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ORIGINAL ARTICLE

The Influence of a Xanthine-Catechin Chemical Matrix on *in vitro* Macrophage-Activation Triggered by Antipsychotic Ziprasidone

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Abstract— Ziprasidone (ZIP) is an effective antipsychotic with low side effects than other second-generation antipsychotics. Despite this, there are reports of adverse events and previous studies associating the use of ZIP the inflammatory response. It is possible to infer that bioactive molecules present in some foods could attenuate peripheral inflammatory and oxidative stress potentially triggered ZIP. This is the case of guaraná xanthine-catechin chemical matrix (XC-Mix) that presents caffeine, theobromine, and catechin. The *in vitro* protocols using murine RAW 264.7 cell macrophages were ZIP-exposure in culture medium supplemented with chemical isolated and admixture of Caf, The, and Cat. Main results showed that supplementation with isolated and XC-mix had a lowering effect on 72 h macrophages proliferation. XC-mix with 1:1:1 proportion at 25 μg/mL of each caffeine, theobromine, and catechin, molecules present lowering effect on nitric oxide levels, oxidative stress markers (DNA oxidation quantified by 8-hydroxy-2′ –deoxyguanosine), lipoperoxidation, and protein carbonylation. XC-mix also decreased protein levels and downregulated genes of proinflammatory cytokines (IL-1β, IL-6, TNF-α). At contrary, XC-Mix increased levels and upregulated gene of anti-inflammatory IL-10 cytokine. The

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results suggest that XC-matrix could present some beneficial action on peripheral proinflammatory effects ZIP-triggered. Complementary *in vivo* studies could be useful to confirm these *in vitro* findings described here.

KEY WORDS: Caffeine; Theobromine; Catechin, *Paullinia cupana*; Schizophrenia; Cytokine.

BACKGROUND

Neuropsychiatric disturbs, such as schizophrenia, bipolar disorders, and also dementia, as well as psychosis symptoms related with these morbidities, have been associated with chronic neuroinflammation states [1–4]. Paradoxically, the use of most second-generation antipsychotics (SGAs) has been also consistently associated to metabolic side effects, highly related to chronic inflammatory morbidities, such as obesity and diabetes *mellitus* type 2, which are directly associated with low-grade peripheral inflammatory states [2, 3].

Despite these less intense effects in patients that intake ziprasidone (ZIP), previous investigations have described some side reactions immune dysfunctions with ZIP use including allergic events associated to hypersensitive reactions, chest pain and diffuse urticaria [4–6], hypertensive reactions [5], and respiratory failure [6]. Moreover, ZIP has been associated with Kounis syndrome development, which is an allergic reaction preceding and leading to acute coronary syndrome [7].

An *in vitro* study using a non-human RAW 264.7 macrophage cell lines confirmed that ZIP is able to activate inflammatory response inducing higher levels of proinflammatory cytokines, such as interleukin 1 (IL-1), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), and decreasing in the anti-inflammatory interleukin 10 (IL-10) cytokine levels [7]. The effects described in macrophage ZIP-exposed were similar to those found in these cells by exposure of phytohemagglutinin (PHA), a natural pro-inflammatory antigen. Furthermore, previous studies also described potential ZIP cytotoxic effects by increase of oxidative stress molecule on human peripheral blood mononuclear cells [7].

Considering that ZIP is an effective antipsychotic with low side effects than other SGAs, identification of a chemical matrix present in some dietary foods that modulates inflammatory states triggered by this drug could be clinically relevant. This is the case of xanthine molecules that includes caffeine and theobromine and also polyphenols that includes catechins found in some functional foods commonly consumed by population. This is the case of chemical matrix present in beverages, such as green and black tea [8], yerba mate [9] and guaraná [10, 11]. Guarana's (*Paullinia cupana*, Mart) is an Amazonian fruit whose seed toast is richest in caffeine and also has theobromine, another xanthine, and catechin polyphenols [12]. Guaraná powder is broadly used to produce energetic beverages in the world [12, 13]. Previous *in vivo* and *in vitro* studies have described that guaraná extract could have some antioxidant [14, 15] and anti-inflammatory effects [14–17].

Therefore, it is possible that guaraná main XC-mix and their isolated molecules could have some anti-inflammatory action against ZIP-macrophage activation. This hypothesis is based in previous investigation of guarana's XC-mix showed cryoprotectant effect in thawed human sperm by modulation of some oxidative stress markers [15]. Moreover, Barbisan et al. [17] described that similar XC-mix was able to improve anti-inflammatory Lithium effect on PHA activated-macrophages.

MATERIALS AND METHODS

Chemical Reagents and Equipment

In the present study, drugs and reagents used in the experiments, including Dulbecco modified Eagle medium (DMEM), caffeine, ziprasidone, catechin, and theobromine and other chemical reagents, were purchased from Sigma-Aldrich (San Louis, MO, USA). Materials used in all cell culture experiments were purchased from Vitrocell-Embriolife (Campinas, São Paulo, Brazil) and Gibco-Life Technologies (Carlsbad, CA, USA) including fetal bovine serum, heat-inactivated horse serum, penicillin, and streptomycin. Quantikine kits for Elisa immunoassays were purchased from Biomyx Technology (San Diego, Ca, USA). Part of this *in vitro* study used commercial murine RAW 264.7 macrophage cells that were obtained from American Type Culture Collection (ATTC, Manassas, VA, USA) by the Cell Bank of Rio de Janeiro, which thawed and provided aliquots of these cells for the study. This non-profit non-governmental organization also issued a certificate that the line was not contaminated. The analyses involving the measurement of absorbance or fluorescence were conducted using SpectraMax i3x Multi-Mode

Microplate Reader equipment (Molecular Devices, Sunnyvale, California, USA).

Xanthine-Catechin Mixture and ZIP on RAW Macrophages

This protocol was also performed using murine RAW 264.7 macrophages (ATCC TIB-71) as experimental model, and macrophages were cultured as described in Jung et al. [18]. Briefly, all protocols were performed at 37 °C in a 5% CO_2 incubator with cells cultured in DMEM medium supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 mg/mL). Cells (1×10^5) were seeded in six-well plates and allowed to adhere for 24 h before receiving XC-matrix and ZIP supplementation. All analyses were performed in 72-h cell cultures and in triplicate.

