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ORIGINAL ARTICLE

Assessment of Mexican Arnica (*Heterotheca inuloides Cass*) and Rosemary (*Rosmarinus officinalis*) Extracts on Dopamine and Selected Biomarkers of Oxidative Stress in Stomach and Brain of *Salmonella typhimurium* Infected rats

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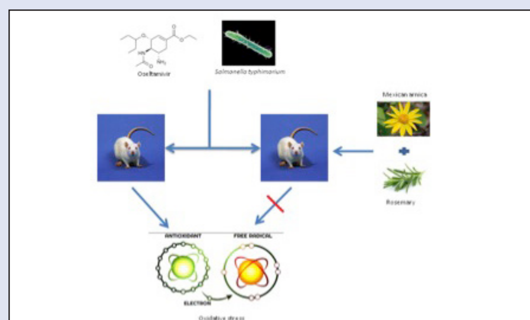
ABSTRACT

Background: The effects of some natural products on dopamine (DA) and 5-hydroxyindole acetic acid (5-HIAA) in brain of infected models are still unclear. **Objective:** The purpose of this study was to measure the effect of Mexican arnica/rosemary (MAR) water extract and oseltamivir on both biogenic amines and some oxidative biomarkers in the brain and stomach of young rats under infection condition. **Methods:** Female Wistar rats (weight 80 g) in the presence of MAR or absence (no-MAR) were treated as follows: group 1, buffer solution (controls); oseltamivir (100 mg/kg), group 2; culture of *Salmonella typhimurium* (*S. Typh*) (1 × 10^6 colony-forming units/rat) group 3; oseltamivir (100 mg/kg) + *S. Typh* (same dose) group 4. Drug and extracts were administered intraperitoneally every 24 h for 5 days, and *S. Typh* was given orally on days 1 and 3. On the fifth day, blood was collected to measure glucose and hemoglobin. The brains and stomachs were obtained to measure levels of DA, 5-HIAA, glutathione (GSH), TBARS, H_2O_2, and total ATPase activity using validated methods. **Results:** DA levels increased in MAR group treated with oseltamivir alone but decreased in no-MAR group treated with oseltamivir plus *S. Typh*. 5-HIAA, GSH, and H_2O_2 decreased in this last group, and ATPase activity increased in MAR group treated with oseltamivir plus *S. Typh*. TBARS (lipid peroxidation) increased in MAR group that received oseltamivir alone. Most of the biomarkers were not altered significantly in the stomach. **Conclusion:** MAR extract alters DA and metabolism of 5-HIAA in the brain of young animals infected. Antioxidant capacity may be involved in these effects.

Key words: DA, glutathione, 5-HIAA, Mexican arnica plant, Mexican rosemary plant, *Salmonella typhimurium*

SUMMARY

- The purpose of this study was to measure the effect of Mexican arnica/rosemary water extract and oseltamivir on both biogenic amines and some oxidative biomarkers in the brain and stomach of young rats under infection condition. Results: Mexican arnica and rosemary extract alter dopamine and metabolism of 5-HIAA in the brain of young animals infected. Antioxidant capacity may be involved in these effects.



Abbreviations used: AS: Automated system, ATP: Adenosine triphosphate, CNS: Central nervous system, CFU: Colony-forming unit, DA: Dopamine EDTA: Ethylenediaminetetraacetic acid, 5-HIAA: Äcido 5-hidroxindolacético (serotonina), GABA: γ-aminobutyric acid, GSH: Glutathione, H2O2: Hidrogen peroxide, HCLO4: Perchloric acid, iNOS: Inducible nitric oxide synthase, LPS: Lipopolysaccharides, MAR: Arnica/Rosemary, NaCl: Sodium Chloride, NOGSH: nitrosoglutathione,NOS: Nitric oxide, OPT: Ortho-phtaldialdehyde, Pbs: Phosphate buffered saline, pH: potential of Hydrogen, Pi: Inorganic phosphate, ROS: Reactive oxygen species, RNSs: Reactive nitrogen species Tba: Thiobarbaturic acid, TBARS: Thiobarbituric aid reactive, Tca: Trichloroacetic, Tris-HCL: Tris hydrochloride, TSA: Trypticasein Soya Agar

