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Anti-inflammatory and antixidant properties of blend formulated with compounds of *Malpighia emarginata D.C* (acerola) and *Camellia sinensis* L. (green tea) in lipopolysaccharide-stimulated RAW 264.7 macrophages

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ABSTRACT

The antioxidant and anti-inflammatory properties of *Malpighia emarginata D.C* (acerola) and *Camellia sinensis* L. (green tea) have been studied, particularly as an alternative in medicinal approach for different physio pathological conditions. Here we develop an powder blend formulated with both *Malpighia emarginata D.C* and *Camellia sinensis* L. which have in the composition higher content of ascorbic acid and epigallatocathechin-3-gallate respectively. Using different conditions for microencapsulation of biocompounds, we performed the powder production through spray-drying process. After, we evaluate the antioxidant and anti-inflammatory properties of blends formulated with *Malpighia emarginata D.C* and *Camellia sinensis* L. in an *in vitro* model of inflammation, using LPS-stimulated RAW-264.7 macrophage cell line. We observed that co-treatment with blends was able to modulate the redox parameters in cells during the *in vitro* inflammatory response. Moreover, the co-treatment with blends were able to modulate inflammatory response by altering the secretion of cytokines IL-1β, IL-6, IL-10, and TNF-α. Taken together, our results demonstrate for the first time the synergistic effects antioxidant and anti-inflammatory of *Malpighia emarginata D.C* and *Camellia sinensis* L. These results warrant further use of the blend powder for use in the products to heath beneficial, principally in terms of prevention of chronic diseases.

1. Introduction

*Malpighia emarginata D.C.*is a native plant of South America and Central America which is commonly known as acerola. The acerola fruit is consumed worldwide, principally due the health benefits that are related with ascorbic acid (AA) and other bioactive compounds [1] present on the fruit [2]. In terms of bioactive compounds, additionally to AA, acerola fruit have a significant number of others secondary metabolites such as phenolic compounds. Moreover, is known that phenolic compounds represent a rich class of molecules that present a diversity of biological properties, such as antioxidant, anti-inflammatory, antimicrobial, antihypertensive, and anticarcinogenic activities [3]. Considering the increase in the production of foods based in new technologies and functional food, the acerola fruit have gained evidence in production of both foods and medicinal products [1,2,4]. In this way, acerola is explored principally due the high content of AA.

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However, for industrial process acerola yet present innumerous challenges. The main challenge is maintaining the bioactive compounds, such as AA stables in new innovative products.

In the same way that acerola, the traditional *Camellia sinensis* L. is used to produce one of most popular beverage consumed in the world know as green tea [5]. The tea from *Camellia sinensis* L. present a diversity of metabolites such as phenolic compounds, saponins, alkaloids, and polysaccharides in the composition which are associated with several biological properties [6,7]. In this terms, different studies have reported the health benefits of green tea. Antioxidant, bactericidal, cardiovascular-protection, anti-diabetes, and neuroprotective effects are some of biological actions related with green tea [8,9]. In terms of biological compounds catechins represent a significant amount of water-soluble components of the green tea, and one of the most abundant is the epigallatocathechin-3-gallate [10,11].

Biologically, *Camellia sinensis* L and *Malpighia emarginata* D.C share some properties that have been associated with positive effects for health. Several studies reveal the modulation of pathways involved in regulation of inflammatory mechanism such as mitogen-activated protein kinases (MAPKs) p38, ERK ½, and JNK which are related with metabolites present in both *Camellia sinensis* L and *Malpighia emarginata D.C*. Secretion of citokynes and stimulation of transcription factors such as nuclear factor kappa B (NF-κB), nuclear E2 related factor 2 (Nrf2), and p53 also are mechanism which the main metabolites of the *Camellia sinensis* L and *Malpighia emarginata D.C* can regulates different biological systems. These metabolic and signaling pathways have been extensively studied, principally due their roles in pathophysiological diseases. Furthermore, the antioxidant properties reported to metabolites of both *Camellia sinensis* L and *Malpighia emarginata* D.C are described as responsible for modulating cellular signaling pathways. These modulations regulate the level of oxygen reactive species (ROS) or reactive nitrogen species (RNS) and plays important role in maintenance of cellular homeostasis.