Initially, the effect of isolated guarana's chemical matrix molecules on cellular proliferation of ZIP-activated macrophages was evaluated in 72-h cell cultures. The range concentration of isolated molecules tested here was similar to Barbisan et al. [17], which also studied XC-mix effect on activated-macrophage lithium-exposed: 0, 0.25, 50, 75, 100, and 200 μg/mL. From these results were produced and tested three potential XC-mix. The XC-mix reference concentration was based in the quantification of these bioactive molecules in a hydroalcoholic guaraná extract that was previously published by Bittencourt et al. [10] (caffeine = 12.240 mg/g, theobromine = 6.733 mg/g, and total catechins = 4.336 mg/mg). In this reference concentration, the estimated proportion was (3) caffeine, (2) theobromine, and (1) total catechins. Therefore, first XC-1mix (3:2:1) of these molecules where added 75 caffeine, 50 theobromine, and 25 catechin μg/mL concentrations in culture medium of macrophages. The second XC-2mix (2:1:1) was produced with an admixture containing 50 caffeine, 25 theobromine, and 25 catechin μg/mL concentrations. The third XC-3 mix (1:1:1) was produced with an admixture containing similar concentrations of three isolated molecules (25 μg/mL).

Assessment of Cellular Proliferation

One of the characteristics of the activation of mononuclear cells is the increase in the rate of cellular proliferation in relation to a group of non-activated cells. For this reason, in both protocols, the proliferative rate in 72 h cell cultures was performed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction spectrophotometric assay, as described previously by Barbisan et al. [17]. Briefly, MTT was dissolved in 5 mg/mL phosphate-buffered saline (PBS) and was added to a 96-well plate containing the sample treatments. Further, the plate was incubated for 1 h at 37 °C. Culture supernatant was removed, and the cells were resuspended in 200 μL of dimethyl sulfoxide (DMSO). Reaction was spectrophotometrically read at 560 nm absorbance.

Nitric Oxide and Others Oxidative Stress Marker Assessments

In the two protocols were quantified the nitric oxide (NO), which is considered a key molecule in the early inflammatory response, which was spectrophotometric quantified by a colorimetric assay used to detect organic nitrate [18]. The Griess assay detects nitrite formed by the spontaneous oxidation of NO under physiological conditions involving azo coupling between diazonium species, which are derived from sulfanilamide and NO_2, and naphthylethylenediamine. A colorimetric produce measure at 540 nm is obtained from this reaction that is proportional of NO level present in the sample [18]. Barbisan et al. [17] described detailed the NO quantification performed here. Other oxidative markers studied here were lipid peroxidation (LPx) and protein carbonylation (PCarb). LPx was spectrophotometrically estimated through the formation of thiobarbituric acid reactive substances (TBARSs) as previously described by Jentzsch et al. [19] and PCarb quantified according to Levine et al. [20], using 532 and 370 nm wavelengths, respectively.

Immunological Assays

The cytokines IL-1, IL-6, TNF-α, and IL-10 levels were quantified in 72-h cell cultures in both protocols conducted with PBMCs and RAW macrophages. The follow variables associated with oxidative metabolism were also quantified just in the PBMC experiment: antioxidant enzyme levels SOD, catalase (CAT), glutathione peroxidase (GPX), and 8-hydroxy-2′-deoxyguanosine (8-OHdG) that is a marker of DNA oxidation (oxDNA). These measures were performed by immunoassay tests using Quantikine Human Immunoassays kits as manufacturer instructions. Briefly, all reagents and working standards were prepared and the excess microplate strips were removed, before adding 50 μL of the assay diluent RD1W to each well. Next these procedures, 100 μL of standard control for our sample was added per well, after which the well was covered with an adhesive strip and incubated for 1.5 h room temperature. Each well was subsequently aspirated and washed twice, for a total of three washes. The antibody of each molecule analyzed here was added to

each well and covered with a new adhesive strip before being incubated for 30 in at room temperature. The aspiration/wash step was repeated, and the conjugate of each antioxidant enzyme (100 μL) was added to each well and incubated for 30 min at room temperature. The aspiration/wash step was repeated before adding 100 μL of substrate solution to each well, followed by incubation at room temperature for additional 20 min. Finally, 50 μL of stop solution was added to each well and the optical density was determined within 30 min using a microplate reader set to 450 nm.

Cytokine Gene Expression Assay

Modulation of cytokine gene expression was conducted here by qRT-PCR analysis using a similar approach to that described by Jung et al. [21]. Briefly, total RNA obtained from each treatment was isolated using TRIzol® reagent and quantified using a NanoDrop™ 1000 Spectrophotometer System® (Thermo Fisher Scientific, Wilmington, DE, USA). Next, a cDNA was obtained using Script™ cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA, USA) following manufacturer's instructions. The qRT-PCR assay was performed with the Quanti Fast SYBR® Green PCR Kit (Qiagen, São Paulo, SP, Brazil) in a Rotor Gene® Q equipment (Qiagen, São Paulo, SP, Brazil). The specific forward and reverse primer sequences are described use here were: IL-1β-F 5′GCGGCATC CAGCTACGAAT3′; R-5′ACCAGCATCTTCCTCA GCTTGT3′; IL-6-f 5′TACCCCCAGGA GAAGATTC CA3′; R-5′CCGTCGAGGATGTACC GAATT3′; TNF-α-F 5′CAACGGCATGGATCTCAAAGAC3′; R-5′ TATGGGCTCATACCA GGGT TTG3′; IL-10-F 5′ GTGATGCCCCA AGCTGAGA3′ R- 5′TGC TCTTG TTTTCACAGGGAAGA3′. The β-actin housekeeping gene was used as an internal control of gene expression analysis. Relative gene expression was calculated using the comparative Ct method and was expressed as fold expression relative to the control.

Statistical Analysis

Data treatments were performed according to *in vitro* good practice presumptions described by Griesinger et al. [22]. As currently is used, all assays were conducted in independent triplicate, and for this, analysis data obtained were normalized by the mean of cell growth and transformed as % of control [23]. Statistical analyses were performed using GraphPad Prism software (6.0 version). Data were presented as % mean ± standard deviation (SD) of negative or positive control group. Treatments were repeated, at least 5 times in each 96-well plate. The upper and lower values of 2-SD range found in these repetitions were considered outliers and excluded of the analysis, because generally, these outliers generate relative SD > 10% indicating presence of some experimental imprecision software. Comparison among treatments were performed by two-way analysis of variance followed by Bonferroni's *post hoc* test. All test comparisons with < 0.05 were considered statistically significant. In results showed in figures different letters identified statistical differences ($p < 0.05$) among treatments.