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INTRODUCTION

Oseltamivir is used for the treatment of influenza virus infections. This drug is well tolerated by adults and the most common adverse effects are nausea and vomiting. In young patients, the drug has been associated with neuropsychiatric behaviors including jumping and falling from balconies.[1] This abnormal behavior could be linked to an increase in DA as a result of treatment with oseltamivir. Probably the administration of oseltamivir phosphate in the presence of inflammation increases the brain concentration of both parent drugs and their active metabolites, which may explain the central nervous system (CNS) side effects observed with this agent,[2] since it was reported that altered activities of

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dopaminergic-serotonergic pathway play a role in the etiopathogenesis of behavioral and psychologic signs and symptoms of dementia.[3]

However, the pharmacologic mechanism of the neuropsychiatric effects of oseltamivir remains unclear not only in adults but also in young pediatric population. Oseltamivir (Tamiflu) is now being stockpiled by Mexican governments as a first-line treatment for an anticipated outbreak of swine influenza caused by AH1N1, which came into being in late March 2009 due to an outbreak of a respiratory illness that was later proved to be caused by H1N1 (S-OIV) virus, a novel swine origin influenza A. Lipopolysaccharides (LPS) or endotoxins activate the hypothalamic-pituitary-adrenal axis and cerebral catecholamine systems and increase mouse brain concentrations of the serotonin catabolite[4] as consequence of LPS-induced inflammation. It has been reported that serotonin can modulate glutamate and GABA release in the CNS.[5]

Salmonella typhimurium (*S.Typh*) enterotoxin has been used to probe generation of reactive oxygen species (ROS), potent mediators of inflammatory disorders, on cellular or animal models, leading to a loss of cell viability,[6] using antioxidant enzymes as biomarkers of oxidative stress. Inducible nitric oxide synthase (iNOS) is the major contributor to initiation/exacerbation of the CNS inflammatory/degenerative conditions through the production of excessive nitric oxide (NO), which generates reactive nitrogen species (RNSs). Activation of iNOS and NO generation has come to be accepted as a marker and therapeutic target in neuroinflammatory conditions.[7] NO is a neuromodulator as well, but an extra amount may lead to cell damage by oxidative stress or by forming nitrosoglutathione (NOGSH) within the cell.[8] Free radicals are known to damage cell components,[9] mainly plasma membrane lipids, where the CNS is particularly susceptible, and extremely dependent on the amount of antioxidants.[10]

Recent studies indicated that the use of antioxidants induces defensive mechanisms to the brain by diminishing free radical-induced lipid peroxidation.[11] Natural products like Arnica (*Heterotheca inuloides Cass*)[12] and Rosemary (*Rosmarinus officinalis*)[13] function as antioxidant and anti-inflammatory agent, respectively. Both plants are widely used in Mexican traditional medicine as secure antioxidant and anti-inflammatory agents.

Free radicals are ROS or RNS with impaired electrons, which may induce oxidative damage to biologically important molecules; CNS is particularly susceptible to this type of damage. Membrane lipids are known to strongly interact with the lipid bilayer structural proteins,[14] such as the Na+-K+ ATPase, which is responsible for ion interchange across the membrane.[15]

Since swine influenza by AH1N1 produces inflammation and oseltamivir, an anti-inflammatory drug is the drug of choice for the treatment; the administration of oseltamivir jointly with natural plants like arnica and rosemary extracts may induce a beneficial effect in the treatment of swine influenza by AH1N1. On the basis of this assumption, it is therefore necessary to determine the effects of oseltamivir and some natural plants commonly used in the treatment of this influenza in order to establish methods for their safe administration using an infection condition. Then, the purpose of this study was to determine the effect of oseltamivir with Mexican arnica and rosemary (MAR) on DA and 5-hydroxyindole acetic acid (5-HIAA) levels, lipid peroxidation, glutathione (GSH), H_2O_2, and ATPase enzyme in brain and stomach of juvenile infected animal models.

