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Antidepressant Drugs Modulate Differentially Anti-inflammatory Lithium's Property: An *in Vitro* and *in Vivo* Study

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Abstract: Li (lithium), a mood stabilizer has anti-inflammatory effect. However, in clinical practice, Li can be administered together with other antidepressants drugs, such as FLX (fluoxetine), IMI (imipramine), NOR (nortriptyline) and ESC (escitalopram). As interaction between Li and these antidepressant drugs on inflammatory modulation has not been investigated yet, we performed an *in vitro* protocol using a non-human macrophage cell line. Oxidative and inflammatory markers, as well as cell cycle analysis and cytokine gene expressions were compared among treatments. An IR (inflammatory ratio) was calculated based on the following oxidative-inflammatory variables: nitric oxide, superoxide anion, reactive oxygen molecules, cytokines IL-1β, IL-6, TNF-α and IL-10. The *in vitro* calculated IR data were validated through an *in vivo* analysis of 154 human subjects with similar IR. Li and control cells presented similar IR values. FLX, NOR and IMI increased slightly IR values indicating some proinflammatory effect, whereas ESC decreased IR values indicating some anti-inflammatory effect. However, cells exposed to Li + ESC triggered a proinflammatory response on macrophages. Thus, IR comparison results suggest that the Li anti-inflammatory effect is not universal and could be influenced by both basal macrophage-inflammatory state and interaction of other psychiatric drugs. These results could be useful to understand some inconsistencies observed in human studies involving Li and other psychiatric drugs.

Key words: Mood disorders, inflammation, selective serotonin reuptake inhibitors, bipolar disorder, lithium.

1. Introduction

Li (lithium) is used to treat bipolar disorder for decades and remains the gold standard in the treatment of this psychiatric disease, presenting efficacy to control manic and depressive symptoms and also to prevent recurrence of mood alterations and suicide risk [1-3]. Studies also reported that Li treatment could reduce prevalence of some neurological and cardiovascular morbidities, including myocardial infarction [4-6].

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Despite the exact biochemical, mechanism by which Li exerts mood stabilization it is not totally elucidated. A potential causal action of Li on bipolar disorder could involve effect on neurotransmitter signalling, adenylyl cyclase system, signalling on inositol phosphate and protein kinase, metabolism of arachidonic acid [1] and also in inflammatory metabolism [7].

In fact, some psychiatric diseases, including bipolar disorder have been associated with chronic neuroinflammation status with increase of proinflammatory cytokines levels in blood peripheral circulation [8, 9]. Li seems to present anti-inflammatory effect by inhibition of GSK-3 (glycogen synthase kinase-3), that when is hyperactivated contributes to establish chronic inflammation patterns [10, 11], and also reducing significantly some proinflammatory cytokines such as interleukin IL-1β, IL-6 and TNF-α produced by macrophages, neutrophils and/or dendritic cells [12]. However, whether Li anti-inflammatory effects have some influence of other environmental and pharmacological factors is an open question.

In clinical practice, Li is often added to antidepressants drugs, mainly as strategy to treat refractory depression and to accelerate antidepressant response of some drugs such as FLX (fluoxetine), IMI (imipramine), NOR (nortriptyline) and ESC (escitalopram) [13-17].

However, there are some controversial results about Li beneficial effects by inflammatory modulation [18], and we cannot exclude that controversies could be associated with potential intervenient variables that could interfere in the results or in their interpretation, such as genetic factors that act on inflammatory modulation [19-21].

Inconsistences between *in vitro* and *in vitro* or *in vitro* and *in vivo* data previously published in the literature also could be associated with the fact that inflammatory metabolism involves a dynamic cascade of events orchestrated by several self-related proinflammatory and anti-inflammatory molecules. Therefore, some drugs could trigger temporal changing in inflammatory cascade, but the final result to be similar making it difficult to interpret data. In some studies, particularly *in vitro* protocols that are not other inflammatory markers to use as reference, such a C-reactive protein produced by liver, oxidative and inflammatory markers are generally analysed in isolation and did not represent the global inflammatory status (or proinflammatory, balanced or anti-inflammatory condition) [22-26].

In this context, the aim of present study was to evaluate potential interference of some antidepressive drugs on Li anti-inflammatory effect. Therefore, we performed an *in vitro* investigation to evaluate the potential interaction between Li and antidepressant drugs (FLX, NOR, IMI and ESC) on inflammatory response, and to avoid interference of the two limiting aspects described above, we use a non-human macrophage cell line (RAW 264.7) to avoid some interference of genetic, lifestyle and healthy conditions factors in the results, and we compared data from an IR (inflammatory ratio) that was calculated base in six oxidative-inflammatory markers [NO (nitric oxide), superoxide anion, ROS (reactive oxygen molecules), cytokines IL-1β, IL-6, TNFα) and IL-10, an anti-inflammatory cytokine. The IR used in the *in vitro* protocol, was also validated by a complementary *in vivo* analysis with subjects who took blood samples to assess laboratorial presence of some body inflammation.

2. Material and Methods

2.1 Cell Culture Conditions and Experimental Design

No-activated murine RAW 264.7 macrophages (ATCC TIB-71) were used as *in vitro* experimental model. We choose to perform the analysis using a non-human cell line to avoid any potential genetic interference in the results obtained from drug treatments. The cells were cultured with DMEM medium supplemented with 10% FBS (fetal bovine

serum), penicillin (100 U/mL), and streptomycin (100 mg/mL). All experiments were performed at 37 °C and 5% CO_2 incubator and were three times replicated. Initially, 1×10^5 cells were seeded in 6-well plates and allowed to adhere for 24 h. All reagents used in cell culture are from Sigma Aldrich (USA), whereas experiments were performed using commercial formulations of each psychiatric drugs tested here. All experiments were performed in triplicate.

Therapeutic concentrations of each drug recognised as effective in the treatment of depressive symptoms [27] were adapted to cell culture conditions. Curve concentration of these drugs was constructed by analysis of two lower and two higher concentration related to therapeutic dose of each drug: Li = 0.17, 0.35, 0.7, 1.4 and 2.8 mEq; IMI = 50, 100, 200, 400 and 800 ng/mL; NOR = 25, 10, 100, 200 and 400 ng/L; FLU = 50, 100, 200, 400 and 800 ng/mL; ESC = 12.5, 25, 50, 100 and 200 μg/L). PHA (phytohemaglutin) as positive control of macrophages inflammatory response at 40 μg/mL concentration. PHA is a lectin antigen found in some plants that is able to induce mitosis and inflammatory response in immune cells [21].

The following parameters were used to evaluate the potential inflammatory modulation by psychiatric drugs on 72 h cell culture RAW macrophages: cell viability and proliferation; quantification of the oxidative markers NO (nitric oxide); superoxide anion, ROS (reactive oxygen species) and IL-1β, IL-6, TNFα and IL-10 cytokines levels. The pharmacogenomics effect of drugs on gene modulation of cytokines studied here was investigated by qRT-PCR.

2.2 IR (Inflammatory Ratio) Analysis

IR was calculated from the follow equation: IR= [% of control of proinflammatory markers] / [% of control of IL-10 cytokine x 6]. The following six proinflammatory markers were included in the equation: NO, Superoxide anion, ROS levels, and cytokines levels IL-1β, IL-6, TNFα. The sum of proinflammatory markers was divided by IL-10 (% of control and multiplicated by six), since were evaluated six proinflammatory markers in order to obtain a 1 ratio. Equation applied to control cells found value 1 since all variables are considered as 100%. Considering standard errors related to some variation on experimental conditions, treatments with values below 1 (> 0.8) were considered with anti-inflammatory effect, whereas treatments with values > 1.2 were considered with proinflammatory. Values between 0.81 and 1.19 were considered similar to untreated cells. The IR use decreased potential variations in the moment of inflammatory cascade decreasing data misinterpretation.

As *in vitro* analysis presents some methodological constraints, we tried to validate IR using a complementary *in vivo* human was performed to confirm the association between inflammatory status of IR values by comparison with CRP (C-reactive protein) levels. We performed this comparison because CRP considering an important marker of inflammation, being synthetized by the liver in response to factors released mainly by macrophages, such as IL-6 [28]. Elevated CRP levels have been associated with psychiatric disorders, including bipolar disorder and some investigations also suggested that CRP could be modulated by Li and antidepressant drugs [29].

The cytokines and CRP levels were analysed from information of 154 subjects previously enrolled in a broader research project in which the gene-environmental interaction associated with the aging processes and chronic diseases (genesis project) developed in free-living populations in southern Brazil region by Biogenomic Lab from Federal University of Santa Maria (UFSM, RS, Brazil). Details are described in Duarte [30, 31], that evaluated association between a single nucleotide polymorphism (SNP, rs 4880) located in the superoxide dismutase manganese dependent enzyme (Val16Ala-SOD2) with no-hypercholesterolemic and hypercholesterolemic subjects and also evaluated the potential pharmacogenetic effect of this polymorphism on

rosuvastatin treatment. IR of each human subject was calculated from the following equation: IR = [IL-1β+IL-6+ TNFα]/[IL-10 × 3]. Human Ethics Committee of each university (UFSM, number 23081.009087/2008) approved the study. In the occasion of prior data collection, all participants gave written informed consent prior to participating in this study.

