersed in ice bath for 5 min and finally centrifuged at 700 g for 15 min (Sorvall RC-5B Dupont). The absorbance of the supernatants tissues were read in triplicate at 532 nm in a spectrophotometer (Helios-α de UNICAM). The concentration of reactive substances to thiobarbaturic acid (TBA-RS) was expressed as µM of malondialdehyde per gram of wet tissue.

Method for measuring total ATPase: The method was carried out by using approximately 1 mg of the brain homogenate in 0.05 M tris-HCl at pH 7.4, which was incubated for 15 min in a solution that contains 3 mM $MgCl_2$, 7 mM KCl, 100 mM NaCl. After the 15 min of incubation, 4 mM of tris-ATP was added to the homogenate and re-incubated for another 30 min at 37°C with agitation in Dubnoff Labconco bath. The reaction was stopped by using 100 µL of trichloroacetic acid at 10%. The samples were centrifuged at 3500 rpm for 5 min at 4°C (Calderon-Guzman *et al*., 2005) and an aliquot of the supernatant was used to measure inorganic phosphate (P_i) using the method proposed by Fiske and Subarrow (1925). The absorbance of the supernatant was measured at 660 nm using Helios of UNICAM spectrophotometer and the difference of this absorbance among the solutions without ouabain was considered as total ATPase activity and was expressed in µm Pi g^{-1} of wet tissue per minute as marker of oxidative stress.

Analysis of results: One way analysis of variance (ANOVA) with Tukey-Kramer contrast or non parametric Kruskal-Wallis with Steel Dwass contrast after being subjected to variances homogeneity test were used. The values of $p<0.05$ were considered statistically significant (Serna, 2011). To carry out the tests, JMP Statistical Discovery Software version 10.0 from SAS was used.

RESULTS

Blood glucose was not altered in the rats treated with zanamivir and pyridoxine in the presence of sucrose (Table 1). Food consumption of the rats lightly decreased in the group treated with zanamivir and pyridoxine in presence of sucrose. This result could be attributed to the intake of sucrose or pyridoxine during treatment (Table 2).

The concentration of dopamine in brain of rats treated with zanamivir and pyridoxine in the presence of sucrose is shown in Fig. 1. In cortex dopamine concentration was unaltered by administration of sucrose, zanamivir, pyridoxine or their combination. In hemispheres, a significant decrement of this biomarker was observed in those animals who received sucrose when compared with control and the groups who received zanamivir or pyridoxine plus sucrose; these groups shows increment in comparison with zanamivir, pyridoxine or sucrose groups being significant only between zanamivir and zanamivir plus sucrose. In cerebellum/medulla oblongata, DA concentration increased significantly in the groups of rats that received zanamivir or pyridoxine plus sucrose when compared with the group that received sucrose. No differences with control group were observed.

Table 1: Levels of glucose in blood of rats treated with zanamivir and pyridoxine in presence of sucrose

Groups*	Glucose (mg dL^{-1})±SD
Control (vehicle)	138.8±4.6
Zanamivir	130.2±8.2
Pyridoxine	145.2±7.9
Sucrose	141.8±9.3
Sucrose+zanamivir	140.2±12.1
Sucrose+pyridoxine	148.8±9.4

*n = 6 animals per group

Table 2: Food consumption per group of rats treated with zanamivir and pyridoxine in presence of sucrose during the study (g)

	Days of treatment				
Groups*	1	2	3	4	5
Control (vehicle)	88	90	84	85	90
Zanamivir	84	80	100	85	80
Pyridoxine	83	80	90	85	82
Sucrose	82	50	80	75	70
Sucrose+zanamivir	86	80	80	75	80
Sucrose+pyridoxine	84	70	72	80	70

*n = 6 animals per group

The GSH concentration was similar for all animals in cortex, while in hemispheres a slight decrement vs control was observed, significant differences were found only in the group of animals treated with sucrose as compared with those who received zanamivir plus sucrose. Decreased concentrations of GSH in cerebellum/medulla oblongata was observed in all groups vs control and were significant for those who received sucrose alone or in combination with zanamivir or pyridoxine, the groups treated with zanamivir or pyridoxine were statistically different when compared with the sucrose group, pyridoxine and pyridoxine plus sucrose were also significantly different (Fig. 2).