MATERIALS AND METHODS

Forty Wistar rats each with a weight of 80 ± 5 g (4 weeks old) were recruited and equally divided into two groups, one for MAR (aqueous extract 15% w/v of assayed plants) and the other for no-MAR (absence of the aqueous extract of plants). Each of these two groups was in turn divided into four groups of five animals each (n = 5). The MAR and no-MAR groups were then treated as follows: Group 1, control, treated only with saline solution; group 2, oseltamivir (100 mg/kg); group 3, inoculated with live culture of *S.Typh* (1×10^6 colony-forming units/rat); and group 4, oseltamivir (same dose) + *S.Typh* (same dose). All treatments were given intraperitoneally every 24 h for 5 days except *S.Typh* administration, which was made orally on first and third day only. The animals were procured from Bioterium of Metropolitan University of Mexico City and housed four or five per cage in clean plastic cages and allowed to acclimatize in the room environment for 1 day. Animals were maintained in a mass air displacement room with a 12-h light: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*. On the fifth day of the treatment, the rats were sacrificed by decapitation 60 min after receiving the last dose of oseltamivir, *S.Typh*, and MAR and their brains and stomachs were extracted and put in NaCl 0.9% at 4°C, idem, and the blood was collected to measure glucose levels.

Brain dissection was carried out by sagittal cutting. The left cut was homogenized in five volumes of Tris-HCl 0.05 M, pH 7.4 for the assessment of lipid peroxidation (TBARS), H_2O_2, and total ATPasa. The right cut was homogenized in five volumes of perchloric acid ($HClO_4$) 0.1 M to measure the levels of GSH, 5-HIAA, and dopamine (DA). 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 procedures were done following national and international rules.

The procedure to measure blood glucose was carried out in all groups of animals at the moment of sacrifice. Ten microliter of nonanticoagulant fresh blood was obtained and smeared on a reactive filter paper in Accu-Chek active (Roche Mannheim Germany) equipment and the concentration was read in milligram per deciliter.

Inoculation of rats

The corresponding animals were inoculated with a live culture of *S.Typh* obtained from strain bank (ceparium) of Experimental Bacteriology Laboratory of National Institute of Pediatrics, Mexico City. The strain was re-identified and an aliquot of maintenance medium was inoculated in SS agar (*Salmonella Shigella* culture medium). The cultures were incubated for 18-24 h at 37°C. The isolated colonies with morphologies suggestive of *S.Typh* were selected and confirmed by conventional biochemical tests. Inoculation preparation was carried out by sowing the strain in TSA (Trypticasein Soya Agar) and incubated at 37°C for 18 h. The bacterial biomass was collected with hyssop, resuspended in buffer PBS, pH 6.8, and adjusted to an $AS_{450nm} = 0.175$ (equivalent to 3×10^8 CFU/mL) using DU 640 spectrophotometer (BECKMAN). It was later diluted to obtain a concentration of 1×10^6 CFU/ml.[16] The inoculation was carried out by oral administration of nonlethal volumes of 1 ml per animal using an orogastric tube.

Technique for the measurement of DA

The DA levels were measured in the supernatant of tissue homogenized in $HClO_4$ after centrifugation at 9000 rpm for 10 min in a microcentrifuge (Hettich Zentrifugen, model Mikro 12-42, Germany), with a version of the technique reported by Calderón *et al*.[17] An aliquot of the $HClO_4$ supernatant and 1.9 mL of buffer (0.003 M octylsulphate, 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 micromole per gram of wet tissue.