The development of innovate products with health claims is a trend that have been observed on pharmaceutical and food companies [12]. Consumers have increased the perception and modified their behavior with relation to functional foods [13]. In this terms, innovative technologies in tea production have been studied, principally in order to improve the maintenance of the biological properties during process of production. The formulation of blends for tea production is a concept that have been explored recently for industry [14–16]. These advance have as main objective prospect the development of products with functional properties that mediates health benefits such as reducing risks for several chronic diseases(*i.e.* cancer, diabetes, hypertension and osteoporosis) [17].

Here in this study, we investigated the antioxidant and anti-inflammatory effects of the powder blend produced with *Camellia sinensis* L and *Malpighia emarginata* D.C in an *in vitro* model of inflammation. First, we produced three different formulation of powder blend through the spray drying method. After we evaluated the biological properties *in vitro* to investigate the role of the blends in modulate redox parameters in an *in vitro* model of inflammation which was mediated by lipopolysaccharides (LPS)-stimulated macrophages. Markers of macrophage response for inflammation such as secretion of cytokines IL-1β, IL-6, IL-10, and TNF-α in the medium were measured. Our data demonstrate for the first time the potential anti-inflammatory and antioxidant of the powder blend produced with *Camellia sinensis* L and *Malpighia emarginata* D.C. The blend of *Camellia sinensis* L and *Malpighia emarginata* D.C. here produce also was able to inhibit the effect mediated by LPS in our model of inflammation.

2. Material and methods

Chemicals - Chemicals and culture reagents were purshased from Sigma Chemical Co. (St.Louis, MO, USA). Protease inhibitor cocktail was purchased from Roche Products Limited (Welwyn Garden City, UK). Indirect ELISA reagents were purchased from Bio-Rad Laboratories (Hercules, CA, USA).

Blend preparation – The fresh acerola fruits (*Malpighia emarginata* D.C.) were purchased at local supermarket. The acerola fresh fruits were sanitized with chlorine-base 200 p.p.m. to prepare the acerola juice. Then, they were homogenized with an industrial blender (fruit/water, 255 g/150 mL) for 3 min. After this, the material was sieved through a 0.39 mm mesh to collect the juice. The green tea (*Camellia sinensis* L.) extract was purchased at local supermarket to make the infusion. The extract was submitted to infusion with boiling water (75 °C, w/v, 100 g/ 1 L, with homogenization at 30 rpm). After this, the material was sieved through a 0.39 mm mesh to collect the green tea aqueous extract. To finish the preparation of the blend, the fresh juice of acerola and green tea aqueous extract were blended in one proportion of 80% of acerola juice and 20% of green tea aqueous extract (v/v). The blend thus was separated into three formulations: the first composed of the blend and maltodextrin (10%), the second composed of the blend and maltodextrin (15%), and the third composed of the blend and maltodextrin (20%); in accordance with described. Each formulation was homogenized at 500 rpm for 10 min. No other additives were used. The main difference between formulations was the use of maltodextrin, and these amounts of maltodextrin were set because of glass transition temperature (Tg) estimations, as previously described [18].

Phenolic and ascorbic acid content determination

The total phenolic content of the acerola juice and green tea aqueous extract was determinate through the modified Folin-Ciocoateu method [19]. Tannic acid was used as standard and results were expressed as mg gallic acid equivalent/100 mL of sample. The ascorbic acid content was evaluated as previously described by [20] and results were expressed as mg ascorbic acid equivalent/100 mL of sample. For the blends we evaluate the percentage of retention of both biological compounds in the powder produced through spray drying method.

Spray drying – To produce the powder blend was used at present work an industrial spray drying unit with a capacity to evaporate 10 kg of water per hour was available for product production. Immediately after blends preparation, it was spray dried using a Labmaq SD10 spray dryer, equipped with a two-fluid nozzle (2 mm orifice), with atmospheric air as drying gas in open cycle mode (no air-recirculation). Atomization gas flow rate (F_{atom}) was at 2 kg/h and was combined with drying gas inlet temperature (T_{in}) 110 °C. Both formulations were dried using equal conditions. Other conditions were kept constant such as drying gas flow rate (F_{drying}) was 288 kg/h, and product flow rate (F_{feed}) of 2 kg/h. The experimental was selected for outlet temperature be at least 10 °C below the estimated glass transition temperature. This approach was used to avoid extensive agglomeration and stickiness to the dryer walls and thus keep the yield as high as possible [21].