RESULTS

Initially, a concentration curve of three isolated bioactive molecules tested here was performed in ZIP-activated macrophages evaluating the effect on cellular proliferation of 72-h cell cultures. All caffeine concentrations tested here showed inhibitory effect on cellular proliferation than just ZIP-exposed cultures (Fig. 1a). Theobromine exposure caused decreasing in the cellular proliferation just in the lower concentration (25 μg/mL) (Fig. 1b). Catechin triggered lower inhibitory cellular proliferation than caffeine and theobromine ($p < 0.001$) presenting a concentration-response effect. However, catechin at lower concentration (25 μg/mL) was able to revert cellular proliferation to same levels than untreated control group (Fig. 1c). Results from three XC-mix proportions (Fig. 1d) showed that lower concentrations of each bioactive molecules were able to decrease ZIP-exposed macrophage proliferation in 72-h cell cultures (Fig. 1d).

The analysis of macrophage spreading in monolayer indicating some proinflammatory response was compared among treatments. An example of this evaluation is showed in Fig. 2a, b. From these results, a second complementary analysis was performed evaluating the effect of XC-Mix in 1:1:1 proportion on cellular proliferation and NO levels of 72-h cell cultures. In the present analysis, all isolated molecules and when put together (XC-Mix) in the ZIP-activated-macrophage cultures were able to reduce cellular proliferation (Fig. 2c). Similar results were observed on NO levels that decreased significantly in cultures exposed to X-C isolated and admixture molecules (Fig. 2d).

Considering that cellular proliferative inhibition could be caused by a cytotoxic effect, and not by an

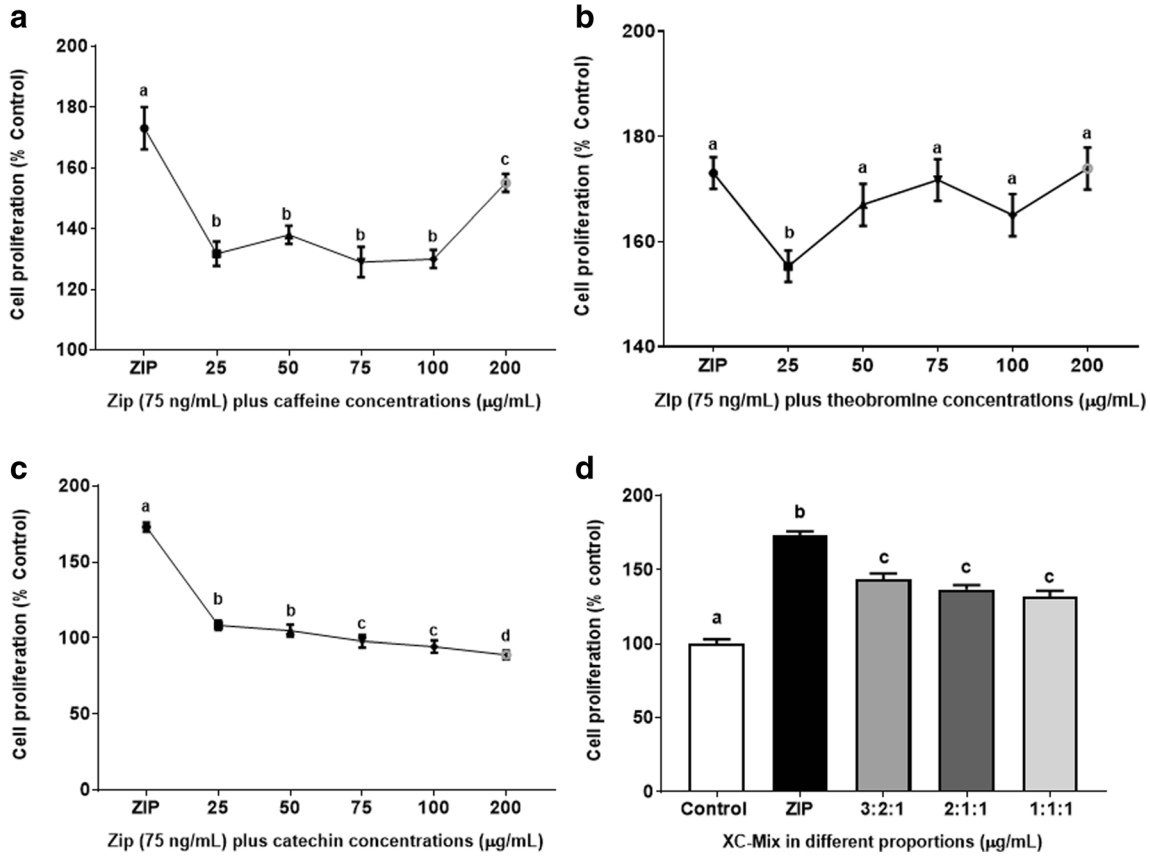

Fig. 1. Effect of different concentrations of ziprasidone and/or caffeine, theobromine, catechin, xanthine, and catechin mixture on macrophage proliferation in 72-h cell cultures. Effect of different ZIP concentrations on macrophage proliferation was analyzed using one-way analysis of variance (ANOVA), followed by the Tukey *post hoc* test. Effect of different XC molecule concentrations on macrophage proliferation was analyzed by performing two-way ANOVA followed by the Bonferroni *post hoc* test. The different letters (a, b, c, and d) indicate statistical differences in each treatment at $p < 0.05$. *Ziprasidone—ZIP. Xanthine and catechin = XC mixture = Mix.*

anti-inflammatory effect, some oxidative markers were also analyzed here. All markers tested here (oxDNA, LPX, PCarb) presented significant lower levels in macrophages ZIP-exposed that were cell cultures were XC-mix supplemented (Fig. 3).