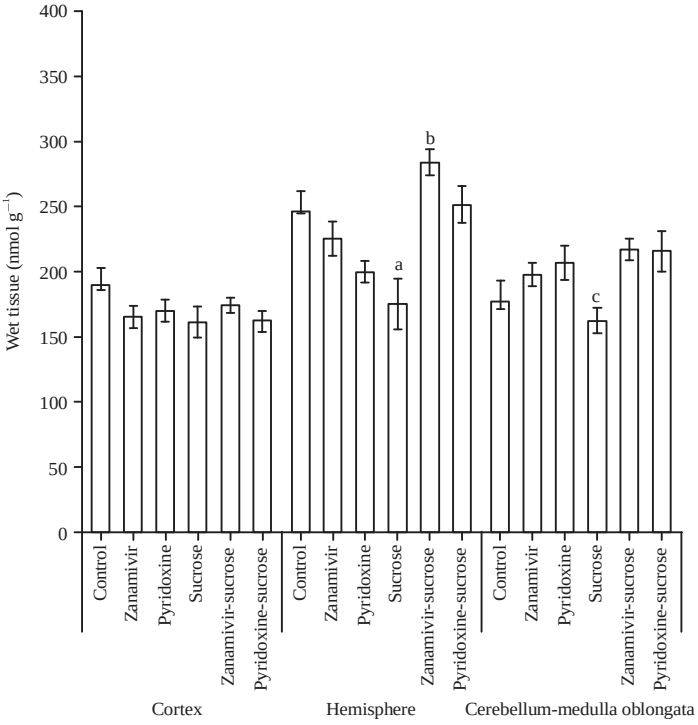

Fig. 1: Levels of dopamine in brain regions of rats treated with zanamivir and pyridoxine in presence of sucrose. No differences in cortex. In hemispheres, a: Sucrose vs control, zanamivir+sucrose, pyridoxine+sucrose $p<0.01$, b: Zanamivir vs zanamivir+sucrose $p = 0.02$, cerebellum/medulla oblongata, c: Zanamivir+sucrose and pyridoxine+sucrose vs sucrose $p = 0.02$

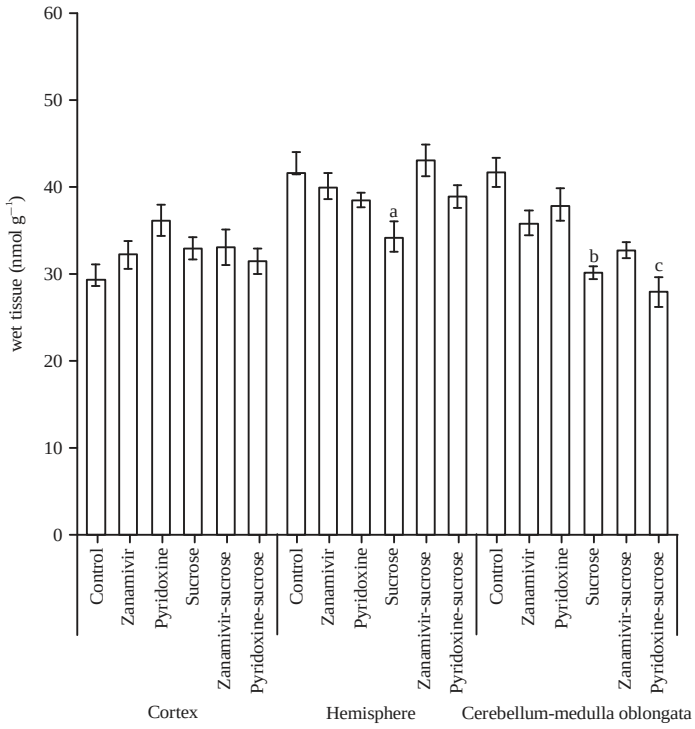

Fig. 2: Levels of glutathione (GSH) in brain regions of rats treated with zanamivir and pyridoxine in presence of sucrose. Hemispheres, a: Sucrose vs zanamivir+sucrose, p = 0.02, cerebellum/medulla oblongata, b: Control vs sucrose, zanamivir+sucrose and pyridoxine+sucrose p<0.01, c: Pyridoxine+sucrose vs pyridoxine p = 0.008