Measurement of 5-HIAA

The levels of 5-HIAA 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 modified version of the technique reported by Beck *et al.*[18] An aliquot of the $HClO_4$ supernatant and 1.9 mL of acetate buffer 0.01 M, pH 5.5 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 296 nm excitation and 333 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 nanomole per gram of wet tissue.

Technique for the measurement of GSH

The levels of GSH were measured from a sample of the floating tissue homogenized in $HClO_4$ which was obtained after being centrifuged at 9000 rpm for 5 min (in a microcentrifuge Mikro 12-42, Germany), according to the technique reported by Hissin and Hilf.[19] Phosphate buffer, 1.8 mL, at pH 8.0 with EDTA at 0.2%, an aliquot of 20 µL of the floating tissue in $HClO_4$, and 100 µL of *ortho*-phtaldialdehyde (OPT) in concentration of 1 mg/mL in methanol were put in an assay tube and incubated for 15 min at ambient temperature in total darkness. At the end of incubation, the samples were read in a PERLIN ELMER LS 55 spectrofluorometer with excitation longitude of 350 nm and emission of 420 nm. FL Win Lab version 4.00.02 software was used. The values were inferred in a previously standardized standard curve and reported in nanomole per gram of wet tissue.

Measurement of lipid peroxidation (TBARS)

The determination of TBARS was carried out using the modified technique of Gutteridge and Halliwell,[10] 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), containing 1.25 g of Tba, 40 g of trichloroacetic acid (Tca), and 6.25 mL of concentrated chlorhydric acid (HCl) diluted in 250 mL of deionized H_2O, was added to it. The mixture was heated to boiling point for 30 min (Thermomix 1420). The samples were later put in ice bath for 5 min and centrifuged at 700 g for 15 min (Sorvall RC-5B Dupont). The absorbance of the floating tissues was read in triplicate at 532 nm in a spectrophotometer (Helios-α of UNICAM). The concentration of reactive substances to the thiobarbaturic acid (TBARS) was expressed in micromole of malondialdehyde per gram of wet tissue.

Technique for the measurement of ATPase

The technique was carried out by using approximately 1 mg of the brain homogenate in 0.05 M Tris-HCl at pH 7.4. This was incubated for 15 min in a medium, which contained 3 mM $MgCl_2$, 7 mM KCl, and 100 mM NaCl, with 4 mM of Tris-ATP, which was added to the homogenate after 15 min of incubation and again incubated for 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,[20] and an aliquot of the floating tissue was used to measure inorganic phosphate (P_i) using the method proposed by Fiske and Subbarow.[21] The absorbance of the floating was measured at 660 nm using Helios-α of UNICAM spectrophotometer. ATP ase dependent of calcium and magnesium was expressed in µg Pi/g tissue/min

Measurement of H_2O_2

The determination of H_2O_2 was made using the modified technique of Sinha.[22] Each brain region (cortex, hemispheres, cerebellum/medulla oblongata) and stomach was homogenized in 3 mL of Tris-HCl 0.05 M pH 7.4 buffer. From the diluted homogenates, 100 µL was taken and 1 mL of potassium dichromate solution ($K_2Cr_2O_7$) and anhydride acetic acid was added to it and the mixtures were heated to boiling point for 15 min (Thermomix 1420). The samples were later placed in an ice bath for 5 min and centrifuged at 3000 rpm for 5 min (Sorvall RC-5B Dupont). The absorbance of the floating was read by triplicate at 570 nm in a spectrophotometer (Helios-α of UNICAM). The concentration of H_2O_2 was expressed in micromoles.

Analysis of results

Kruskal–Wallis statistical test and two-way analysis of variance (ANOVA) with their respective contrasts after being subjected to variances homogeneity test were used. The values of P less than 0.05 were considered statistically significant.[23] To carry out the tests, JMP Statistical Discovery Software version 8.0.0 from SAS was used.