Finally, the effect of XC-mix in cytokine protein and gene expression levels of ZIP-activated-macrophages was evaluated, and results are presented in the Fig. 4. Again, XC-Mix presented a significant lowering effect on proinflammatory cytokines, IL-1, IL-6, and TNF-α of ZIP-activated macrophages. At contrary, this exposure was able to elevate the IL-10 levels, an anti-inflammatory cytokine (Fig. 4a). The genes of proinflammatory cytokines were also downregulated, whereas IL-10 gene was upregulated in ZIP-activated macrophages concomitantly exposed to XC-Mix.

DISCUSSION

Despite ZIP to be an antipsychotic with attenuated metabolic alteration side effects, a previous study showed that this drug could induce some peripheral inflammatory response as previously demonstrated by Duarte et al. [8]. However, it is possible that peripheral inflammatory triggered by ZIP antipsychotic seems to be modulated by bioactive molecules presented in some food beverages including guaraná. The results presented here corroborated this hypothesis, showing the inhibition of cellular proliferation in ZIP-activated macrophages and lowering effect on proinflammatory cytokines.

Before analysis of main results found here is important to comment some pharmacological properties of ZIP antipsychotic drug. This is a benzoxazole-piperidine

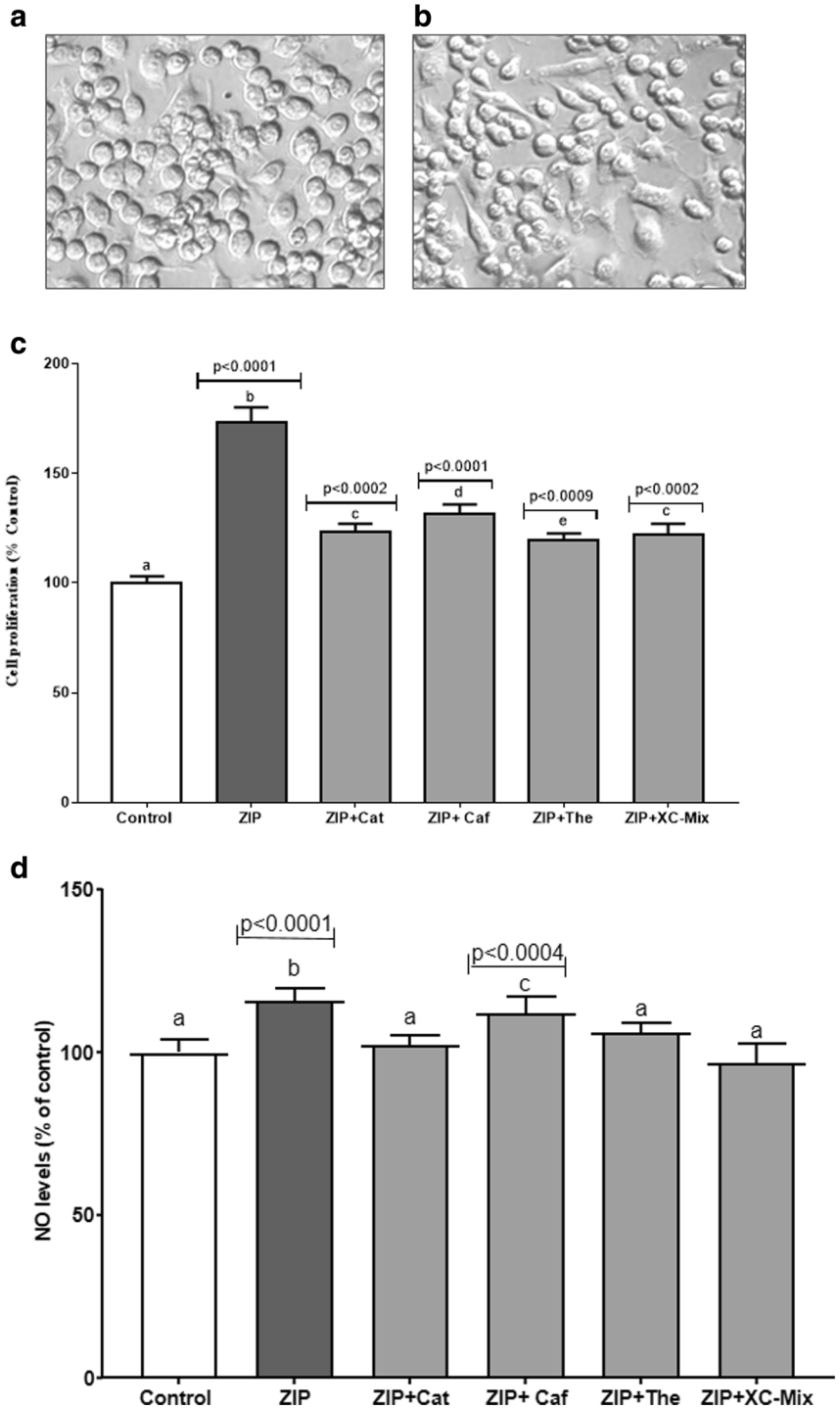

Fig. 2. Macrophage activation and proliferation. **a** RAW 264.7 monocyte inactivated cells (× 40), scale bar = 20 μm. **b** Macrophage activation observed by change of cell morphological patterns (arrow) (× 40). **c** Cell proliferation analyzed by MTT test after 72 h of the cell culture. **d** Nitric oxide levels determined by a modified Griess method quantifying nitrite/nitrate concentrations, after 72 h of the cell culture. Results are presented as percentage of the untreated control group. Samples were statistically compared by two-way ANOVA analysis followed by the Bonferroni *post hoc* test. The different letters (a, b, c, and d) indicate statistical differences in each treatment at $p < 0.05$. *Ziprasidone—ZIP. Xanthine and catechin = XC mixture = Mix.*