2.3 Laboratorial Analysis

All analyses involving measurement of absorbance or fluorescence were performed with SpectraMax i3x Multi-Mode microplate reader (Molecular Devices-*Sunnyvale*, *CA- USA*). Cell viability and proliferation were firstly determined by the MTT (3-[4,5dimethylthiazol-2-yl] -2,5-diphenyltetrazolic bromide) reduction spectrophotometric assay [32] and flow cytometry (Accuri BD C6, Franklin Lakes, NJ-USA) described in Azzolin [33].

The quantification of NO and Superoxide anion was performed by spectrophotometry using methods previously described in the literature respectively for Jung and Morabito [34, 35]. The ROS production was determined using the 2′-7′-dichlorofluorescein diacetate (DCFDA) fluorimetric assay according to Barbisan [36].

The cytokines (IL-1β, IL-6, TNFα and IL-10) in the cell culture supernatants were measured, according to the manufacturer's instructions; following previously described methodological details [34] in Jung [34], 2016. The gene expression of these cytokines was determined by quantitative real time PCR (qRT-PCR) analysis by Rotor-Gene Q 5plex HRM System (QIAGEN, Hilden-Germany). Total RNA of each treatment was extracted using Trizol (Thermo Fisher Scientific *Waltham, MA-USA*). To perform reverse transcription, RNA was added to the samples of RNA (1,000 ng/μL) with 0.2 μL of DNAase (Invitrogen Life Technologies, USA) at 37 °C for 5 minutes, followed by heating at 65 ºC for 10 minutes. The cDNA was generated with 1 μL of Iscript cDNA and 4 μL of Mix Iscript (Thermo Fisher Scientific *Waltham, MA-USA*) according to the following reaction conditions: 5 ºC for 10 minutes, 25 ºC for 5 minutes, 85 ºC for 5 minutes and finally 5 °C for 60 minutes.

The qRT-PCR was conducted under the following reaction conditions: 95 °C for 3 min followed by 40 cycles of 95 °C for 10 s, 60 °C for 30 s followed by a melt curve of 60 °C to 90 °C in 0.5 °C increments for 5 s. The q RT-PCR reactions of each sample were made in triplicate, using 1 μM of each primer, 1,000 ng/μL of cDNA, RNAase-free water and 2× QuantiFast SYBR® Green PCR Master Mix (QIAGEN Biotechnology, Germany), in a final volume of 20 μL. The beta-actin gene was used as a housekeeping gene, and its expression level was used as an internal control. The relative expression was calculated using the comparative CT (Citosine-Timine) and was expressed as the fold expression compared to the control.

The specific primer pairs of cytokines are presented used in this study were: IL-1β Forward GCGGCATCCAGCTACGAAT and Reverse ACC AGCATCTTCCTCAGCTTGT; IL-6 Forward TACCCCCAGGAGAAGATTCCA and Reverse CCGTCGAGGATGTACCGAATT; TNF-α Forward CAA CGGCATGGATCTCAAAGAC and Reverse TATGGGCTCATACCAGGGTTTG; IL-10 Forward GTGATGCCCCAAGCTGAGA and Reverse TGCTCTTG TTTTCACAGGGAAGA.

The effect of the treatments on gene modulation was also evaluated comparing the rate of pro-inflammatory cytokine gene expression (PI) in relation to anti-inflammatory IL-10 expression (AI). The maximum value of PI/AI rate considered was 10 times higher than the control group, in order to construct an elucidative graph. However, when the rate was higher than 10 times, this information was cited in the results section, below.

2.4 Statistical Analysis

The *in vitro* statistical analyses were conducted employing GraphPad Prism 5 software. Data from each

variable in the treatments and from IR were compared using one-way ANOVA (analysis of variance), followed by the Tukey or Dunnet *post hoc* tests. The results of these analyses were expressed as mean ± standard deviation. In order to confirm that IR could represent an actual information of human blood inflammatory status, this ratio was calculated a sample subjects that are categorized in two groups: NI (non-inflammation) and with some IC (inflammatory condition) using CRP > 0.6 µl/L as cut-off point reference. IR values comparison between NI and IC subjects was used to determine sensitivity, specificity, predictive value of IR values by ROC (receiver operating characteristic) curve analysis. The value of area under the curve (AUC) consider predictive was ≥ 0.70. From this analysis and percentile distribution an IR cut-off point was determined and frequency distribution between NI and IC was compared by chi-square analysis. OR (odds ratio) and respective 95% confidence interval to IC present higher IR values was also calculated. A multivariate logistic regression analysis (Backward Wald method) was also used to determine the potential influence of sex and age on association of IR values and IC estimated by higher CRP levels. Results from *in vitro* and *in vivo* analyses with $p < 0.05$ were considered statistically significant.

3. Results

Initially, cytotoxic effects of Li and antidepressant drugs tested here were evaluated. Any drug decreased significantly macrophage viability except when cells were exposed to higher ESC concentrations (Fig. 1). PHA-activated macrophages did not change the viability when compared to no-activated control group (data no shown).

All Li concentrations tested here did not change cellular proliferation in 72 hours cultures when compared to control group (Fig. 2) using MTT assay. Similar to Li, isolated antidepressant drugs at estimated therapeutic concentrations did not change significantly the cell proliferation pattern. However, higher FLX concentrations triggered increase of macrophage proliferation. At contrary, a lowering effect on cell proliferation was observed when macrophages were

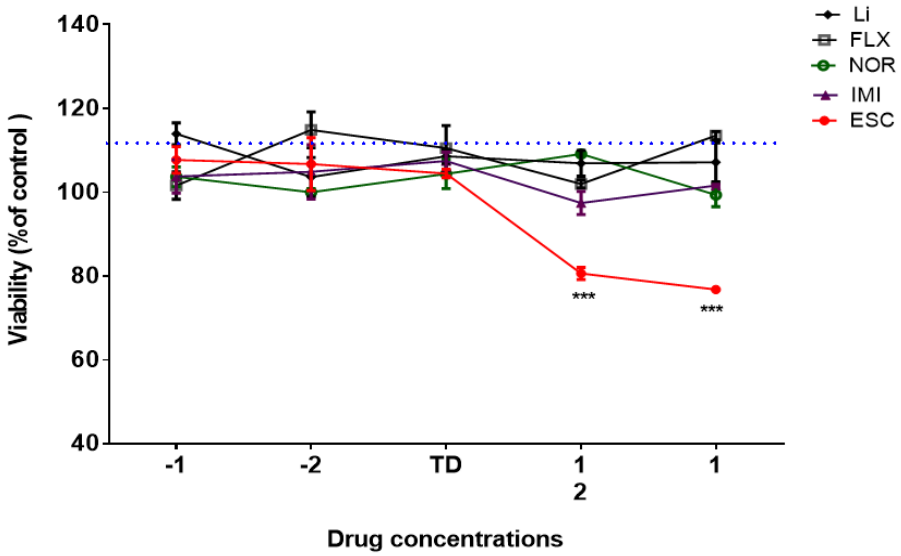

Fig. 1 Li (lithium) and antidepressant drugs (FLX = fluoxetine; NOR = nortriptyline; IMI = imipramine; ESC = escitalopram) effect on RAW macrophages in 24 hours cell cultures. Concentrations of each drug were determined by estimative of plasmatic TD (therapeutic dose) (LI = 0.7 ME/L; FLX = 100 ng/L, NOR = 100 ng/L, IMI = 200 ng/mL and ESC = 50 ng/L) and two lower (-2, -1) and two higher (1 -2) concentrations, that represented under and super dosing. Treatments were statistically compared by Anova One-Way followed by Tukey *post hoc* test. Concentrations with significant differences were calculated by *post hoc* test ($p \leq 0.05$). *** $p < 0.0001$.