Lipid peroxidation decrement was observed in cortex for the animals treated with zanamivir or sucrose in comparison with control, zanamivir plus sucrose and pyridoxine plus sucrose. For the rats treated with pyridoxine and pyridoxine plus sucrose, lipid peroxidation was similar; but when compared with zanamivir, pyridoxine group shows significantly more lipid peroxidation but less than zanamivir plus sucrose group. In hemispheres a significant decrement in lipid peroxidation was found for all groups as compared with control group, comparison carried out between all treated groups shows significant difference between the pyridoxine group and the other groups, except sucrose group.

In cerebellum/medulla oblongata a significant reduction in lipid peroxidation was observed for all treatments when they were compared with control group being zanamivir plus sucrose and pyridoxine the groups with significant differences between treatments (Fig. 3).

The activity of ATPase remains unaltered in cortex for all treatments, in hemispheres a slight but not significant increment in the activity for the zanamivir, pyridoxine and pyridoxine plus sucrose vs control was observed, between treatments analysis reveals significant differences for zanamivir plus sucrose group in comparison with zanamivir and pyridoxine plus sucrose groups. A decrement in the activity of ATPase in cerebellum/medulla oblongata was seen for all treatments in comparison with control group but statistical differences were only between this group and zanamivir and sucrose groups (Fig. 4).

DISCUSSION

Central nervous system plays an important role in orchestrating glucose metabolism, with accumulating evidence linking dysregulated central nervous system circuits to the failure of normal glucoregulatory mechanisms. The metabolism of brain glucose is crucial for CNS function, mainly because glucose is critical for the brain (Shi and Liu, 2006) and because the changes in the homeostasis of glucose are associated with the action of ATPase enzymes (Torlinska *et al*., 2006). In the present study, ATPase concentration decreased by the effect of zanamivir, probably as consequence of biochemical changes of the enzyme in the brain (Neault *et al*., 2001).

Studies carried out by Isaev *et al*. (2007), indicated that the effect of hypoglycemia is to increase calcium charge in the nerve tissue and this in turn alters the effect of ATPase enzymes. Other studies suggest that ATPases decreased in crude synaptosomal membranes of the diabetic mice. These animals exhibit a decreased capacity for glucose oxidation and