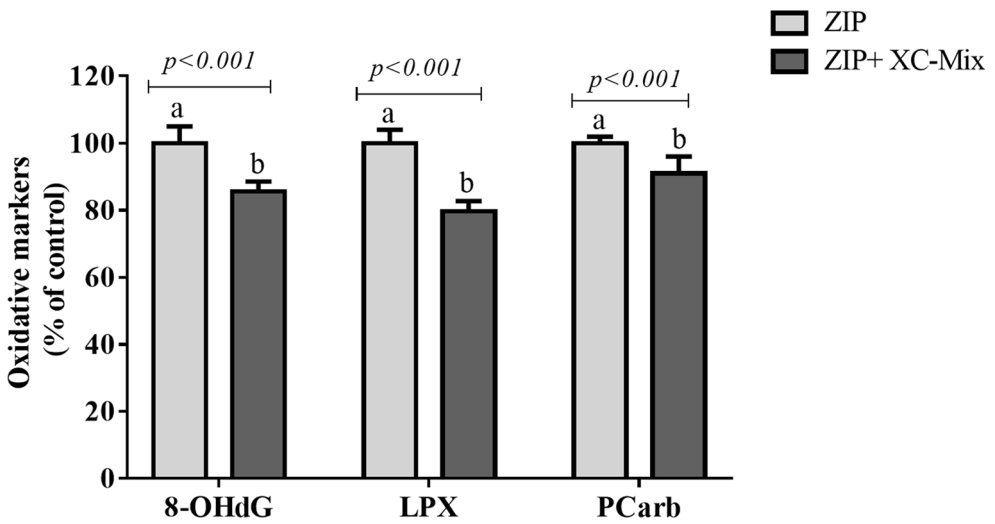

Fig. 3. Comparison of changes in the levels of oxidative markers. 8-OHdG = DNA oxidation, LPX = lipoperoxidation, Pcarb = carbonylation of proteins in macrophages treated with ziprasidone (ZIP) isolated and zip + xanthine-catechin (XC) mixture, incubated for 72 h. Statistical analysis was performed using two-way analysis of variance followed by the Bonferroni *post hoc* test. The different letters (*i.e.*, a, b) indicate statistical differences in each treatment at $p < 0.05$.

(piperazine) derivative with relative recent approval for clinical use due to effective action on positive and negative symptoms in the schizophrenia. The ZIP presents a pleiotropic neural pathway action since it has selective monoaminergic antagonism by dopamine D2 and 5-HT2A receptors and presents affinity for 5-HT1A, 2C, 1D receptors and by 1- and 2- adrenergic and H1-histaminergic receptors. Thereby, ZIP is able to inhibit synaptic reuptake of serotonin and norepinephrine [24].

Moreover, it is relevant to consider that macrophages are cells able to sense and respond to a great variety of stimuli and rapidly change their functional repertoire to meet the

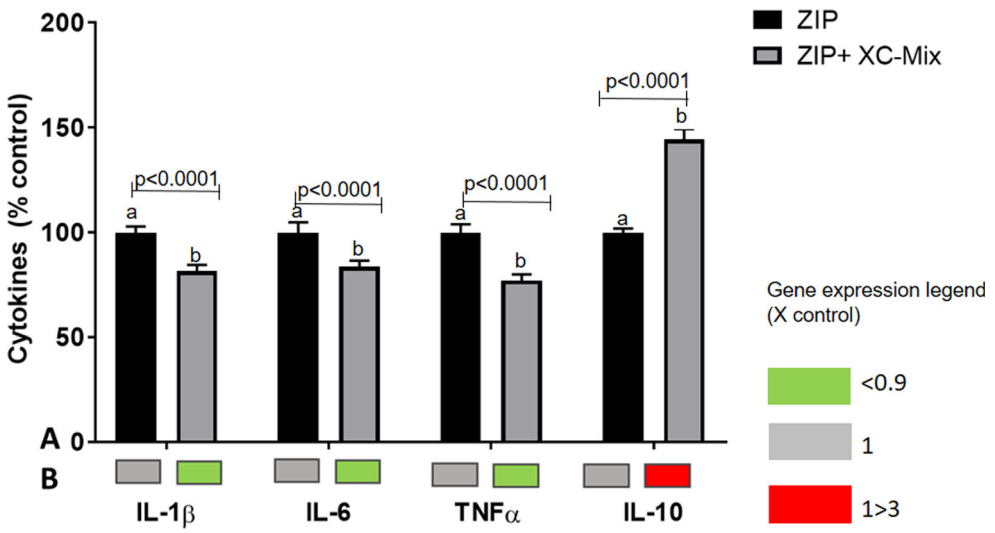

Fig. 4. Effect of ziprasidone and xanthine-catechin mixture cytokine protein levels and gene expression. **a** Cytokines protein level expression levels after 72-h treatments. **b** Gene expression levels after 24-h treatments. Treatments were statistically compared by ANOVA two-way followed by Bonferroni *post hoc* test. Different letters identified concentrations with significant differences calculated by *post hoc* test ($p < 0.05$). Gene expression of each cytokine are represented by colored squares and were determined using untreated control group as reference to calculate the relative mRNA expression. The expression level of beta-actin was used as an internal control (housekeeping gene). *Ziprasidone—ZIP. Xanthine and catechin = XC mixture = Mix.*

demands of the current microenvironment [24]. Due to this complex nature, macrophages present a diverse phenotype according its ontogeny and the tissue that these cells are located. Therefore, evidence have showed that mature macrophages retain a remarkable plasticity in their functional repertoires. This plasticity is a key of macrophage's function since these cells contribute directly and actively to restore homeostasis under different microenvironmental conditions [25]. In these terms, it is highly likely that the presence of bioactive molecules with antioxidant and anti-inflammatory properties could act on macrophage activation.

Other important comment to be made here is respect of RAW 264.7 used here as experimental model of pro-inflammatory and anti-inflammatory agents. This model is originated from Abelson leukemia virus-transformed cell line BALB/c mice derived that has been used for more than 40 years. The RAW use in experimental *in vitro* studies is based in its capacity of performing pinocytosis and phagocytosis. Upon antigen stimulation, specially LPS, these cells increase NO production, other inflammatory cytokines, and cellular proliferation [26]. Taciak et al. [26] suggested that RAW cells closely mimic bone marrow-derived macrophages in terms of cell surface receptors. However, the RAW 264.7 use needs caution since their functional characteristics remains stable until 30th passage. In the present study, RAW macrophages are between the 18 and 24 cell culture passages.