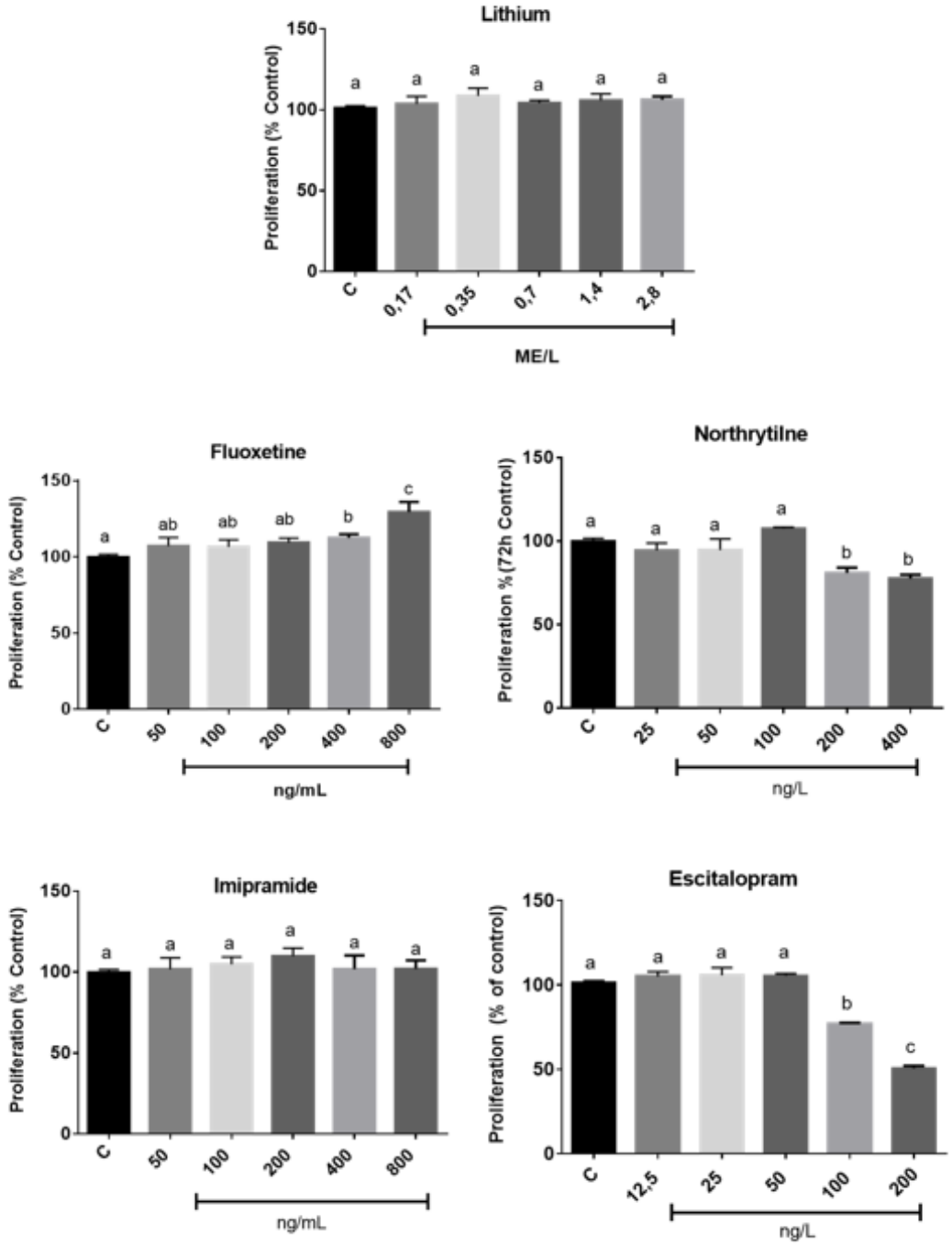

Fig. 2 Effect of Li and antidepressant drugs on 72 hours cell culture proliferation of macrophages RAW 264.7 measured by MTT assay. Concentrations of each drug were determined by estimative of plasmatic therapeutic dose (Li = 0.7 ME/L; FLX = 100 ng/L, NOR = 100ng/L, IMI = 200 ng/mL and ESC = 50 ng/L) and two equal and lower and two equal and higher concentrations, that represented under and super dosing, respectively. Treatments were statistically compared by Anova One-Way followed by Tukey *post hoc* test. Concentrations with significant differences calculated by *post hoc* test ($p \leq 0.05$) were identified by different letters.

treated with higher NOR and ESC concentrations ($p \leq 0.01$). Not all concentrations of IMI affected macrophages proliferative rate.

As expected PHA-activated macrophages presented a significant increase of cellular proliferation (127.3 ± 3.2%) than untreated cells. However, any treatment showed similar proliferative rate than activated macrophages PHA exposed when compared to Control group.

From these results, we performed all complementary analysis using just estimated therapeutic concentrations of Li (0.7 ME/L) and each antidepressant drug (FLX = 100 ng/L, NOR = 100 ng/L, IMI = 200 ng/mL and ESC = 50 ng/L).

Interaction between Li and antidepressant drugs on cell proliferation was also evaluated by flow cytometry analysis in 72 h cell cultures. In this analysis, we compared Li plus antidepressant drugs in relation to untreated and PHA-activated macrophages. Li presented similar frequency of S-phase cells than Control group. However, as can see in Fig. 3, FLX, IMI and ESC increased frequency of S phase cells that indicate a proliferative state. NOR did not change this frequency when compared to C group.

Interaction between Li+FLX and Li+IMI decreased the frequency of S-phase cells, whereas interaction between Li+NOR or Li+ESC triggered increase in the frequency of S-phase cells.

The levels of oxidative metabolism markers related to inflammatory response were also compared among treatments and results are presented in Fig. 4. NO levels of Li, Li+NOR and Li+IMI were similar to Control group. FLX, Li+FLX and ESC induced a slight increase of NO levels. Cells Li+ESC exposed showed moderate NO levels, whereas NOR and IMI exposure triggered higher NO levels than Control group. In relation to superoxide, cells Li or Li+ESC treated presented a slight increase of this oxidative molecule.

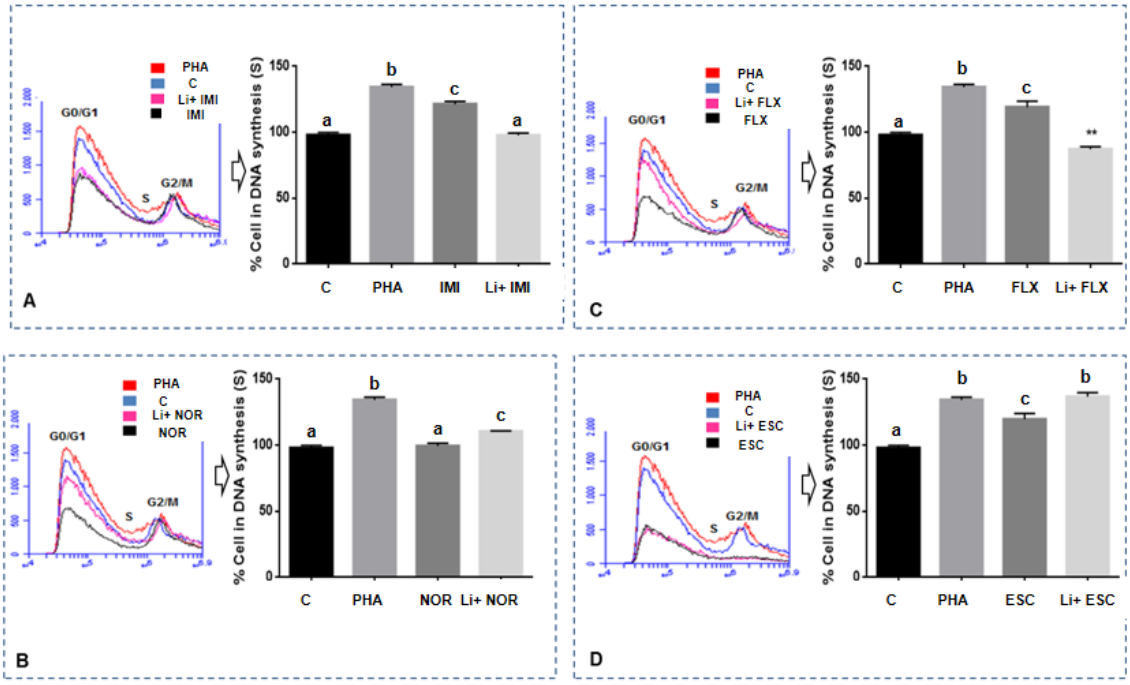

Fig. 3 Effect of Li and antidepressant drugs on macrophage cell cycle evaluated by flow cytometry. A representative flow cytometry cell cycle analysis of each antidepressant drug is showed plus graphic bars representing % of cells in S-phase cycle. Concentrations of each drug were determined by estimative of plasmatic therapeutic dose (Li = 0.7ME/L; FLX = 100 ng/L, NOR = 100ng/L, IMI = 200 ng/mL and ESC = 50 ng/L. Treatments were statistically compared by Anova One-Way followed by Tukey *post hoc* test. * $p < 0.05$; ** p <001, *** $p < 0001$.