Cell culture - The mouse macrophage cell line RAW 264.7 were grown in RPMI-1640, 10% FBS and maintained at 37 °C in an atmosphere containing 5% CO_2. The media was supplemented with 1% penicillin/streptomycin.

Treatments – The powder blend from three different formulation was dissolved in medium. Concentrated stocks were prepared immediately before experiments by dissolved powder blend into medium. The solution was kept protected from light to kept blend stable. Cells were treated with different concentrations of blend (0.05 μg/mL, 0.5 μg/mL, 5 μg/mL, 50 μg/mL, and 500 μg/mL). All treatments were initiated by adding concentrated solutions to reach final concentrations in the well.

MTT assay and Sulphorhodamine B (SRB) assay - After 24 h of blends treatments, RAW 264.7 cells viability was assessed by the MTT assay [22]. This colorimetric assay was performed to assess growth of the cells. It estimates cell numbers indirectly by staining total cellular protein with SRB [22].

Lactic Dehydrogenase release (LDH assay) - The presence of LDH in a medium of cultured cells is a useful tool to evaluate cell necrosis as a result of cell membrane disruption. LDH activity was measured spectrophotometrically in the culture medium at λ = 340 nm by measuring

β-Nicotinamide-adenine dinucleotide (NADH) reduction [22].

Thiobarbituric acid-reactive species (TBARS), protein carbonyls, and protein thiol content - We measured the formation of TBARS during an acid-heating reaction, which is widely adopted for measurement of lipid peroxidation, as previously described [23]. Results are expressed as nmol of TBARS/mg of protein. Protein carbonyls, the measure was based on the reaction with dinitrophenylhydrazine as previously described [24]. Results are expressed as nmol of carbonyl/mg of protein. For protein thiol content the samples were analyzed to estimate oxidative alterations in proteins [25]. Results are expressed as mmol of SH/mg of protein. We performed the methods that also were used in previously study [26].

Estimation of antioxidant enzyme activities - Catalase (EC 1.11.1.6) (CAT) activity was assayed by measuring the rate of decrease in H_2O_2 absorbance in a spectrophotometer at $\lambda = 240$ nm, and the results are expressed as units of CAT/mg of protein [27] Superoxide dismutase (EC 1.15.1.1) (SOD) activity was assessed by quantifying the inhibition of superoxide-dependent adrenaline auto-oxidation in a spectrophotometer at $\lambda = 480$ nm, as previously described, and the results are expressed a units of SOD/mg of protein [28].

Indirect ELISA - IL-1β, IL-6, IL-10, and TNF-α were quantified by indirect ELISA. IL-1β was detected by Abcam AB9722; IL-6 by Abcam AB6672; IL-10 by Abcam AB9969, TNF-α by Abcam AB6671. Briefly, cells medium was placed in ELISA plate (220 μL /well). After 24 h incubation, plates were washed three times with Tween-Tris buffered saline (TTBS, 100 mM Tris −HCl, pH 7.5, containing 0.9% NaCl, and 0.1% Tween-20). Subsequently, 200 μL of primary antibody against IL-1β, IL-6, TNF-α, or IL-10 (1:1.000) were added and incubation was carried for 24 h at 4 °C. The plates were washed three times with TTBS and incubated with IgG peroxidase-linked secondary antibody (1:1.000) for 2 h. After washing the plate three times with TTBS, 200 μL of substrate solution (TMB spectrophotometric ELISA detection kit) were added to each well and incubated for 15 min. The reaction was terminated with 50 μL/well of 12 M sulfuric acid stopping reagent and the plate read at $\lambda = 450$ nm in a microplate.

Statistical analysis - Results are expressed as mean values ± standard error of the mean (S.E.M.); p values were considered significant when $p < 0.05$. Differences in experimental groups were determined by one-way ANOVA followed by the post-hoc Tukey's test whenever necessary.