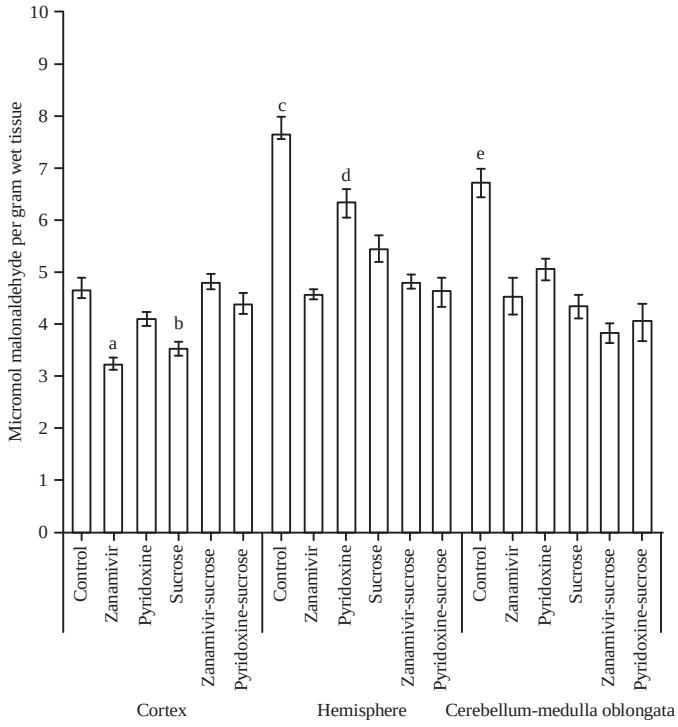

Fig. 3: Lipid peroxidation in brain regions of rats treated with zanamivir and pyridoxine in presence of sucrose. Cortex, a: Zanamivir, b: Sucrose vs control, zanamivir+sucrose and pyridoxine+sucrose and zanamivir+sucrose $p<0.03$, hemispheres, c: Control vs zanamivir, pyridoxine, sucrose, pyridoxine+sucrose, zanamivir+sucrose $p = 0.001$, d: Pyridoxine vs pyridoxine+sucrose $p = 0.001$, cerebellum/medulla oblongata, e: control vs all groups $p<0.001$

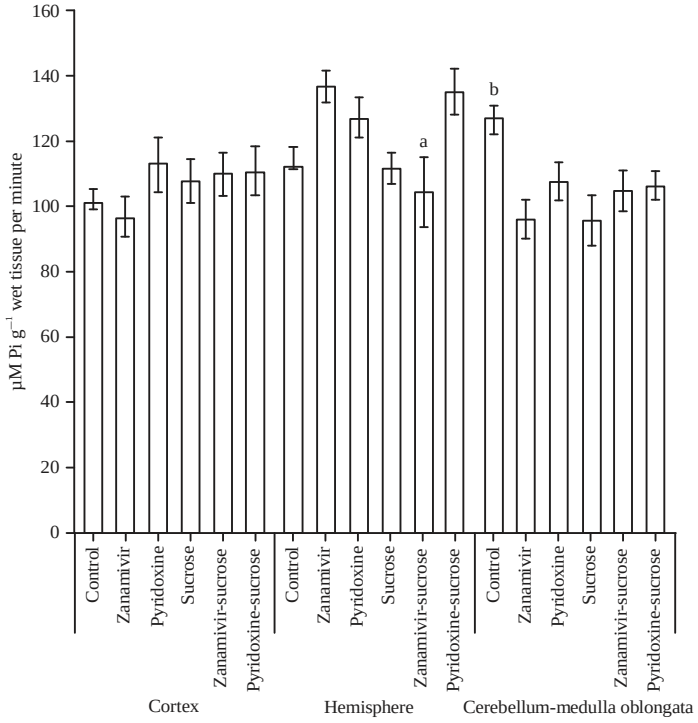

Fig. 4: Ca^{+2}, Mg^{+2} ATPase in brain regions of rats treated with zanamivir and pyridoxine in presence of sucrose. Hemispheres, a: Zanamivir+sucrose and pyridoxine+sucrose vs zanamivir $p = 0.01$, cerebellum/medulla oblongata, b: Control vs zanamivir and sucrose $p<0.006$

increased capacity for fatty acid oxidation (Makar *et al*., 1995), because glucose determines fatty acid oxidation by controlling the rate of long-chain fatty acid entrance into the mitochondria (Sidossis *et al*., 1996). In the present study, it is found that increased lipid peroxidation in brain cortex on consumption of sucrose in combination with zanamivir and pyridoxine and opposite effect were found on cerebellum/medulla oblongata and hemispheres regions, where zanamivir intake alone or combined decreased lipid peroxidation.

In the present study, the concentration of dopamine increased in brain hemispheres of rats that received zanamivir plus sucrose. These results are in accordance with Johnson *et al*. (2011), who suggest that the chronic effects of excessive sugar intake may lead to alterations in mesolimbic dopamine signaling, even though the mechanism of action is not clearly known. However, Anitha *et al*. (2012), suggested that the potential role of pyridoxine supplementation in ameliorating diabetes mediated dysfunctions in striatal dopaminergic receptor expressions.

With respect to the concentration of GSH, there was an increase in the biomarkers in cortex and cerebellum/medulla oblongata regions principally by the administration of zanamivir or pyridoxine. These results indicate that pyridoxal 5'-phosphate may be associated with neuroprotection effect, as referred Hwang *et al*. (2007), due that pyridoxine diet induces slight elevation in brain the concentration of pyridoxal 5'-phosphate (Masisi *et al*., 2012). Probably these effects could be due to decrease of nitric oxide levels, because to its extremely important role in cellular free radical detoxification (Zhu *et al*., 2006).