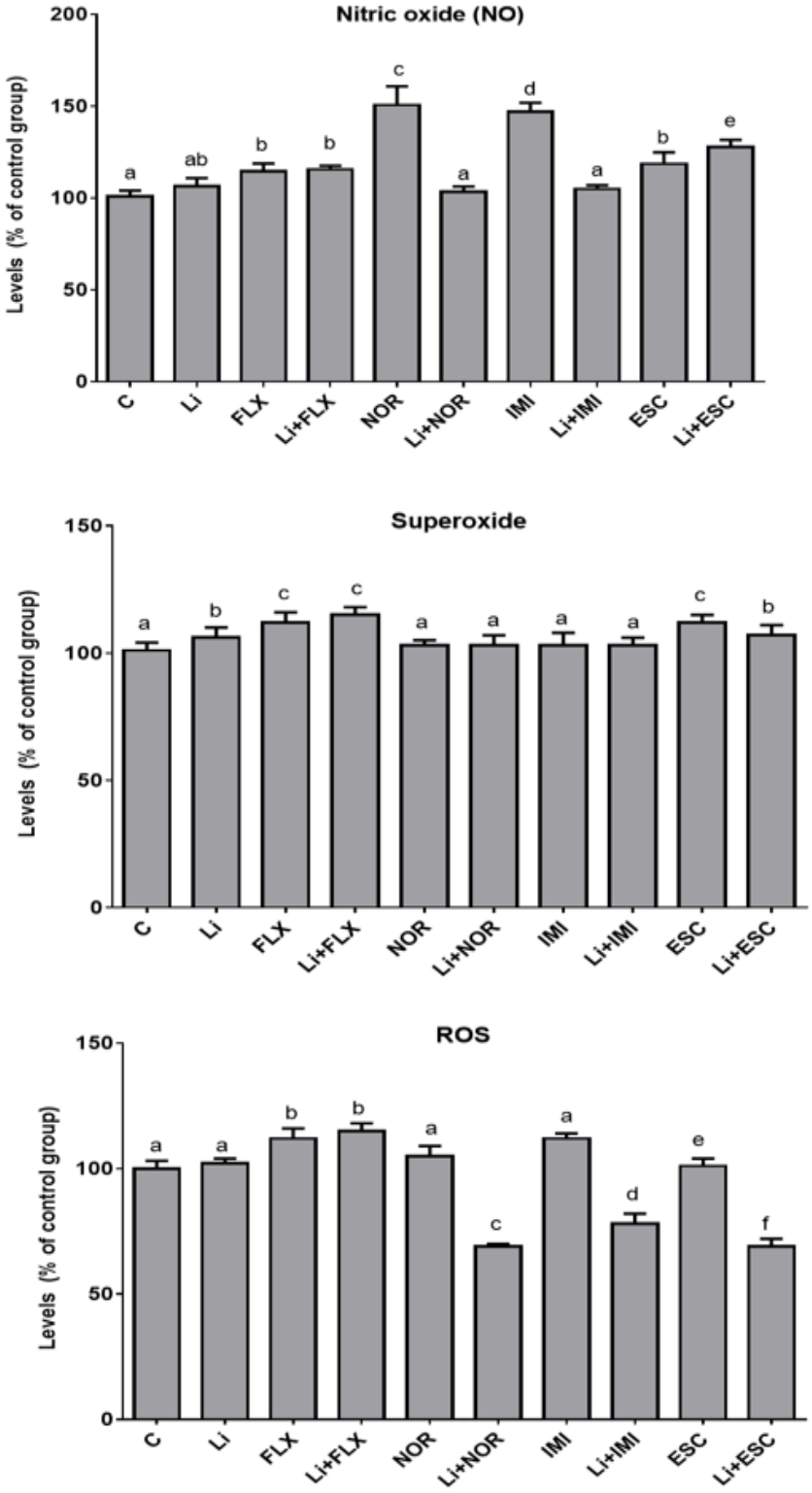

Fig. 4 Effect of Li and antidepressant drugs on 72 hours cell culture proliferation of macrophage RAW 264.7 on three oxidative markers: NO (nitric oxide), superoxide and ROS (reactive oxygen species). Concentrations of each drug were determined by estimative of plasmatic therapeutic dose (Li = 0.7 ME/L; FLX = 100 ng/L, NOR = 100 ng/L, IMI = 200 ng/mL and ESC = 50 ng/L) Treatments were statistically compared by ANOVA One-Way followed by Tukey *post hoc* test. Different letters identified concentrations with significant differences calculated by *post hoc* test ($p < 0.05$).

Cells NOR, Li+NOR, IMI and Li+IMI exposed did not change superoxide levels when compared to Control group. FLX, Li+FLX and ESC exposure triggered higher superoxide levels than Control group. ROS levels were similar to Control group when exposed to Li, NOR and IMI treatments. Li+NOR, Li+IMI and Li+ESC had a lowering effect on ROS levels. At contrary, FLX, Li+FLX present a slight increase in ROS levels than Control group.

As expected PHA-activated cells presented higher levels of NO (123.4 ± 4.5 % of control), superoxide (133.7 ± 5.2% of control) and ROS (147.6 ± 3.8% of control).

Results of cytokine levels and gene expression analysis are shown in Fig. 4, and presented differential pattern according to treatment with and without Li exposure.

Cells treated With Li presented similar IL-1β levels than Control group. All antidepressant drugs increased the levels of this proinflammatory cytokine. Li exposed concomitantly with FLX, IMI and ESC decreased partially IL-1β levels in relation to cells just treated with these drugs. However, this effect was not observed in cells NOR exposed, that in the Li presence remained with higher IL-1β levels. IL-1β gene was downregulated in cultures Li, Li+FLX and Li+ESC exposed. All treatments triggered up regulation of this gene. However, the intensity of over expression was attenuated in Li+NOR and Li+IMI than cells treated with these isolated antidepressant drugs (NOR and IMI) (Fig. 5A).

A higher concentration of IL-6 in comparison of Control group was observed in cells Liu-exposed. However, these levels were lower than cells exposed to antidepressant drugs. Concomitant treatment with Li decreased the IL-6 levels of all drugs tested here. Except for Li+NOR, all treatments triggered up regulation of IL-6 gene when compared to Control group. However, the high gene expression was attenuated when cells were concomitantly treated with Li+FLX, Li+IMI and Li+ESC (Fig. 5B).

A lowering effect on TNF-α levels was observed when cells were just Li exposed, whereas all antidepressant drugs presented higher levels of this proinflammatory cytokines when compared to Control group. Concomitant Li exposure decreased partially TNF-α levels triggered by FLX, IMI and ESC drugs. Again, in the presence of Li, cells NOR exposed maintained similar high levels of TNF-α. Gene expression analysis showed TNF-α downregulation in cells Li, FLX and Li+FLX treated. NOR and IMI caused up regulation of this gene, but this effect was reverted in the Li presence to levels similar to untreated cells (Control group).

Li+ESC treatment also decreased intensity of TNF-α up regulation found in cells just ESC exposed (Fig. 5C).

Li and antidepressant drugs effect on IL-10, an anti-inflammatory cytokine was also investigated. When cells were just Li exposed IL-10 levels remained similar to Control group. FLX was the drug that trigger higher IL-10 levels than other treatments, that was slight attenuated in the presence of Li. The other drugs presented lowering effect on IL-10 levels, that was attenuated in the presence of Li. All treatments triggered up regulation of IL-10 gene, except when Li+NOR exposed, that showed downregulation of this gene (Fig. 5D).

Activated-macrophages showed higher levels of all proinflammatory cytokines and lower IL-10 levels than control group. This treatment also triggered up regulation of IL-1β, IL-10 and TNF-α cytokines genes and downregulation of IL-10 gene. In relation of PHA treatment, any Li and antidepressant drugs did not present similar results or these effects presented an intermediary pattern (data no shown).

As isolated results did not show a clear pro-inflammatory or anti-inflammatory pattern related to Li plus antidepressant drugs, IR was calculated and compared among treatments. In the *in vitro* protocol PHA-activated macrophages were used as reference of a pro-inflammatory response in 72 hours cell cultures,