3. Results

The phytochemical determination of total phenolic compounds and ascorbic acid were made in both *in nature* materials such as juice of *Malpighia emarginata* D.C. and extract from *Camellia sinensis* L. (Fig. 1). These characterizations also were performed in all formulations of blend here studied (Fig. 1). Our results reveal that acid ascorbic had a significant decrease in the content in the formulated blend with 5% of maltodextrin. The formulations with 10% of maltodextrin and 15% of maltodextrin were able to retain the ascorbic acid in the blends powder. In terms of total phenolic compounds, we observed that formulations with 10% of maltodextrin and 15% of maltodextrin present higher levels of retention of those compounds. These results showed that the production of the powder through the spray drying process was able to maintain the bioactive compounds in the blend powder produced. Although, there are lowest in the levels of the phytochemical evaluated, the retention of these compounds was efficient. The loss of phytochemical compounds during spray drying process here observed can be associated to thermal conditions that samples are submitted during the process.

After determination of phytochemical retention in the powder blend, we first decided to evaluate the potential of blend produced from *Camellia sinensis* L and *Malpighia emarginata* D.C. in modulate the viability of the RAW 264.7 macrophage cells. The cells were treated with formulations of blend at concentrations of 0.05 μg/mL, 0.5 μg/mL, 5 μg/mL, 50 μg/mL and 500 μg/mL (Fig. 2) for determination of viability. The formulations here studied, and the concentration of blends used in treatments did not mediate alterations in the cell viability. To confirm these effects in the cell viability we perform three different assays such as MTT, SRB and LDH release. At the same time, we analyzed the effects of co-treatment during the inflammatory response mediated by treatment with LPS at 1 μg/mL. In the co-treatment with formulation of 5% of maltodextrin, all concentrations of blend used to co-treatment were able to inhibit the decrease in LPS-mediated cell viability (Fig. 2). Interestingly, formulations with 10% and 15% of maltodextrin did not present effects in inhibition of the decrease in LPS-mediated cell viability.

After evaluate cell viability, we decide analyzed parameters of oxidative stress in cells treated with blend in the three formulations produced here in our study. All concentrations of blend used to treatment of the cells did not alter the protein carbonylation levels (Table 1). Although the blend did not alter protein carbonyl levels in the cells that receive the blend treatment, in the cells that were co-treated during inflammatory response the blend did not block the alterations in protein carbonylation levels induced by treatment with LPS at 1 μg/mL. Interestingly, in terms of protein thiol content our results reveal that blend concentrations used to in co-treated during inflammatory response blocking the alterations in protein thiol levels induced by treatment with LPS at 1 μg/mL (Table 1). These results confirm that blends produced from *Camellia sinensis* L and *Malpighia emarginata* D.C.here studied can modulate oxidative alterations onto an inflammatory model.

Blend treatment also modified the activity of antioxidant enzymes. We observed an increase in SOD activity in all concentrations of the blend used to treatment in the cells (Table 1). Similarly, we observed an increase in SOD activity in the cells that were co-treated during inflammatory response by treatment with LPS at 1 μg/mL, however SOD activities were like LPS treatment alone (Table 1). In CAT activity the treatment of the cells with blends decrease the activity of the CAT in both cells treated alone with blend and in cells co-treated with LPS at 1 μg/mL. Our results reveal that the treatment with blends were able to modulate oxidative parameters and antioxidant enzymes activities in our "*in vitro*" model of inflammation.

Secretion of cytokines involved in inflammatory process such as IL-1β, IL-6, IL-10, and TNF-α can occurs when macrophage cells are LPS-stimulated. Is known that these cytokines regulate cellular fate. Moreover, the modulation of the expression of these cytokines is regulated through different transcription factors such as nuclear factor kappa B (NF-κB), nuclear E2 related factor 2 (Nrf2). Here, our results reveal that the co-treatment with the blend mediated a reduction in the secretion of both cytokines IL-1β, IL-6, IL-10, and TNF-α when macrophage cells were LPS-stimulated (Fig. 3). These suggest that the antioxidant properties presented for the blends also corroborate to regulate the expression and secretion of cytokines here evaluated. Taken together, our data suggesting that blend produced from *Camellia sinensis* L and *Malpighia emarginata* D.C. has anti-inflammatory properties.