CONCLUSION

There are important findings as novel study, however, there are limitations in the results presented, in particular, on examining non-pathological animal models. In view of this, recommend further studies to investigate the possible relationship between chronic sugar intake and neuraminidase inhibitors with pyridoxine in different pathological animal models.

Alterations of dopaminergic system were seen in animals treated with zanamivir and pyridoxine. Reduction of oxidative stress may be involved in these effects.

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REFERENCES

Anitha, M., P.M. Abraham and C.S. Paulose, 2012. Striatal dopamine receptors modulate the expression of insulin receptor, IGF-1 and GLUT-3 in diabetic rats: Effect of pyridoxine treatment. Eur. J. Pharmacol., 696: 54-61.

Arancibia, H.F., U.S. Ugarte, F.R. Soto, A. Hernandez and R. Alonzo *et al*., 2011. [Impact of the obesity in patient with severe influence by virae A/H1N1]. Revista Chilena Medicina Intensiva, 26: 7-16, (In Spanish).

Calderon-Guzman, D., J.L. Hernandez-Islas, I. Espitia-Vazquez, G. Barragan-Mejia, E. Hernandez-Garcia, A.D. Santamaria-Del and H. Juarez-Olguin, 2004. Pyridoxine, regardless of serotonin levels, increases production of 5-hydroxytryptophan in rat brain. Arch. Med. Res., 35: 271-274.

Calderon-Guzman, D., I. Espitia-Vazquez, A. Lopez-Dominguez, E. Hernandez-Garcia and B. Huerta-Gertrudis *et al*., 2005. Effect of toluene and nutritional status on serotonin, lipid peroxidation levels and NA^+/K^+-ATPase in adult rat brain. Neurochem. Res., 30: 619-624.

Calderon-Guzman, D., N. Osnaya-Brizuela, R. Garcia-Alvarez, E. Hernandez-Garcia, A. Guille-Perez and H. Juarez-Olguin, 2008. Levels of glutathione and some biogenic amines in the human brain putamen after traumatic death. Proc. West. Pharmacol. Soc., 51: 27-29.

El-Sayed, W.M. and M.A. Al-Kahtani, 2011. Potential Adverse Effects of Oseltamivir in Rats: Males are More Vulnerable Than Females. Can. J. Physiol. Pharmacol., 89: 623-630.

Fiske, C.H. and Y. Subbarow, 1925. The colorimetric determination of phosphorus. J. Biol. Chem., 66: 375-400.

Gutteridge, J.M. and B. Halliwell, 1990. The measurement and mechanism of lipid peroxidation in biological systems. Trends Biochem. Sci., 15: 129-135.

Hanslik, T., P.Y. Boelle and A. Flahault, 2010. Preliminary estimation of risk factors for admission to intensive care units and for death in patients infected with A (H1N1) 2009 influenza virus, France, 2009-2010. PLoS Curr. 10.1371/currents.RRN1150

Heneghan, C.J., I. Onakpoya, M. Thompson, E.A. Spencer, M. Jones and T. Jefferson, 2014. Zanamivir for influenza in adults and children: Systematic review of clinical study reports and summary of regulatory comments. Br. Med. J. 10.1136/bmj.g2547

Hissin, P.J. and R. Hilf, 1976. A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal. Biochem., 74: 214-226.

Hwang, I.K., K.Y. Yoo, D.H. Kim, B.H. Lee, Y.G. Kwon and M.H. Won, 2007. Time course of changes in pyridoxal 5'-phosphate (vitamin B6 active form) and its neuroprotection in experimental ischemic damage. Exp. Neurol., 206: 114-125.

Isaev, N.K., E.V. Stelmashuk and D.B. Zorov, 2007. Cellular mechanisms of brain hypoglycemia. Biochemistry, 72: 471-478.