RESULTS

Table 1 shows that the levels of glucose did not increase significantly with respect to control group not exposed to plants.

Table 2 shows the levels of DA, 5-HIAA, and some biomarkers of oxidative stress in the cortex region of young rats (*S.Typh* and non-*S.Typh* infected) treated with oseltamivir in the presence or absence of MAR. DA levels were found to increase significantly ($P < 0.001$) in the ANOVA two-way statistical test in the group that was treated with oseltamivir alone and decrease in the group that was treated with oseltamivir + MAR + *S.Typh*. However, the same effect was obtained with decreased significance ($P < 0.001$) in the levels of 5-HIAA, GSH, and H_2O_2 in this last group, and opposite results were obtained with increased significance ($P < 0.01$) in total ATPase activity in the groups that received oseltamivir combined with *S.Typh* and MAR with respect to the control groups.

The concentration of lipid peroxidation in the cortex region of young rats infected with *S.Typh* and treated with oseltamivir alone or in combination with and MAR increased significantly ($P < 0.001$) in the ANOVA two-way statistical test when compared with the control groups.

Table 3 shows the levels of DA, 5-HIAA, and the biomarkers of oxidative stress in the hemisphere regions. DA levels decreased significantly ($P < 0.001$) in the ANOVA two-way statistical test in the groups that received oseltamivir alone or combination with *S.Typh* and MAR with respect to the control group. 5-HIAA, GSH, and H_2O_2 levels in hemisphere regions decreased significantly ($P < 0.001$) in the ANOVA two-way statistical test in the groups that received oseltamivir, *S.Typh*, and MAR with respect to the control group. For the activity of total ATPase in the hemisphere regions, it could be seen that there was a decreased significance ($P < 0.01$) in the ANOVA two-way statistical test in the groups that received oseltamivir alone, *S.Typh* alone, or oseltamivir + *S.Typh* when compared with the control groups.

Table 1: Glucose levels in blood of *S.Typh*-infected rats treated with oseltamivir and Mexican plant extract

Groups		mg/dL
E	(a)	165 ± 37
E + *S.Typh*	(b)	157 ± 8
E + Osel	(c)	157 ± 8
E + Osel + *S.Typh*	(d)	152 ± 6
PBS Ctrl	(e)	140 ± 14
Osel	(f)	158 ± 2
S.Typh	(g)	152 ± 14
Osel + *S.Typh*	(h)	159 ± 8

Mean values ± SD. Plant extract (E) = Mexican arnica + Mexican rosemary; *S.Typh* = *Salmonella typhimurium*; Osel = Oseltamivir

Table 2: Biogenic amines and some oxidative stress markers in *S.Typh*-infected cortex rats treated with oseltamivir and Mexican plant extract

Groups		Dopamine (nM/g)	5-HIAA (nM/g)	TBARS (μM malondialdehyde/g)	GSH (nM/g)	H_2O_2 (μM/g)	Total ATPase (μM Pi/g/min)
E	(a)	1.65 ± 0.38	44.2 ± 20	5.39 ± 0.6	486.4 ± 72	0.107 ± 0.01	31.09 ± 6.9
E + *S.Typh*	(b)	1.58 ± 0.31	30.6 ± 18*	6.33 ± 1.1	519.2 ± 84	0.101 ± 0.03	22.60 ± 9.6
E + Osel	(c)	1.88 ± 0.30	30.8 ± 21*	7.12 ± 1.3*	456.6 ± 55	0.114 ± 0.02	28.43 ± 17
E + Osel + *S.Typh*	(d)	1.30 ± 0.27*	40.6 ± 08	6.36 ± 0.7	427.9 ± 64*	0.104 ± 0.2*	43.40 ± 20*
PBS Ctrl	(e)	2.67 ± 0.70	95.4 ± 18	5.35 ± 1.1	335.4 ± 64	0.043 ± 0.01	12.98 ± 10
Osel	(f)	3.22 ± 0.60*	91.4 ± 34	7.14 ± 0.9*	287.9 ± 80*	0.042 ± 0.01	11.09 ± 08
S.Typh	(g)	3.33 ± 0.85	96.4 ± 35	6.05 ± 0.7	346.6 ± 100	0.043 ± 0.01	20.13 ± 16
Osel + *S.Typh*	(h)	3.38 ± 0.5	99.4 ± 27	5.85 ± 0.9	299.4 ± 98	0.038 ± 0.01	21.64 ± 15*