4. Discussion

The properties of secondary metabolites produced from different biochemical pathways in plants have been extensively studied, principally due the antioxidant, anti-inflammatory, and anti-tumoral properties [29–31].In these terms, both *Camellia sinensis* L and *Malpighia emarginata* D.C. are species that have higher importance to public health, principally because these plants are used therapeutically with medicinal objectives for treatment of different diseases. Additionally, products derived from *Camellia sinensis* L and *Malpighia emarginata* D.C. are also widely consumed in the foods and supplements, which can use directly metabolites isolates such as AA, epigallatocathechin-3-gallate or all metabolites extracted from plants by different methods. Tea is mostly commonly beverage consumed in the world, and *Camellia sinensis* L represent one of main plants used. Beside, is known that in

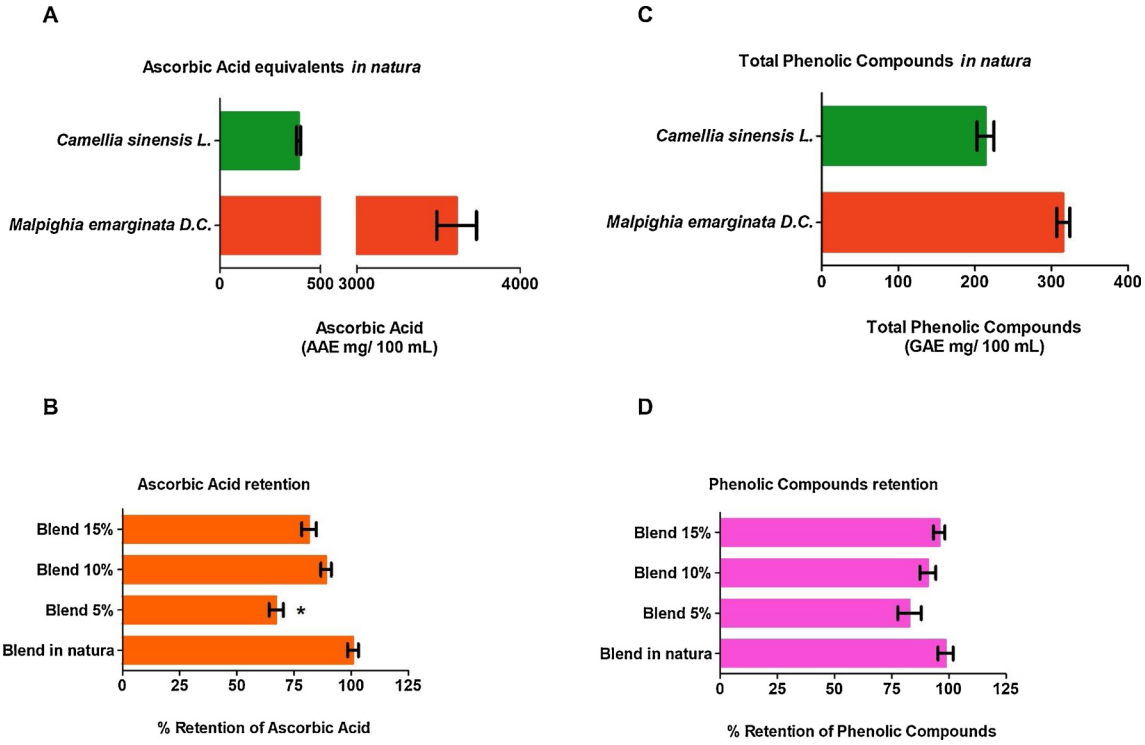

Fig. 1. Contents of ascorbic acid (AA) and total phenolic compounds of *in nature* juice of *Malpighia ermaginata* D.C., extract of *Camellia sinensis* L.., and blends formulated. **(A)** Ascorbic acid equivalent content *in nature* juice of *Malpighia ermaginata* D.C., extract of *Camellia sinensis* L.. **(B)** Retention ratio of ascorbic acid in different blend formulated. **(C)**Total phenolic compounds content *in nature* juice of *Malpighia ermaginata* D.C., extract of *Camellia sinensis* L.. (D) Retention ratio of total phenolic compounds in different blend formulated. Blend *in nature* is represented through the preparation of the fresh juice of acerola and green tea aqueous extract in one proportion of 80% of acerola juice and 20% of green tea aqueous extract (v/v). Data represent mean ± S.E.M. from three independent experiments (n = 6 per group). One-way ANOVA followed by the post hoc Tukey's test, $^{*}p < 0.05$ *vs* the blend *in nature* (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

plants the content of secondary metabolites can vary in accordance with plant tissues and stages of the plant [32]. Here we developed a group of blend formulation with *Camellia sinensis* L and *Malpighia emarginata* D.C., moreover we investigate and elucidate the evidences of these blends in exhibit antioxidant, anti-inflammatory activities in a biological model of inflammation.