Results described here showed that polyphenols, such as catechins, could be useful to attenuate pro-inflammatory states triggered by ZIP on macrophages. These results are relevant since previous evidence has suggested that polyphenols are directly involved in modulation of mental health including action on behavior, mood, brain plasticity, and cognition. Specially in schizophrenia, a devastating mental disorder that causes chronic oxidative stress and neuroinflammatory states, direct interference of polyphenols could be useful. However, schizophrenia is a devastating mental disorder, with oxidative stress involved in its pathophysiology. The direct interference of polyphenols with schizophrenia pathophysiology has not been reported yet. However, increased oxidative stress caused by haloperidol was inhibited *ex vivo* by different polyphenols. Curcumin, extract from green tea and from *Ginkgo biloba*, may have benefits on serious side effects associated with administration of neuroleptics to patients suffering from schizophrenia. Polyphenols in the diet have the potential to become medicaments in the field of mental health after a thorough study of their mechanism of action. The broad use of RAW 264.7 cells includes a relatively large number of studies involving effect of polyphenols present in several plant extracts or food. This is the case of study performed by Palacz-Wrobel et al. [27] that investigated the effect apigenin, kaempferol, and resveratrol on TNFα gene expression and protein secretion and IL-10. These bioactive molecules are present in several functional foods showing antioxidant and anti-inflammatory action. The study showed that all molecules were able to reduce the intensity of inflammatory processes by inhibition the secretion of pro-inflammatory TNF-α cytokine and increase the levels of an anti-inflammatory IL-10 cytokine. A previous investigation also described that epigallocatechin gallate (EGCG) and other catechins have important anti-inflammatory activity on LPS-stimulated RAW macrophages [12, 28]. Duarte et al. [8] previously described that ZIP was able to induce inflammatory states similar to PHA-antigen exposure. Therefore, the results found here showing that isolate total catechin or in XC-mix could attenuate inflammatory response of ZIP-activated macrophages corroborate these previous investigations. Unfortunately, we are not able to find in the literature reviewed previous studies involving the effect of polyphenols, in general on inflammatory response triggered by antipsychotic drugs.

In relation to xanthine, both caffeine and theobromine seems to present some anti-inflammatory effect. These results have been published in the literature, as a subclinical study performed by Kempf et al. [29]. In this investigation, habitual coffee drinkers refrained for 1 month from coffee drinking, and in the second month, they consumed four cups of filtered coffee each day. In the third month, these volunteers consumed 8 cups of coffee (150 mL/cup). Biochemical marker analyses suggested that coffee consumption appears to present beneficial effects on subclinical inflammation, by cytokine modulation and HDL-cholesterol.

However, there are some controversies in relation to caffeine especially considering the intake of beverages richest in this substance by subjects with psychotic symptoms. Biological effects linked to caffeine consumption is most determined from blocking action exerted by this molecule in all adenosine receptors located at the neurons and brain glial cells. Some animal studies described that caffeine plays a role opposite to dopamine in the striatum [21], and this action could be explained previous reports that have suggested potential association between schizophrenia and high caffeine, but not with low and moderate intake [12].

Some other studies reported that caffeine consumption was associated with smoking habit that is highly prevalent in schizophrenic patients, but not to symptomatology of this psychiatric disorder, including psychosis [30]. This is the case of an investigation performed in Spain was performed

to clarify whether or not, after controlling for intervenient variables, such as tobacco smoking, the association between schizophrenia and caffeine intake would be consistent. The authors showed high frequency of caffeinated beverages in schizophrenia patients than controls. However, smoking was directly associated with caffeine. The authors considered that lack of independent association between caffeine consumption and schizophrenia was surprising. Based in these controversies, as well as in general attenuating effect of the macrophage inflammatory response found in the three XC-Mix tested here, we decided to focus subsequent analyses on the matrix with similar concentration of caffeine, theobromine, and catechin [30].

Therefore, it is possible to infer that the role of caffeine in psychotic symptoms could be dependent on the concentrations of this molecule, and perhaps its interaction with other bioactive molecules present in a given food matrix. In contrast, this latter inference is still quite speculative and should be better investigated from complementary studies. However, in the present investigation, it is relevant to comment the general properties of theobromine, the other main xanthine present in guaraná matrix and also added in XC-matrix tested here.

It is well established that caffeine has several beneficial effects range from alertness to reducing the risk of neurodegenerative diseases [31]. However, besides caffeine, other xanthines such as theobromine. This is the case of cocoa and guarana, the biological effect of theobromine being less studied than that of caffeine. Growing evidence obtained from previous studies suggest that theobromine has psychoactive actions in humans that are qualitatively different from those of caffeine [32]. For example, theobromine's effect on blood pressure is different from caffeine by not clarified reasons [33]. In fact, results described by Costa Krewer et al. [14] showed less prevalence of hypertension in Amazonian riparian elderly people that habitually intake guarana than others that never consumed this beverage containing guarana.

Another important physiological theobromine effect is its vasodilator effect useful to treat asthma and other respiratory problems linked with high NO levels produced by inducible oxide nitric synthase 1 (NOS1) enzyme. In this investigation, theobromine supplement in macrophage ZIP-exposure is able to revert NO levels for similar concentrations found in the control group. This effect was also found in cells just catechin-supplemented, whereas caffeine supplementation reverts just partially NO levels in comparison with ZIP group. Due its properties, there are reports that pharmacological use of pentoxifylline, a synthetic analogue of theobromine, presents attenuating effect on inflammation and stress oxidative states associated to acute lung injury by nitrogen mustard exposure [34]. The anti-inflammatory effect of pentoxifylline seems to be associated with its capacity to competitively inhibit phosphodiesterases in a range of cells and tissues increasing intracellular cAMP, a second messenger which acts *via* protein kinase A. Subsequently, this biochemical reaction suppresses gene transcription of pro-inflammatory mediators including TNFα. Furthermore, evidence has suggested that pentoxifylline inhibitory phosphodiesterase effect could act directly on Toll-like receptor (TLR) that is an important molecule involved in the inflammatory response. The study that described this effect suggested that pentoxifylline could be used to prevent clinical conditions such as neonatal sepsis [35].

Furthermore, previous evidence also reported that theobromine has higher lung diffusion than other drugs used in the therapy of respiratory diseases and this is a differential aspect in relation to caffeine. Considering reports that ZIP could trigger side effects including hypersensitive and allergic reactions and respiratory failure [36], it possible to infer that supplements richest in theobromine could be beneficial to attenuate these negative symptoms. It is also possible to speculate that XC-mix that present theobromine in its composition could act on important side effects triggered by other antipsychotics drugs. For example, olanzapine is SGA highly related to diabetes risk by apoptosis induction of beta-pancreatic cells. Pentoxifylline is considered an immunomodulatory and anti-inflammatory agent used to treat vascular disorders. This molecule inhibits proinflammatory cytokine production, and experimental studies such as that performed by Malekifard et al. [37] described that pentoxifylline has therapeutic effect against the autoimmune destruction of the pancreatic beta-cells induced by streptozotocin.