Mean values ± SD. g = g of wet tissue; Plant extract (E) = Mexican arnica + Mexican rosemary; *S.Typh* = *Salmonella typhimurium*; Osel = Oseltamivir
Cortex $^*P < 0.05$. Dopamine: d vs. a, f vs. e; 5-HIAA: b, c vs. a; TBARS: c vs. a, f vs. e; GSH: d vs. a, f vs. e; H_2O_2: d vs. a; total ATPase: d vs. a, h vs. e

Table 3: Biogenic amines and some oxidative stress markers in *S. Typh*-infected hemispheres rats treated with oseltamivir and Mexican plant extract

Groups		Dopamine (nM/g)	5-HIAA (nM/g)	TBARS (μM malondialdehyde/g)	GSH (nM/g)	H_2O_2 (μM/g)	Total ATPase (μM Pi/g/min)
E	(a)	2.20 ± 0.35	43.5 ± 26	7.96 ± 1.0	448.3 ± 93	0.162 ± 0.04	40.74 ± 22
E + *S.Typh*	(b)	1.78 ± 0.44*	35.8 ± 11	9.08 ± 1.0	501.9 ± 82	0.139 ± 0.04*	44.31 ± 26
E + Osel	(c)	2.35 ± 0.58	40.9 ± 18	10.7 ± 1.5*	505.1 ± 79	0.161 ± 0.03	56.39 ± 27
E + Osel + *S.Typh*	(d)	1.84 ± 0.72*	30.7 ± 9*	10.8 ± 1.3*	424.1 ± 67*	0.165 ± 0.03	45.73 ± 22
PBS Ctrl	(e)	6.03 ± 2.4	154.4 ± 21	6.55 ± 1.1	307.0 ± 66	0.087 ± 0.02	37.34 ± 28
Osel	(f)	3.90 ± 1.0*	138 ± 23	8.42 ± 1.5*	246.2 ± 50	0.046 ± 0.01*	8.58 ± 6*
S.Typh	(g)	5.26 ± 2.8	137 ± 21	7.54 ± 1.1	227.4 ± 65*	0.056 ± 0.02	13.31 ± 10*
Osel + *S.Typh*	(h)	4.34 ± 1.0	126 ± 17*	7.16 ± 1.1	257.5 ± 44	0.064 ± 0.02	25.35 ±21

Mean values ± SD. g = g of wet tissue; Plant extract (E) = Mexican arnica + Mexican rosemary; *S.Typh* = *S.Typh*; Osel = Oseltamivir
Hemispheres $^*P < 0.05$; Dopamine: b, d vs. a, f vs. e; 5-HIAA: d vs. a, h vs. e; TBARS: c, d vs. a, f vs. e; GSH: d vs. a, g vs. e; H_2O_2: b vs. a, f vs. e; total ATPase: f, g vs. e

Table 4: Biogenic amines and some oxidative stress markers in *S. Typh*-infected cerebellum/medulla oblongata rats treated with oseltamivir and Mexican plant extract