Johnson, R.J., M.S. Gold, D.R. Johnson, T. Ishimoto, M.A. Lanaspa, N.R. Zahniser and N.M. Avena, 2011. Attention-deficit/hyperactivity disorder: Is it time to reappraise the role of sugar consumption? Postgrad. Med., 123: 39-49.

Kumar, P., M. Khanna, V. Srivastava, Y.K. Tyagi, H.G. Raj and K. Ravi, 2005. Effect of quercetin supplementation on lung antioxidants after experimental influenza virus infection. Exp. Lung Res., 31: 449-459.

Lee, E., J.H. Seo, H.Y. Kim, S. Na and S.H. Kim *et al*., 2011. Clinical characteristics and outcomes among pediatric patients hospitalized with pandemic influenza A/H1N1 2009 infection. Korean J. Pediatr., 54: 329-334.

Makar, T.K., B.L. Hungund, G.A. Cook, K. Kashfi and A.J.L. Cooper, 1995. Lipid metabolism and membrane composition are altered in the brains of type II diabetic mice. J. Neurochem., 64: 2159-2168.

Masisi, K., S. Suidasari, P. Zhang, Y. Okazaki, N. Yanaka and N. Kato, 2012. Comparative study on the responses of concentrations of B_6-vitamers in several tissues of mice to the dietary level of pyridoxine. J. Nutr. Sci. Vitaminol. (Tokyo), 58: 446-451.

Neault, J.F., A. Benkiran, H. Malonga and H.A. Tajmir-Riahi, 2001. The effects of anions on the solution structure of Na,K-ATPase. J. Biomol. Struct. Dyn., 19: 95-102.

Rani, P.J.A. and C. Panneerselvam, 2001. Carnitine as a free radical scavenger in aging. Exp. Gerontol., 36: 1713-1726.

Rogers, K.S. and C. Mohan, 1994. Vitamin B_6 metabolism and diabetes. Biochem. Med. Metab. Biol., 52: 10-17.

Serna, L.S., 2011. [Practical Manual of Statistics for Health Sciences]. 1st Edn., Castilla Serna Luis, Mexico, DF., ISBN-13: 978-6071708137, Pages: 167, (In Spanish).

Shi, H. and K.J. Liu, 2006. Effects of glucose concentration on redox status in rat primary cortical neurons under hypoxia. Neurosci. Lett., 410: 57-61.

Sidossis, L.S., C.A. Stuart, G.I. Shulman, G.D. Lopaschuk and R.R. Wolfe, 1996. Glucose plus insulin regulate fat oxidation by controlling the rate of fatty acid entry into the mitochondria. J. Clin. Invest., 98: 2244-2250.

Toovey, S., E.P. Prinssen, C.R. Rayner, B.T. Thakrar and R. Dutkowski *et al*., 2012. Post-marketing assessment of neuropsychiatric adverse events in influenza patients treated with oseltamivir: An updated review. Adv. Ther., 29: 826-848.

Torlinska, K., A. Grochowalska, J. Kupsz, J. Skoracka and S. Kojo, 2006. *In vivo* and *In vitro* effects of hyperglycemia on Na^{+}-K^{+}, Ca^{+2}, Mg^{+2}-dependent ATPases activity in brain synaptosomes of aging rats. J. Physiol. Pharmacol., 57: 145-158.

Valko, M., D. Leibfritz, J. Moncol, M.T.D. Cronin, M. Mazur and J. Telser, 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol., 39: 44-84.

Wang, K., M. Shun-Shin, P. Gill, R. Perera and A. Harnden, 2012. Neuraminidase inhibitors for preventing and treating influenza in children. Cochrane Database Syst. Rev., Vol. 18.

Wang, W., H. Chen, Q. Li, B. Qiu and J. Wang *et al*., 2011. Fasting plasma glucose is an independent predictor for severity of H1N1 pneumonia. BMC Infect Dis. 10.1186/1471-2334-11-104.

Zhu, Y., P.M. Carvey and Z. Ling, 2006. Age-related changes in glutathione and glutathione-related enzymes in rat brain. Brain Res., 1090: 35-44.