CONCLUSION

In conclusion, despite methodological constrains related to *in vitro* studies, the whole of results described here suggested that XC-matrix initially based in guaraná powder could present some beneficial action on peripheral proinflammatory effects ZIP-triggered. These results opened the perspective potential development of supplements or other pharmacological forms, including transdermal patches to minimize proinflammatory and oxidative stress effects associated with ZIP intake. It is possible to infer that this action could be also beneficial to attenuate other metabolic side effects related to SGAs use. Additional *in vitro* and *in vivo* investigations could test further this hypothesis.

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Availability of Data and Materials. All data generated or analyzed during the current study are included in this article.

AUTHORS' CONTRIBUTIONS

TD and FB designed the research and analyzed and interpreted the data; TD, FB, BSNC, VFA, BOT, and IBMC interpreted the data and wrote the paper; TD, FB, MMMFD, IECJ, and IBM analyzed and interpreted the data; EER and PASPL performed the research and analyzed the data; TD, FB, BSNC, VFA, BOT, and IBMC directed, designed, analyzed, and interpreted the data. All the authors read and approved the final manuscript.

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COMPLIANCE WITH ETHICAL STANDARDS

Competing Interests. The authors declare that they have no competing interests.

Ethics Approval. It is an *in vitro* work with the use of cell lines, there is no need for approval by the Ethics Committee.

REFERENCES

1. Khoury, R., and H.A. Nasrallah. 2017. Inflammatory biomarkers in individuals at clinical high risk forpsychosis (CHR-P): State or trait? *Schizophrenia Research* 199: 31–38.
2. Chen, J., F.H. Xu, R. Shao, C. Chen, and C. Deng. 2017. Molecular mechanisms of antipsychotic drug-induced diabetes. *Frontiers in Neuroscience* 11: 643.
3. Rajiv, R., K. Muzaffer, and S. Guloksuz. 2017. The link between the immune system, environment, and psychosis. *Schizophrenia Bulletin* 43: 693–697.
4. Ku, H.L., T.P. Su, and Y.H. Chou. 2006. Ziprasidone-associated pedal edema in the treatment of schizophrenia. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 30: 963–964.
5. Phillips, E.J., W.H. Chung, M. Mockenhaupt, J.C. Roujeau, and S.A. Mallal. 2011. Drug hipersensivity: Pharmacogenetics and clinical syndromes. *Journal of Allergy and Clinical Immunology* 127 (3 Suppl): 60–66.
6. Gresham, C., and A.M. Ruha. 2010. Respiratory failure following isolated ziprasidone ingestion in a toddler. *Journal of Medical Toxicology* 6: 41–43.
7. Hamera, L., and B.F. Khishfe. 2017. Kounis syndrome and ziprasidone. *American Journal of Emergency Medicine* 35: 493–494.
8. Duarte, T., F. Barbisan, P.A.S. do Prado-Lima, V.F. Azzolin, I.E. da Cruz Jung, M.M.M.F. Duarte, C.F. Teixeira, M.H. Mastella, and I.B.M. da Cruz. 2018. Ziprasidone, a second-generation antipsychotic drug, triggers a macrophage inflammatory response *in vitro*. *Cytokine* 106: 101–107.
9. Pan, J., Y. Jiang, Y. Lv, M. Li, S. Zhang, J. Liu, Y. Zhu, and H. Zhang. 2018. Comparison of the main compounds in Fuding white tea infusions from various tea types. *Food Science and Biotechnology* 27: 1311–1318.
10. Souza, A.H.P., R.C.G. Corrêa, L. Barros, R.C. Calhelha, C. Santos-Buelga, R.M. Peralta, A. Bracht, M. Matsushita, and I.C.F.R. Ferreira. 2015. Phytochemicals an bioactive properties of Ilex paraguariensis: An *in vitro* comparative study between the whole plant leaves and stems. *Food Research International* 78: 286–294.
11. Bittencourt, L.S., D.C. Machado, M.M. Machado, G.F. Dos Santos, T.D. Algarve, D.R. Marinowic, E.E. Ribeiro, F.A. Soares, F. Barbisan, M.L. Athayde, and I.B.M. Cruz. 2013. The protective effects of guarana extract (*Paullinia cupana*) on fibroblast NIH-3T3 cells exposed to sodium nitroprussiade. *Food and Chemical Toxicology* 53: 119–125.
12. Schimpl, F.C., J.F. da Silva, J.F. Gonçalves, and P. Mazzafera. 2013. Guarana: Revisiting a highly caffeinated plant from the Amazon. *Journal of Ethnopharmacology* 150 (1): 14–31.
13. Pandiaraj, P., A. Gnavavelbabu, and P. Saravanan. 2015. Synthesis of CuO nanofluids and analysis of its increased effective thermal conductivity for flat plate heat pipe. *International Journal of ChemTech Research* 8 (4): 1972–1976.
14. Krewer, C.C., L. Suleiman, M.M.F. Duarte, E.E. Ribeiro, C.P. Mostardeiro, M.A.E. Montano, M.I.U. Marques da Rocha, T.D. Algarve, G. Bresciani, and I.B.M. da Cruz. 2014. Guaraná, a supplement rich in caffeine and catechin, modulates cytokines: Evidence from human in vitro and in vivo protocols. *European Food Research and Technology* 239: 49–57.
15. Werner, C., F.C. Cadoná, I.B.M. da Cruz, E.R.D.S. Flôres, A.K. Machado, M.R. Fantinel, G.C.C. Weis, C.E. Assmann, A.O. Alves, B.D.S.R. Bonadiman, E.E. Ribeiro, and M.A.E. Montano. 2017. A chemical compound based on methylxanthine-polyphenols lowers nitric oxide levels and increases post-thaw-human sperm viability. *Zygote* 25: 719–730.
16. Higgins, J.P., B. Kavita, P.A. Deuster, and J. Shearer. 2018. Energy Drinks: A Contemporary Issues Paper. *Currents Sports Medicine Report* 17: 65–72.
17. Barbisan, F., V.F. Azzolin, C.F. Teixeira, M.H. Mastella, E.E. Ribeiro, P.A.S. do Prado-Lima, R.S. Praia, M.M.F.D. Duarte, and

I.B.M. Cruz. 2017. Xanthine-catechin mixture enchances lithium-induced anti-inflammatory response in activated macrophages *in vitro*. *BioMed Research International* 2017: 4151594.