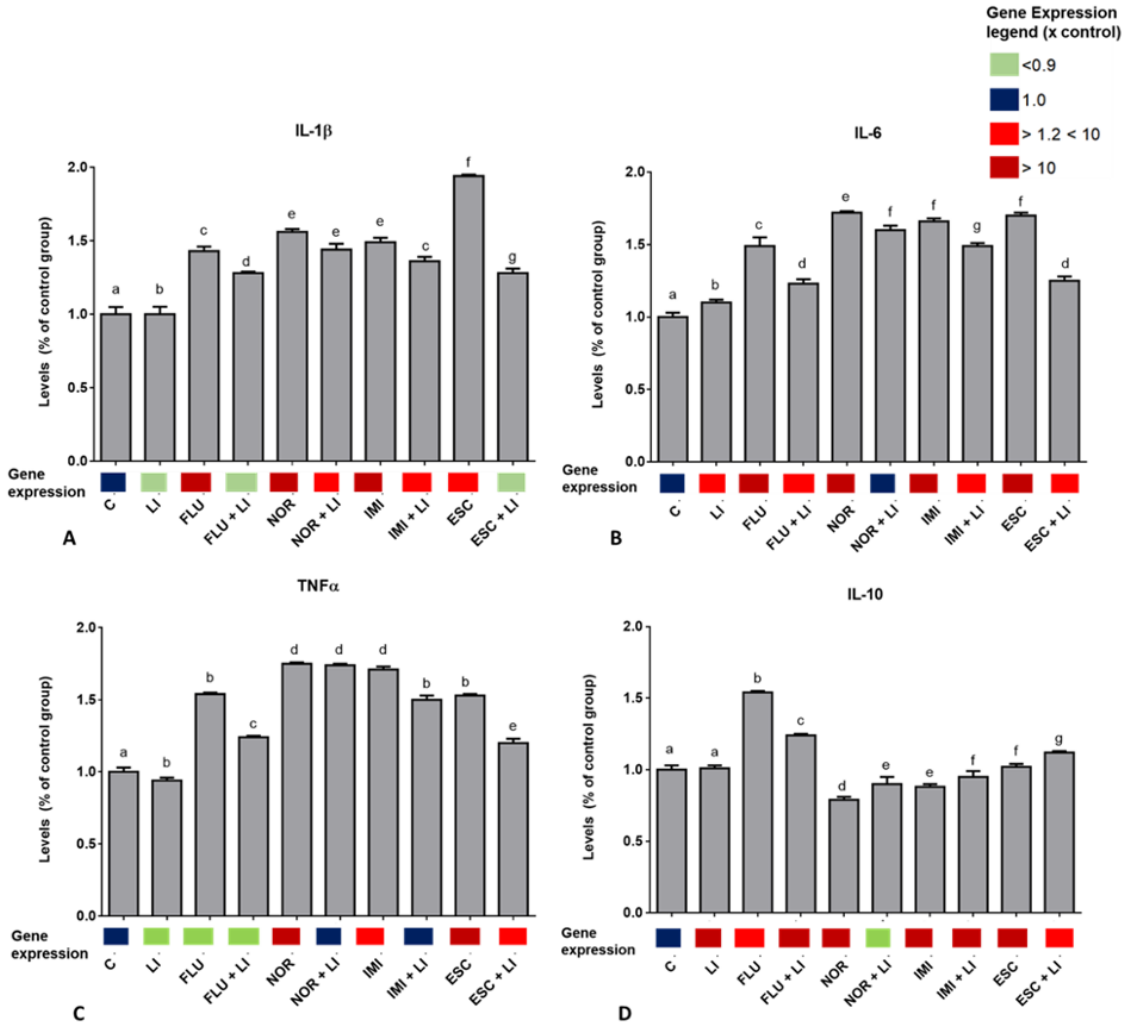

Fig. 5 Effect of Li and antidepressant drugs on 72 hours cell culture proliferation of macrophage RAW 264.7 on four cytokines: IL-1β, IL-6, TNFα, IL-10. Concentrations of each drug used here were estimated from an expected plasmatic therapeutic dose (Li = 0.7 ME/L; NOR = 100 ng/L, FLX = 100 ng/L, IMI = 200 ng/mL and ESC = 50 ng/L). Treatments were statistically compared by ANOVA One-Way followed by Tukey *post hoc* test. Different letters identified concentrations with significant differences calculated by *post hoc* test ($p < 0.05$). Gene expressions of each cytokine are represented by coloured 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).

and as expected PHA-activated macrophages presented higher (IR = 2.97 ± 0.04) than control group. However, other treatments did not show similar results found in PHA exposure.

In non-activated macrophages tested here, Li exposure showed IR similar to untreated cells—Control group (IR = 1.02 ± 0.02). In general, cells treated with isolated antidepressant drugs presented a slight elevation of IR levels, which did not exceed 1.5 times their value in relation to control group. Concomitant Li and FLX, NOR and IMI decreased significantly IR values when compared with cells exposed with these isolated antidepressants. On the other hand, higher IR values were observed in Li+ESC when compared with control group and cells just ESC exposed (Fig. 6).

A complementary analysis using *in vivo* data was performed to evaluate if a similar (but not equal) IR could represent an actual inflammatory state of human

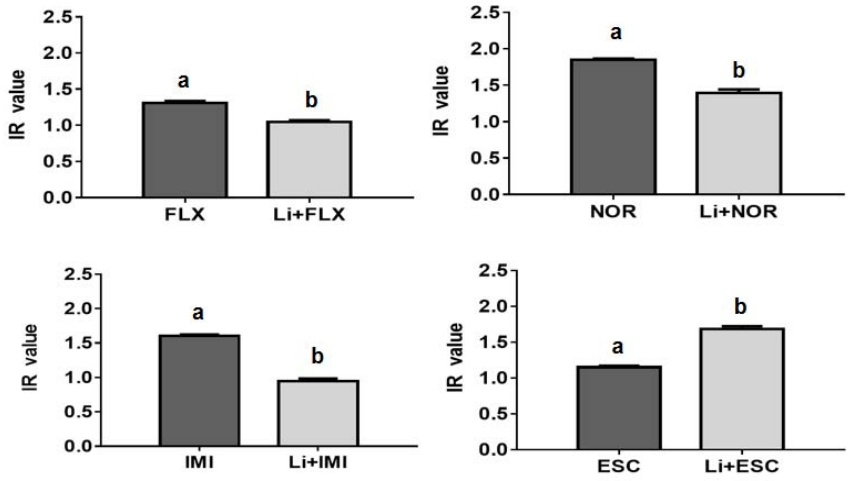

Fig. 6 Comparison of IR (inflammatory ratio) calculated by equation: [NO+Superoxide+ROS+IL1β+IL6+TNFα]/ [IL-10 × 6] in cells exposed to Li and four antidepressant drugs. Concentration of each drug used here was estimated from an expected plasmatic therapeutic dose (Li = 0.7 ME/L; NOR = 100 ng/L, FLX = 100 ng/L, IMI = 200 ng/mL and ESC = 50 ng/L) Treatments were statistically compared by Student t test.

Table 1 Characteristic baselines of sample subjects used to calculate IR from blood peripheral cytokines levels categorized by CRP levels.

Variables	CRP groups			
	NI Group		IC Group	
	Mean ± SD	Median	Mean ± SD	Median
CRP (μl/L)	0.37 ± 0.14	0.35	1.99 ± 1.6	1.15
IL-1β (ng/mL)	93.7 ± 74.0	83.0	199.7 ± 80.5	165.5
IL-6 (ng/mL)	117.3 ± 78.7	105.0	224.7 ± 87.9	179.0
TNFα (ng/mL)	137.9 ± 83.5	134.0	247.9 ± 92.5	213.0
IL-10 (ng/mL)	64.3 ± 18.7	59.0	56.19.9	53.0
IR value[#]	2.2 ± 2.0	3.0	4.9 ± 3.3	1.5

SD = standard deviation; IR = inflammatory ratio calculated by equation: IR = [IL-β+IL-6+TNFα]/[IL-10*3]. Comparison between two groups showed that all variables had $p < 0.001$ determined by p by Mann-Whitney non-parametric test since values did not present a normal distribution calculated by Kolmogorov-Smirnoff test.

patients. Initially we categorized sample by CRP using a cut-off point of 0.6 μl/L in NI and IC groups, and we compared the main cytokines levels analysed here and IR values (Table 1). Results showed that proinflammatory cytokine levels and IR values were higher in IC group, whereas IL-10 levels were lower than NI group.

An ROC analysis was applied to evaluate the accuracy of diagnosis based on the identified potential association between CRP inflammatory marker and IR values. The AUC (area under the curve) of ROC to IR was 0.803 (0.715-890 95%CI) (Fig. 7A). From ROC curve and percentile distribution we established an IR cut-off point = 2. This IR value was related to a sensibility = 0.915 and specificity = 0.486. Comparison of frequencies showed higher association between NI and IC groups (Fig. 7B). Odds ratio showed that subjects with IR > 2.0 presented a chance belongs to IC than NI group. The calculated OR showed 4.041 (1.687-9.681 95%CI) times higher chance of IC group has higher IR higher than 2 values than NI group. A multivariate analysis showed that association between higher CRP levels and IR values was independent of sex and age of sample.

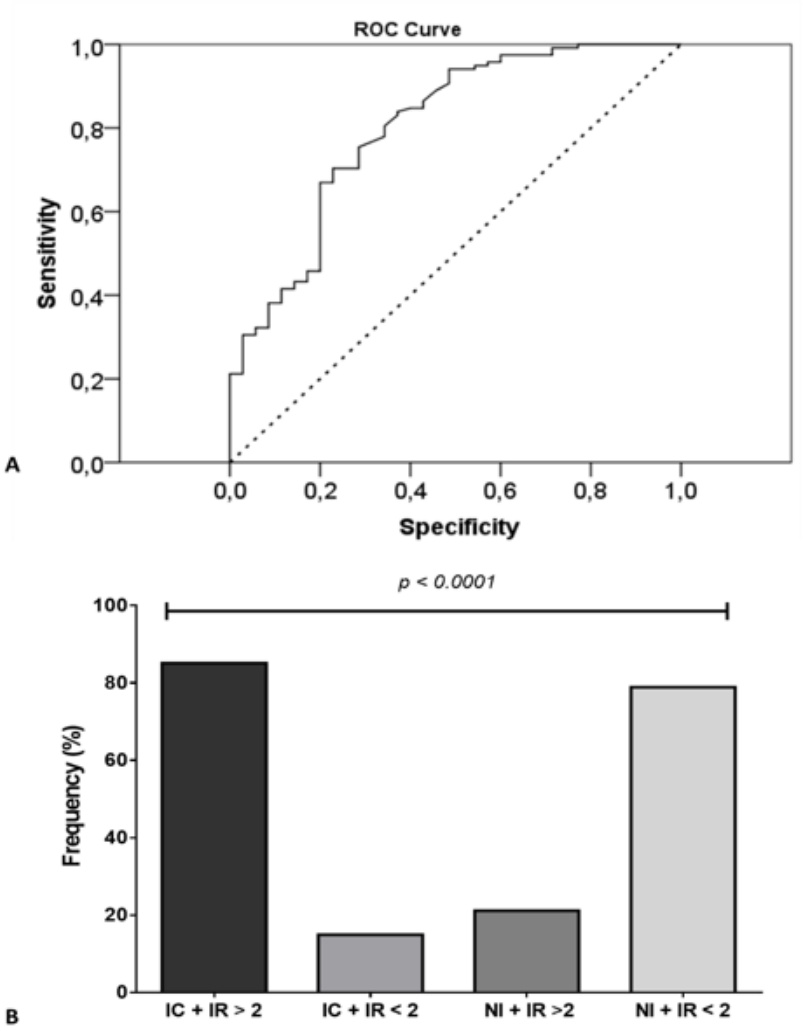

Fig. 7 Analysis of IR values in human subjects. (A) ROC curve showing that accuracy of IR values to predict inflammatory status using as reference CRP levels, constructed from data of 154 human subjects (AUC = area under de curve > 0.8); (B) Comparison of PCR categories (IC = inflammatory condition and NI = non-inflammatory condition) frequency and inflammatory ratio (IR) < and ≥ 2 value by chi-square test.