Groups		Dopamine (nM/g)	5-HIAA (nM/g)	TBARS (μM malondialdehyde/g)	GSH (nM/g)	H_2O_2 (μM/g)	Total ATPase (μM Pi/g/min)
E	(a)	2.21 ± 0.9	24.04 ± 14	8.85 ± 1.6	346.5 ± 28	0.155 ± 0.01	43.15 ± 24
E + *S.Typh*	(b)	1.74 ± 0.3*	21.9 ± 9	9.24 ± 1.2*	411.1 ± 87	0.160 ± 0.02*	33.37 ± 14
E + Osel	(c)	1.95 ± 0.6	29.1 ± 10	10.7 ± 1.9*	333.4 ± 42*	0.152 ± 0.04	43.19 ± 13
E + Osel + *S.Typh*	(d)	1.68 ± 0.4	29.6 ± 7	9.86 ± 1.2	345.1 ± 61	0.154 ± 0.02	14.85 ± 11*
PBS Ctrl	(e)	4.09 ± 0.9	53.0 ± 21	7.47 ± 1.3	154.2 ± 46	0.028 ± 0.01	24.51 ± 15
Osel	(f)	3.21 ± 1.1	55.1 ± 14	8.60 ± 0.55*	133.7 ± 38	0.041 ± 0.02*	4.54 ± 3.0*
S.Typh	(g)	2.65 ± 0.5*	40.3 ± 26	7.31 ± 0.7	101.4 ± 27*	0.054 ± 0.02	21.70 ± 18
Osel + *S.Typh*	(h)	2.67 ± 0.8*	40.8 ± 10	7.33 ± 1.2	105.0 ± 26*	0.043 ± 0.03	25.64 ± 20

Mean values ± SD. g = g of wet tissue; Plant extract (E) = Mexican arnica + Mexican rosemary; *S.Typh* = *Salmonella typhimurium*; Osel = Oseltamivir
Cerebellum/medullaoblongata $^*P < 0.05$; Dopamine: b vs. a, g, h vs. e; TBARS: b, c vs. a, f vs. e; GSH: c vs. a, g, h vs. e; H_2O_2: b vs. a, f vs. e; total ATPase: d vs. a, f vs. e

Table 4 shows the levels of DA, 5-HIAA, and biomarkers of oxidative stress in the cerebellum/medulla oblongata region. DA and GSH levels decreased significantly ($P < 0.001$) in the ANOVA two-way statistical test in the groups that received *S.Typh* alone or in combination with oseltamivir or MAR. The same effect was seen in the activity of total ATPase in this region, which decreased significantly ($P < 0.001$) in the ANOVA two-way statistical test in the groups that received oseltamivir alone or in combination with MAR and *S.Typh* with respect to the control groups.

Lipid peroxidation (TBARS) and H_2O_2 levels in the cerebellum/medulla oblongata region increased significantly ($P < 0.001$) in the ANOVA two-way statistical test in the groups that received oseltamivir alone or in combination with MAR or *S.Typh* in comparison with the control groups.

Table 5 shows the levels of DA, 5-HIAA, and biomarkers of oxidative stress in the stomach of rats. DA and GSH levels showed no significant differences in stomach tissue. However, the levels of 5-HIAA, H_2O_2, and lipid peroxidation decreased significantly ($P < 0.001$) in the ANOVA two-way statistical test in the groups that received *S.Typh* alone or in combination with MAR and oseltamivir.

With respect to the activity of total ATPase, this biomarker increased significant ($P < 0.001$) in the ANOVA two-way statistical test in the groups that received oseltamivir or *S.Typh* alone or in combination. While opposite effects were seen in the same groups that were treated with combination of MAR with respect to the control groups.