To explore the properties of blends we first investigate the viability of cells treated with blend formulation of *Camellia sinensis* L and *Malpighia emarginata* D.C. Through MTT, SRB, and LDH release assays we evaluate the viability/toxicity of blend formulation in RAW 264.7 macrophage cells. The blends, when used in treatment of the RAW 264.7 macrophage cells did not present cytotoxicity in terms of alteration in cell viability. When used in the co-treatment the formulation of 5% of maltodextrin at all concentrations was able to inhibit the decrease in LPS-mediated cell viability. In this term, is known that phytochemical composition of the plant Camellia *sinensis* L and *Malpighia emarginata* D.C. did not present cytotoxic effects, principally when consumed by dietary route, such as juice and tea products [33–35]. The presence of phenolic compounds, ascorbic acid, and carotenoids in both *Camellia sinensis* L and *Malpighia emarginata* D.C., are related with preventive effects in biological systems such as cytotoxic effects [3,36]. Moreover, those compounds exhibit biological activities, including antioxidants, apoptotic, anti-apoptotic, and anti-inflammatory activities [37–39].. Previous studies have demonstrated that metabolites of *Camellia sinensis* L and *Malpighia emarginata* D.C. also present protective effects in genotoxicity, preventing the DNA damage and consequently cellular death [33,40]. Also is know that treatment with LPS in macrophages cells induces DNA damage [41–43]. Our results reveal that blend formulated with of *Camellia sinensis* L and *Malpighia emarginata* D.C inhibited the decrease in cell viability of LPS-stimulated cells. The evidences in the previous studies suggesting that the effect observed in cell viability can be associated with different compounds present in the blend, which can relate with antioxidant, and anti-inflammatory properties. Moreover, the association of two plants for blend production can be improved potentially the beneficial effects observed here in the cell viability. Interestingly, these effects were better observed in the formulation with 5% of maltodextrin, which was the blend that presented a consider lowest of AA during the process of blend production. The lowest of AA during the production can be associated to heat treatment that blend was submitted during spray drying process. Is known that thermal process can alter the structure and stability of secondary metabolites [44,45] However, this can be associated with the higher content of beneficial compounds when compared with the other formulations that have more maltodextrin in the composition.

The production of oxygen reactive species (ROS) or reactive nitrogen species (RNS) is well stablished process that occur naturally during cellular metabolism. However, during inflammatory process occur an imbalance in ROS/RNS production. As consequence there are production of higher levels of ROS/RNS in the tissues with inflammatory process, and there is an impairment of metabolic functions [46,47]. *Camellia sinensis* L and *Malpighia emarginata* D.C present a diversity secondary metabolites with important biological properties. The antioxidant properties, are characteristics that stimulates the intake of both, principally due health beneficial helps to maintain the homeostasis in biological systems [48,49]. The involvement of ROS/RNS with increased risk and incidence of chronic diseases such as arteriosclerosis, respiratory diseases, diabetes, and neurodegenerative disorders is widely studied [50–52]. For instance, several findings reinforce the use of plant extracts through tea, juice, or food supplementation can be a mechanism to improve the treatment and/or prevention of pathophysiological conditions [53–55]. Therefore, we decide to investigate the synergistic antioxidant and anti-inflammatory