4. Discussion

The present investigation analysed if the Li anti-inflammatory effect could be maintained on Li interaction with four antidepressant drugs used in the psychiatric clinical practice. The whole of results evaluated mainly by IR values, suggested that Li maintained similar inflammatory balance that found in untreated non-activated macrophage cells, but this effect was substantially changed in the presence of antidepressant drugs. When Li interacted with FLX, NOR or IMI drugs an anti-inflammatory effect was detected. At contrary, the interaction between Li and ESC triggered increase in the IR values suggesting a proinflammatory effect.

Before we discuss more deeply these results it is important to point out that data described here were obtained using a novel methodological strategy, in order to decrease misinterpretation of results and clarify the whole of data obtained in the experiments.

In this strategy, we used a murine RAW 264.7 macrophage cells. Its cell line is popular mainly in exploratory experiments about putative biological agents or drugs [37]. However, there are some

investigations that consider this cell line not so good to evaluate inflammatory response, since it is usually slow to respond to stimulation and often constitutively exhibit high levels of cytokines in the absence of stimulant, including high levels of IL-1β and TNF-α [38]. Despite these limitations we believe that RAW cells could be useful in the *in vitro* studies considering some important methodological points, such as conducted analysis just in 72 hours cell cultures and considering as basal reference of cytokines and other molecules levels as found in untreated control cells cultured concomitantly to treatments and in the same conditions. We also believe that use of IR analysis can help to decrease methodological concerns related to use of macrophage RAW 264.7 cells *in vitro* protocols.

We also avoid using PBMCs (peripheral blood mononuclear cells), because human beings present some genetic variations that could affect inflammatory response, such as Val16Ala-SOD2 SNP that changes the efficiency of superoxide dismutase manganese dependent (SOD2) that is key enzyme in the control of superoxide levels produced into mitochondria. Particularly, VV genotype present is associated with metabolic diseases, such as hypercholesterolemia, obesity diabetes mellitus complications [19], and resistance anti-inflammatory and antioxidant effect related to rosuvastatin intake by hypercholesterolemic subjects [31]. A recent *in vitro* study performed by Barbisan [21] suggested that basal superoxide-hydrogen peroxide imbalance associated with Val16Ala-SOD2 SNP could affect the intensity of immunosenescence markers, and that especially VV-cells were associated with higher proinflammatory cytokine levels. The genetic variation that causes superoxide-hydrogen peroxide imbalance or other biochemical alterations could affect the immune cells response and have some impact on results obtained and its interpretation. Moreover, there are *in vitro* studies showing differential response of PBMCs carrying different Val16Ala-SOD2 genotypes to different environmental, nutritional and pharmacological agents [36, 39-43].

In the *in vitro* protocol, the macrophage activation was confirmed by use of PHA-antigen to trigger inflammatory response and increase the oxidative and cytokine levels, cellular proliferation, upregulation of proinflammatory cytokines genes and down regulation of IL-10 gene. For this reason, PHA-cells presented higher IR values (> 2.5) when compared with untreated cells and cells Li and antidepressant drugs exposed. As results observed from cell proliferation and gene modulation were heterogeneous, we solved to not include these variables in IR equation, and just use them to confirm macrophage inflammatory status.

Moreover, the use of these would make it impossible to validate *in vitro* IR data by similar analysis in human beings. In fact, we performed a complementary *in vitro* study using similar IR equation and CRP as reference of subject's inflammatory condition.

IR was organized based in similar analytic strategies used to evaluate other biological, ecological and health biomarkers parameters such as: apoptosis/cell proliferation balance by BAX/Bcl-2 gene expression ratio (x), and Jaccard index, also known as Intersection over Union and the Jaccard similarity coefficient (Jaccard, 1991). We considered important to use an IR to compare antidepressive effect on anti-inflammatory action triggered by Li exposure, because sometimes there are minor differences in the inflammatory cascade that could interfere in analysis of results or increase a subjective interpretation about inflammatory modulation.

The results found in the protocol using IR showed AUC > 0.8 that indicates accuracy and predictability of IR as variable to indicate inflammatory status, since it was used as reference CRP levels. CRP is a blood marker of inflammatory states. This molecule is synthetized by the liver in response to signal factors released by macrophages and fat cells, upon stimulation by the cytokines IL-1β, IL-6 and TNF-α during acute-phase inflammatory response, and elevate levels of this protein have been observed in BP patients

[8]. For this reason, we believe to have found an important association between CRP and IR levels. On the other hand, these results corroborate the use of IR values in the *in vitro* evaluation of psychiatric drugs, since in these conditions it is not possible to quantify CRP as inflammatory marker.

Considering the experiments performed by IR analysis, we organized Fig. 8 that shows a synthesis of

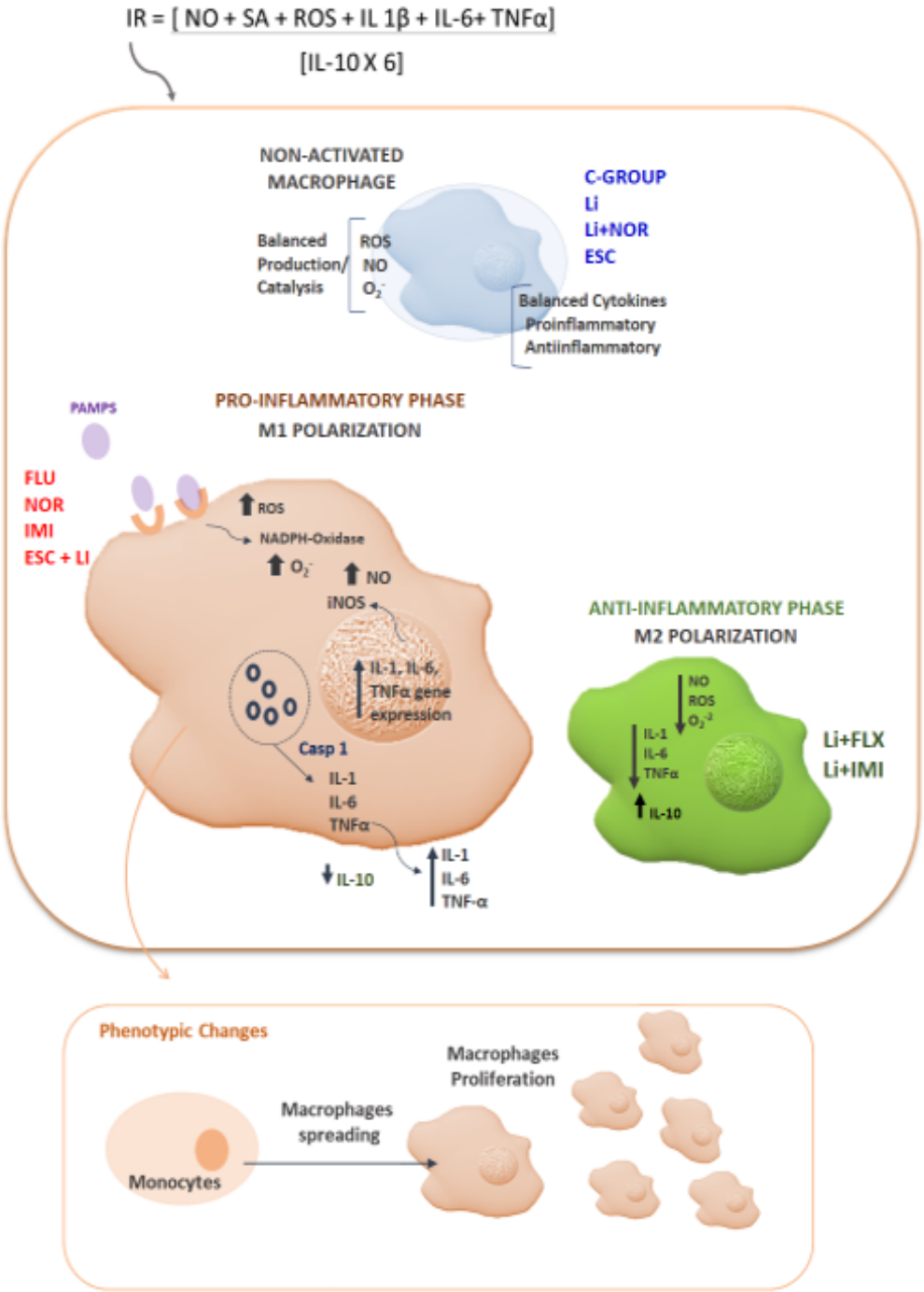

Fig. 8 Scheme of main results associated with Li (lithium) effect in interaction of four antidepressant drugs (FLX = fluoxetine; NOR = nortriptyline; IMI = imipramine; ESC = escitalopram) on inflammatory status of 72 hours macrophages RAW 264.7 cultures by IR (inflammatory ratio) equation. ROS: reactive oxygen molecules, NO = nitric oxide, C group = Control group, O_2^- = Superoxide, PAMPs: Pathogen-associated molecular pattern, iNOS = Nitric oxide synthase.

markers studied here, especially cytokines and oxidative molecules that compound IR equation and main results obtained from cells Li and antidepressants treated.