18. da Cruz Jung, I.E., A.K. Machado, I.B.M. Cruz, F. Barbisan, V.F. Azzolin, T. Duarte, M.M.F. Duarte, P.A.S. do Prado-Lima, G.V. Bochi, G. Scola, and R.N. Moresco. 2016. Haloperidol and Risperidone at high concentrations activate an in vitro inflammatory response of RAW 264.7 macrophage cells by induction of apoptosis and modification of cytokine levels. *Psychopharmacology* 233: 1715–1723.
19. Jentzsch, A.M., H. Bachmann, P. Fürst, and H.K. Biesalski. 1996. Improved analysis of maldondialdehyde in human body fluids. *Free Radical Biology & Medicine* 20: 251–256.
20. Levine, R.L., D. Garland, C.N. Oliver, A. Amici, I. Climent, A.G. Lenz, B.W. Ahn, S. Shaltiel, and E.R. Stadtman. 1990. Determination of carbonyl content in oxidatively modified proteins. *Methods in Enzymology* 186: 464–478.
21. Voiculescu, M., I. Ghiţă, A. Segărceanu, I. Fulga, and O. Coman. 2014. Molecular and pharmacodynamic interactions between caffeine and dopaminergic system. *Journal of Medicine and Life* 7 (Spec Iss 4): 30–38.
22. Griesinger, C., B. Desprez, S. Coecke, W. Casey, and V. Zuang. 2016. Validation of Alternative *In Vitro* Methods to Animal Testing: Concepts, Challenges. *Processes and Tools. Advances in Experimental Medicine and Biology* 132: 856–865.
23. Schott, K.L., C.E. Assmann, C.F. Teixeira, A.A. Boligon, S.R. Waechter, M.M.F. Duarte, and I.B.M. da Cruz. 2018. Brazil nut improves the oxidative metabolism of superoxide-hydrogen peroxide chemically-imbalanced human fibroblasts in a nutrigenomic manner. *Food Chemical and Toxicology* 121: 519–526.
24. Chiara, M., M.P. Rapagnani, and S.M. Stahl. 2011. Ziprasidone hydrocloride: what role in the management of schizophrenia? *Journal Of Central Nervous System Disease* 3: 1–16.
25. Weigert, A., A. von Knethen, D. Fuhrmann, N. Dehne, and B. Brüne. 2018. Redox-signals and macrophage biology. *Molecular Aspects of Medicine* 63: 70–87.
26. Taciak, B., M. Białasek, A. Braniewska, Z. Sas, P. Sawicka, L. Kiraga, T. Rygiel, and M. Król. 2018. Evaluation of phenotypic and functional stability of RAW 264.7 cell line through serial passages. *PLoS One* 13: e0198943.
27. Palacz-Wrobel, M., P. Borkowska, M. Paul-Samojednv, M. Kowalczyk, A. Fila-Danilow, R. Suchanek-Raif, and J. Kowalski. 2017. Effect of apigenin, kaempferol and resveratrol on the gene expression and protein secretion of tumor necrosis factor alpha (TNF-α) and interleukin-10 (IL-10) in RAW-264.7 macrophages. *Biomedicine and Pharmacoterapy* 93: 1205–1212.
28. Zhong, Y., Y.S. Chiou, M.H. Pan, and F. Shahidi. 2012. Anti-inflammatory activity of lipophilic epigallocatechin gallate (EGCG) derivatives in LPS-stimulated murine macrophages. *Food Chemistry* 134: 742–748.
29. Kempf, K., C. Herder, I. Erlund, H. Kolp, S. Martin, M. Carstensen, W. Koenig, J. Sundvall, S. Bidel, S. Kuha, and T. Jaakko. 2010. Effects of coffee consumption on subclinical inflammation and other risk factors for type 2 diabetes: a clinical trial. *The American Journal of Clinical Nutrition* 91: 950–957.
30. Gandhi, K.K., J.M. Williams, M. Menza, M. Galazyn, and N.L. Benowitz. 2010. Higher serum caffeine in smokers with schizophrenia compared to smoking controls. *Drug and Alcohol Dependence* 110: 151–155.
31. Ribeiro, J.A., and A.M. Sebastião. 2010. Caffeine and adenosine. *Journal of Alzheimer's Disease* 20 (Suppl 1): S3–S15.
32. Martínez-Pinilla, E., A. Oñatibia-Astibia, and R. Franco. 2015. The relevance of theobromine for the beneficial effects of cocoa consumption. *Frontiers in Pharmacology* 20: 6–30.
33. Mitchell, E.S., M. Slettenaar, N. vd Meer, C. Transler, L. Jans, F. Quadt, and M. Berry. 2011. Differential contributions of theobromine and caffeine on mood, psychomotor performance and blood pressure. *Physiology and Behaviour* 104: 816–822.
34. Sunil, V.R., K.N. Vayas, J.A. Cervelli, R. Malaviya, L. Hall, C.B. Massa, A.J. Gow, J.D. Laskin, and D.L. Laskin. 2014. Pentoxifylline attenuates nitrogen mustard-induced acute lung injury, oxidative stress and inflammation. *Experimental and Molecular Pathology* 97: 89–98.
35. Almario, B., S. Wu, J. Peng, D. Alapati, S. Chen, and I.R. Sosenko. 2012. Pentoxifylline and prevention of hyperoxia-induced lung - injury in neonatal rats. *Pediatric Research* 71: 583–589.
36. Ashraf, S., W. Graham, and H. Hayreh. 2018. A Case of Ziprasidone-Induced Hypersensitivity Pneumonitis: A Previously Unreported Side Effect of an Atypical Antipsychotic. *American Journal of Respiratory and Critical Care Medicine* 197: A6941.
37. Malekifard, F., N. Delirezh, R. Hobbenaghi, and H. Malekinejad. 2015. Immunotherapic effects of pentoxifylline in type 1 diabetic mice and its role in the response of T-helper lymphocytes. *Iranian Journal of Basic Medical Sciences* 18: 247–252.