DISCUSSION

Lung inflammation is a critical determinant of influenza infection, and oseltamivir reduces the inflammatory response to influenza when given before or after infection.[24] The influenza virus (influenza) infection causes an intense infiltration of pulmonary tissues by macrophages, which abundantly generate a free radical, the nitric oxide (NO), resulting in oxidative stress damage.[25] For this reason, a novel inflammation animal model with *S.Typh* was used in this study to produce exactly

Table 5: Biogenic amines and some oxidative stress markers in *S. Typh*-infected stomach rats treated with oseltamivir and Mexican plant extract

Groups		Dopamine (nM/g)	5-HIAA (nM/g)	TBARS (μM malondialdehyde/g)	GSH (mM/g)	H_2O_2 (μM/g)	Total ATPase (μM Pi/g/min)
E	(a)	7.26 ± 8.0	131.31 ± 100	76.05 ± 37	0.81 ± 0.2	1.77 ± 0.3	58.57 ± 30
E + *S.Typh*	(b)	6.67 ± 1.9	64.48 ± 34*	25.36 ± 11*	0.81 ± 0.1	0.55 ± 0.2*	15.64 ± 6*
E + Osel	(c)	9.40 ± 4.4	117.68 ± 55	38.24 ± 23*	0.59 ± 0.1	0.80 ± 0.4	22.89 ± 13*
E + Osel + *S.Typh*	(d)	8.86 ± 2.7	126.33 ± 86	45.96 ± 14	0.53 ± 0.08	0.86 ± 0.3	23.05 ± 13*
PBS Ctrl	(e)	7.93 ±1.8	245.63 ± 114	9.76 ± 2	1.08 ± 0.7	0.13 ± 0.08	15.26 ± 14
Osel	(f)	9.35 ± 2.5	184.75 ± 29	15.18 ± 6*	0.94 ± 0.3	0.05 ± 0.01*	27.96 ± 26*
S.Typh	(g)	13.34 ± 6.1	161.85 ± 73*	16.38 ± 6*	0.80 ± 0.2	0.10 ± 0.08	35.56 ± 14*
Osel + *S.Typh*	(h)	9.94 ± 4.0	252.99 ± 85	11.88 ± 4	1.28 ± 0.7	0.06 ± 0.03*	20.75 ± 13

Mean values ± SD. g = g of wet tissue; Plant extract (E) = Mexican arnica + Mexican rosemary; *S.Typh* = *Salmonella typhimurium*; Osel = Oseltamivir
Stomach $^{*}P < 0.05$; 5-HIAA: b vs. a, g vs. e; TBARS: b, c vs. a, f, g vs. e; H_2O_2: b vs. a, f, h vs. e; Total ATPase: b, c, d vs. a, f, g vs. e

the same effect of swine virus AH1N1 and the results obtained were perfectly the same.

In cortex region, the DA levels increased in the group that was treated with oseltamivir alone and decrease in the same group in hemispheres region and in the group that was treated with oseltamivir plus plant extract plus *S.Typh* in cortex and cerebellum/medulla oblongata regions. This results provide insight into the turnover of DA due the affect of dopaminergic pathway in young animals and coincide with Marín-Valencia *et al*.,[26] who suggested that defects in biogenic amine metabolites of DA are the hallmark of DA deficiency, which may provide not only a clue for diagnosis but also information about prognosis and treatment monitoring. Machado *et al*.[27] suggested that a constituent from *Rosmarinus officinalis* (rosemary) presented an interaction with the dopaminergic system through the activation of DA, D(1) and D(2), receptors.

The decrease in 5-HIAA, lipid peroxidation, GSH, and H_2O_2 levels in brain regions and stomach in the groups that received oseltamivir combined with *S.Typh* and plant extract probe the antioxidant capacity of Mexican arnica[28] and rosemary extract.[29] However, the results underlying the antioxidant and anti-inflammatory effects of this combination of MAR need more studies in order to establish the authenticity of these effects.

Complementary and alternative medicine have become increasingly popular and several botanical ingredients, many of which have long histories of traditional or folk medicine usage,[30] are the common options for people without health services and those who live far away from towns and are exposed to sanitary risks due to their conditions.

CONCLUSIONS

The results of the present study suggest that MAR extract alter brain DA and 5-HIAA metabolism in young infected animals. Probably, the antioxidant capacity may be involved in these effects.

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Conflicts of interest

There are no conflicts of interest

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