In our study, we tested Li effects on non-activated macrophages (i.e. RAW monocyte-like cells) and observe that this drug presents inflammatory balance similar to untreated cells. This result suggests that Li anti-inflammatory effect could be potentially amplified by inflammation patterns observed in some health conditions including psychiatric diseases, such as Bipolar disorder. In our study, Li was able to revert some slight inflammatory pattern depending of type of antidepressant drug. FLX, NOR and IMI concomitantly Li exposure present a clear decreasing in IR values whereas, concomitant ESC exposure caused an inverse effect.

Fluoxetine and Escitalopram antidepressant drugs that are SSRIs (selective serotonin reuptake inhibitors), Imipramine and Nortryptilne, tricyclic antidepressants were investigated here. Several studies have indicated that SSRIs play a modulatory role on immune system, especially in major depressive patients, that present normalization of plasma proinflammatory cytokines levels [23]. In fact, IR values was not so high to FLX, NOR and IMI and interaction of Li had a clear anti-inflammatory effect suggesting that concomitant use of these drug would not negative in terms of inflammatory metabolism.

However, it seems that such effects are not universal and are dependent on several baseline conditions of the study and have been influenced by SSRIs type, since we observed a proinflammatory effect when macrophages cells were concomitantly treated with Li+ESC. ESC is considered most selective of the SSRIs, and some prior studies, described that its SSRIs increased the levels of IL-receptor agonist and IL-2 in major depressive patients and have some anti-inflammatory effect [44, 45].

A protocol-evaluated effect of ESC on TNF-α and IL-10 levels in mice with inflammatory condition induced by LPS (lipopolysaccharide) administration. Authors reported that ESC had anti-inflammatory and antidepressant effects on LPS-induced mice suggesting that is probable that serotonergic system plays a crucial role in the pathophysiology of inflammation-induced depression [46].

On the other hand, a recent study conducted by Avitsur [47] described contrary results related to ESC action on inflammation LPS-induced in mice. In this study, ESC augmented the secretion of lipopolysaccharide-induced TNFα and induced a faster kinetics of IL-1β indicating a proinflammatory effect. Considering human clinical studies, it is interesting to comment results from an investigation that evaluated the effect of a single daily dose of 10 mg ESC or placebo for four weeks intake by fist degree relatives to patients with depression on cytokines modulation. The results did not show any differences among cytokine levels between subjects ESC or placebo treated suggesting that there is not a global anti-inflammatory effect of ESC [48].

Human investigations also described that immune effect of some antidepressant drugs, such as ESC and NOR could be dependent of basal inflammatory markers. Patients with higher CRP, IL-6 and TNF-α levels showed treatment resistance [49]. Therefore, currently data are confusing about ESC effects on inflammatory modulation. In this way, our results showing proinflammatory action of Li+ESC interaction cannot be discarded and deserve complementary *in vivo* investigations.

5. Conclusions

Despite methodological constraints related to *in vitro* protocols, results from our novel IR analysis suggest that Li does not have a universal anti-inflammatory effect that could be influenced by basal macrophage-inflammatory state and by the interaction of other psychiatric drugs. Although the investigation to be performed by *in vitro* protocol, results described here could have relevance in

psychiatric clinical practice, in respect to variation of response on inflammatory metabolism related to combination of Li and antidepressant drugs. Results described here suggest relevance to perform future in vivo complementary study in order to clarify impact of antidepressive drugs on anti-inflammatory effect triggered by Li.

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References

[1] Marmol, F. 2008. "Lithium: Bipolar Disorder and Neurodegenerative Diseases: Possible Cellular Mechanisms of the Therapeutic Effects of Lithium." *Prog. Neuropsychopharmacol. Biol. Psychiatry.* 32: 1761-71.

[2] Holder, S. D. 2017. "Psychotic and Bipolar Disorders: Bipolar Disorder." *F. P. Essent.* 455: 30-5.

[3] Freeland, K. N., Cogdill, B. R., Ross, C. A., Sullivan, C. O., Drayton, S. J., VandenBerg, A. M., Short, E. B., and Garrison, K. L. 2015. "Adherence to Evidence-based Treatment Guidelines for Bipolar Depression in an Inpatient Setting." *Am J Health Syst Pharm.* 72: S156-61.

[4] Prosser, J. M., and Fieve, R. R. 2016. "Patients Receiving Lithium Therapy Have a Reduced Prevalence of Neurological and Cardiovascular Disorders." *Prog Neuropsychopharmacol Biol Psychiatry* 71: 39-44.

[5] Decker, M., and Muñoz-Torrero, D. 2016. "Special Issue: Molecules against Alzheimer." *Molecules*. 6: 21-32.

[6] Guttuso, T. Jr. 2016. "Low-dose Lithium Adjunct Therapy Associated with Reduced Off-time in Parkinson's Disease: A Case Series." *J Neurol Sci*. 368: 221-2.

[7] Ayorech, Z., Tracy, D. K., Baumeister, D., and Giaroli, G. 2015. "Taking the Fuel out of the Fire: Evidence for the Use of Anti-inflammatory Agents in the Treatment of Bipolar Disorders." *J Affect Disord*. 174: 467-78.

[8] Chang, H. H., Chen, P. S., Wang, T.Y., Lee, S. Y., Chen, S. L., Huang, S. Y., Hong, J. S., Yang, Y. K., and Lu, R. B. 2017. "Effect of Memantine on C-reactive Protein and Lipid Profiles in Bipolar Disorder." *J Affect Disord.* 22: 151-7.

[9] Sayana, P., Colpo, G. D., Simões, L. R., Giridharan, V. V., Teixeira, A. L., Quevedo, J., and Barichello, T. 2017. "A Systematic Review of Evidence for the Role of Inflammatory Biomarkers in Bipolar Patients." *J Psychiatr Res.* 92: 160-82.

[10] Luca, A., Calandra, C., and Luca, M. 2016. "Gsk3 Signalling and Redox Status in Bipolar Disorder: Evidence from Lithium Efficacy." *Oxid Med Cell Longev*. doi: 10.1155/2016/3030547.

[11] Muneer, A. 2017. "Wnt and GSK3 Signaling Pathways in Bipolar Disorder: Clinical and Therapeutic Implications." *Clin Psychopharmacol Neurosci.* 15 (2): 100-14.

[12] van den Ameele, S., van Diermen, L., Staels, W., Coppens, V., Dumont, G., Sabbe, B., and Morrens, M. 2016. "The Effect of Mood-stabilizing Drugs on Cytokine Levels in Bipolar Disorder: A Systematic Review." *J Affect Disord*. 203: 364-73.

[13] Zullino, D., and Baumann, P. 2001. "Lithium Augmentation in Depressive Patients Not Responding to Selective Serotonin Reuptake Inhibitors." *Pharmacopsychiatry* 34 (4): 119-27.

[14] Nierenberg, A. A., Petersen, T. J., and Alpert, J. E. 2003. "Prevention of Relapse and Recurrence in Depression: The Role of Long-Term Pharmacotherapy and Psychotherapy." *J Clin Psychiatry* 15: 13-7.

[15] Freemantle, N. 2007. "No Difference in Depression Relapse Rates between Maintenance Electroconvulsive Therapy and Lithium plus Nortriptyline." *Evid Based Ment Health* 10 (3).

[16] Edwards, S. J., Hamilton, V., Nherera, L., and Trevor, N. 2013. "Lithium or an Atypical Antipsychotic Drug in the Management of Treatment-Resistant Depression: A Systematic Review and Economic Evaluation." *Health Technol*. 17 (54): 1-190.

[17] Saini, R., Raju, M. S., Chaudhury, S., and Srivastava, K. 2016. "Accelerated Antidepressant Response to Lithium Augmentation of Imipramine." *Ind Psychiatry J*. 25 (1): 93-100.

[18] Ayorech, Z., Tracy, D. K., Baumeister, D., and Giaroli, G. 2015. "Taking the Fuel out of the Fire: Evidence for the Use of Anti-inflammatory Agents in the Treatment of Bipolar Disorders." *J Affect Disord.* 174: 467-78.

[19] Bresciani, G., Cruz, I. B., de Paz, J. A., Cuevas, M. J., and González-Gallego, J. 2013. "The MnSOD Ala16Val SNP: Relevance to Human Diseases and Interaction with Environmental Factors." *Free Radic Res.* 47 (10): 781-92.

[20] Bresciani, G., da Cruz, I. B., and González-Gallego, J. 2015. "Manganese Superoxide Dismutase and Oxidative Stress Modulation." *Adv Clin Chem.* 68: 87-130.

[21] Barbisan, F., Azzolin, V. F., Ribeiro, E. E., Duarte, M. M. M. F., and da Cruz, I. B. M. 2017. "The In Vitro Influence of a Genetic Superoxide-Hydrogen Peroxide Imbalance on Immunosenescence." *Rejuvenation Res.* doi: 10.1089/rej.2016.1892.

[22] Szuster-Ciesielska, A., Tustanowska-Stachura, A., and

Słotwińska, M. 2003. "In Vitro Immunoregulatory Effects of Antidepressants in Healthy Volunteers." *Pol J Pharmacol*. 55: 353-62.