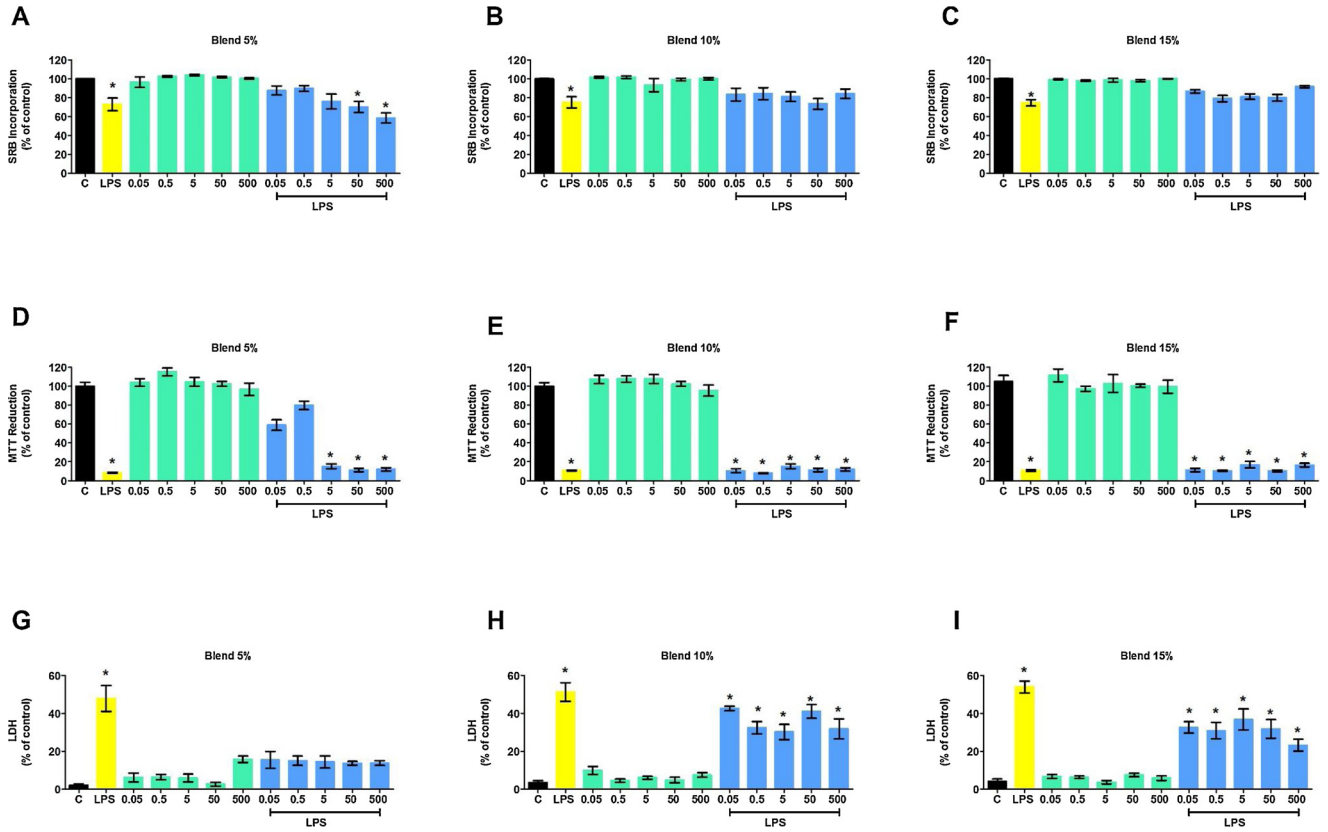

Fig. 2. Parameters of cell viability in RAW 264.7 cells lipopolysaccharides (LPS)-stimulated (1 μg/mL) and co-treated with blends for 24 h. RAW 264.7 cells were treated with blends at 0.05 μg/mL, 0.5 μg/mL, 5 μg/mL, 50 μg/mL and 500 μg/mL. Different assays were performed to evaluate cell viability after incubation; newly lipopolysaccharides LPS (1 μg/mL) was used as a positive control for loss of viability. **(A, B, C)** SRB–incorporation assay; **(D, E, F)** MTT reduction assay; and **(G, H, I)** LDH release assay. Control group is represented in all graphs by "Control". Data represent mean ± S.E.M. from three independent experiments (n = 6 per group). One-way ANOVA followed by the post hoc Tukey's test, $*p < 0.05$ *vs* the control group.

effects of blends formulated and produced through spray drying process into inflammatory macrophage cells model.