[23] Hannestead, J., DellaGioia, N., and Bloch, M. 2011. "The Effect of Antidepressant Medication Treatment on Serum Levels of Inflammatory Cytokines: A Meta-analysis." *Neuropsychopharmacology* 36: 2452-9.

[24] Mondelli, V., Cattaneo, A., and Belvederi Murri, M. 2011. "Stress and Inflammation Reduce Brain-derived Neurotrophic Factor Expression in First-Episode Psychosis: A Pathway to Smaller Hippocampal Volume." *J. Clin Psychiatry* 72: 1677-84.

[25] Beurel, E., and Jope, R. S. 2014. "Inflammation and Lithium: Clues to Mechanisms Contributing to Suicide-lined Traits." *Translational Psychiatry* 4: 1-7.

[26] Baumeister, D., Ciufolini, S., and Mondelli, V. 2016. "Effects of Psychotropic Drugs on Inflammation: Consequence or Mediator of Therapeutic Effects in Psychiatric Treatment?" *Psychopharmacol.* 233: 1575-89.

[27] Schatzberg, A. F., and Nemeroff, C. B. 2009. *The American Psychiatric Publishing Textbook of psychopharmacology*. 4 ed., Arlington.

[28] Magro, F., Sousa, P., and Ministro, P. 2014. "C-reactive Protein in Crohn's Disease: How Informative Is It?" *Expert Rev Gastroenterol Hepatol*. 28 (4): 393-408.

[29] Hamdani, N., Doukhan, R., Kurtlucan, O., Tamouza, R., and Leboyer, M. 2013. "Immunity, Inflammation, and Bipolar Disorder: Diagnostic and Therapeutic Implications." *Curr Psychiatry Rep.* 15 (9): 387-95.

[30] Duarte, M. M, Moresco, R N, Duarte, T., Santi, A., Bagatini, M. D., Da Cruz, I. B., Schetinger, M. R., and Loro, V. L. 2010. "Oxidative Stress in Hypercholesterolemia and Its Association with Ala16Val Superoxide Dismutase Gene Polymorphism." *Clin Biochem*. 43: 1118-23.

[31] Duarte, T., da Cruz, I. B., Barbisan, F., Capelleto, D., Moresco, R. N., and Duarte, M. M. 2016. "The Effects of Rosuvastatin on Lipid-Lowering, Inflammatory, Antioxidant and Fibrinolytics Blood Biomarkers Are Influenced by Val16Ala Superoxide Dismutase Manganese-Dependent Gene Polymorphism." *Pharmacogenomics J.* 16 (6): 501-6.

[32] Machado, A. K., Cadona, F. C., Azzolin, V. F., Dornelles, E. B., Barbisan, F., Ribeiro, E. E., Manica-Cattani, M. F., Duarte, M. M. F., Saldanha, J. R. P., and da Cruz, I. B. M. 2015. "Guaraná (*Paullinia cupana)* Improves the Proliferation and Oxidative Metabolism of Senescent Adipocyte Stem Cells Derived from Human Lipoaspirates." *Food Res. Intern.* 67: 426-33.

[33] Azzolin, V. F., Cadoná, F. C., Machado, A. K., Berto, M. D., Barbisan, F., Dornelles, E. B., Glanzner, W. G., Gonçalves, P. B., Bica, C. G., and da Cruz, I. B. M. 2016. "Superoxide-hydrogen Peroxide Imbalance Interferes with Colorectal Cancer Cells Viability, Proliferation and Oxaliplatin Response." *Toxicol. In Vit*. 32: 8-15.

[34] Jung, I. E. C., Machado, A. K., da Cruz, I. B., Barbisan, F., Azzolin, V. F., Duarte, T., Duarte, M. M., do Prado-Lima, P. A., Bochi, G. V., Scola, G., and Moresco, R. N. 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." *Psychopharmacol.* 233 (9): 1715-23.

[35] Morabito, C., Rovetta, F., Bizzarri, M., Mazzoleni, G., Fanò, G., and Mariggiò, M. A. 2010. "Modulation of Redox Status and Calcium Handling by Extremely Low Frequency Electromagnetic Fields in C2C12 Muscle Cells: A Real-Time, Single-Cell Approach." *Free Radic. Biol. Med.* 48: 579-9.

[36] Barbisan, F., Motta, J. R., Trott, A., Azzolin, V., Dornelles, E. B., Marcon, M., Algarve, T. D., Duarte, M. M, Mostardeiro, C. P., Unfer, T. C., Schott, K. L., and da Cruz, I. B. M. 2014. "Methotrexate-related Response on Human Peripheral Blood Mononuclear Cells May Be Modulated by the Ala16Val-SOD2 Gene Polymorphism." *Plos One.* 9 (10).

[37] Talmadge, J. E, Donkor, M., and Scholar, E. 2007. "Inflammatory Cell Infiltration of Tumors: Jekyll or Hyde." *Cancer Metastasis Rev.* 26 (3-4): 373-400.

[38] Merly, L., Smith, S. L., and Murine, R. A. W. 2017. "264.7 Cell Line as an Immune Target: Are We Missing Something?" *Immunopharmacol Immunotoxicol.* 39 (2): 55-8.

[39] Montagner, G. F. S., Sagrillo, M., Machado, M. M., Almeida, R. C., Mostardeiro, C. P., Duarte, M. M., and da Cruz, I. B. 2010. "Toxicological Effects of Ultraviolet Radiation on Lymphocyte Cells with Different Manganese Superoxide Dismutase Ala16Val Polymorphism Genotypes." *Toxicol in Vitro* 24 (5): 1410-6.

[40] Algarve, T. D., Barbisan, F., Ribeiro, E. E., Duarte, M. M., Mânica-Cattani, M. F., Mostardeiro, C. P., Lenz, A. F., and da Cruz, I. B. M. 2013. "In Vitro Effects of Ala16Val Manganese Superoxide Dismutase Gene Polymorphism on Human White Blood Cells Exposed to Methylmercury." *Genet Mol Res.* 12 (4): 5134-44.

[41] Capeleto D., Barbisan F., Azzolin V., Dornelles E. B., Rogalski F., Teixeira C. F., Machado A. K., Cadoná FC, da Silva T., Duarte T., Duarte M. M., and da Cruz I. B. 2015. "The Anti-inflammatory Effects of Resveratrol on Human Peripheral Blood Mononuclear Cells Are Influenced by a Superoxide Dismutase 2 Gene Polymorphism." *Biogerontology* 16 (5): 621-30.

[42] Dornelles, E. B., Goncalves, B. D., Schott, K. L., Barbisan, F., Unfer, T. C., Glanzner, W. G., Machado, A. K., Cadona,

F. C., Azzolin, V. F., Montano, M. A., Griner, J., and da Cruz, I. B. M. 2017. "Cytotoxic Effects of Moderate Static Magnetic Field Exposure on Human Periphery Blood Mononuclear Cells Are Influenced by Val16Ala-MnSOD Gene Polymorphism." *Environ Sci Pollut Res Int.* 24 (5): 5078-88.

[43] Schott, K. L., Assmann, C. E., Barbisan, F., Azzolin, V. F., Bonadiman, B., Duarte, M. M. M. F., Machado, A. K., and da Cruz, I. B. M. 2017. "Superoxide-hydrogen Peroxide Genetic Imbalance Modulates Differentially the Oxidative Metabolism on Human Peripheral Blood Mononuclear Cells Exposed to Seleno-L-methionine." *Chem Biol Interact.* 273: 18-27.

[44] Chavda, N., and Kantharia, N. D. 2011. "Effects of Fluoxetine and Escitalopram on C-reactive Protein in Patients of Depression." *J Pharmacol Pharmacother.* 2 (1): 11-6.

[45] Ho, T., Pollock, B. G., Mulsant, B. H., Schantz, O., Devanand, D. P., Mintzer, J. E., Porsteinsson, A. P., Schneider, L. S., Weintraub, D., Yesavage, J., Drye, L. T., Munro, C. A., Shade, D. M., Lyketsos, C., and Bies, R. 2016. "R- and S-citalopram Concentrations Have Differential Effects on Neuropsychiatric Scores in Elders with Dementia and Agitation." *Br J Clin Pharmacol*. 82 (3): 784-92.

[46] Dong, Y., Yang, C., Wang, Z., Qin, Z., Cao, J., and Chen, Y. 2016. "The Injury of Serotonin on Intestinal Epithelium Cell Renewal of Weaned Diarrhoea Mice." *Eur J Histochem.* 60 (4): 2689.

[47] Avitsur, R., Paley, S., Franko, M., Wolff, N., Eyal, N., and Doron, R. 2017. "Escitalopram or Novel Herbal Treatments Differentially Alter Cytokine and Behavioral Responses to Immune Challenge. *J Neuroimmunol.* 309: 111-8.

[48] Haastrup, E., Knorr, U., Erikstrup, C., Kessing, L.V., and Ullum, H. 2012. "No Evidence for an Anti-Inflammatory Effect of Escitalopram Intervention in Healthy Individuals with a Family History of Depression." *J Neuroimmunol.* 243 (1-2): 69-72.

[49] Schmidt, C., Leibiger, J., and Fendt, M. 2016. "The Norepinephrine Reuptake Inhibitor Reboxetine Is More Potent in Treating Murine Narcoleptic Episodes than the Serotonin Reuptake Inhibitor Escitalopram." *Behav Brain Res*. 15: 205-10.