The blends here produced inhibited the effects mediated by LPS on redox parameters when used as co-treatment in macrophage cells LPS-stimulated. The main effects observed in redox parameters were in protein thiol content, where all blends were able to block the LPS alteration in protein thiol content. Moreover, antioxidant enzymes activities such as CAT and SOD were also modulated by blends formulated. In terms of CAT, blends were able to decrease the activities of the enzyme. In these terms, is known the potential effects intermediates through epigallatocathechin-3-gallate in modulate the redox environmental in cells. The different authors have described the antioxidant of epigallocathechin-3-gallate, which is one of more important metabolites present in *Camellia sinensis* L. [56,57]. Additionally, the compounds such as AA present in *Malpighia emarginata* D.C also intermediates health beneficial, principally due decrease oxidative damage in biological molecules, such as DNA [58,59]. The importance and properties of secondary compounds produced in plants have been associated with antioxidant, anti-inflammatory, and anti-tumoral effects and likely these compounds contribute to reduce ROS/RNS. Furthermore, compounds such as epigallocathechin-3-gallate are effective in induces inhibition of pro-oxidant enzymes responsible for ROS/RNS production [60]. Epigallocathechin-3-gallate also present chemical properties of chelate divalent metal ions, and therefore can reduce reactions that trigger ROS/RNS production, such as Fenton reaction [60,61]. In LPS-stimulated cells the oxidative damage is a characteristic observed in cells [22,62]. Our results evaluated through redox parameters reveal that LPS induces oxidative damage and that antioxidant enzymes are modulated in the inflammatory model using RAW 264.7 macrophage cells. Interestingly, the co-treatment with blends was able to prevent these alterations, principally in terms of oxidation of thiol protein content and modulation of antioxidant enzymes such as CAT and SOD. Therefore, our data suggesting that blends here produced and evaluated present synergistic effects antioxidant which mediates beneficial response in cellular injury, principally in redox parameters.

During pro-inflammatory response in LPS-stimulated cells is known that occurs characteristically the release of cytokines such as IL-1β, IL-6, IL-10 and TNF-α. Historically, have been demonstrated that the expression and release of these cytokines can be regulated through of different transcription factors activation [63]. Transcription factors such as NF-κB, and Nrf2 are usually associated with these metabolic process. In these terms, is known that the blocked of IL-1β action/secretion have been associated with anti-inflammatory responses [64,65]. Our results demonstrated that co-treatment with blend formulated with 5% of maltodextrin at different doses was able to mediate the inhibition of IL-1β secretion in macrophages LPS-stimulated. The importance in inhibition of IL-1β is associated with decrease in the ROS/RNS production. The cascade of signaling regulates through the ROS/RNS has been associated with IL-1β production and secretion [65]. Here is important to emphasize that ROS/RNS also directly can act through of specific mechanisms and activate the NF-κB, and Nrf2, which are associated with production and secretion of cytokines as IL-1β. The decrease in IL-1β release in our study suggest that blend by antioxidant properties regulates the signaling pathways responsible for the IL-1β production [65]. These effects can be related directly with decrease of ROS/RNS production and consequent modulation of transcription factors as NF-κB, and Nrf2. Our result in the protein thiol content corroborate with this address, principally because the thiol protein are redox sensors responsible for regulates innumerous protein properties, such as activation of different regulatory pathways including inflammatory

Table 1
Redox parameters levels in macrophage cells - RAW 264.7.

			Blend 5%					Blend 5% + LPS 1 μg/mL				
	Control	LPS 1 μg/mL	0.05 μg/mL	0.5 μg/mL	5 μg/mL	50 μg/mL	500 μg/mL	0.05 μg/mL	0.5 μg/mL	5 μg/mL	50 μg/mL	500 μg/mL
Carbonyl groups (nmol/mg prot)	23.77 ± 0.60	34.94 ± 2.85 *	22.11 ± 0.40	19.83 ± 0.09	29.90 ± 1.55	25.90 ± 0.08	22.78 ± 1.34	35.54 ± 0.28 *	29.78 ± 1.35*	30.42 ± 0.81*	31.46 ± 0.42 *	34.69 ± 2.76 *
Thiol groups (μmol/mg prot)	71.33 ± 1.30	61.81 ± 1.07 *	75.51 ± 4.64	70.86 ± 3.82	60.45 ± 2.20	95.35 ± 4.67	80.42 ± 6.80	70.70 ± 2.47	80.96 ± 1.76	75.80 ± 4.42	82.95 ± 5.69 *	70.75 ± 0.92
SOD (U/mg prot)	17.54 ± 1.86	37.88 ± 2.73 *	24.69 ± 2.18	24.12 ± 1.25	23.06 ± 1.17	27.72 ± 2.45	24.57 ± 1.77	21.67 ± 1.77	26.85 ± 2.01	35.54 ± 1.86 *	32.77 ± 2.09 *	25.34 ± 1.37
CAT (U/mg prot)	2.10 ± 0.07	3.80 ± 0.10 *	1.88 ± 0.04	1.49 ± 0.04	1.94 ± 0.05	2.13 ± 0.03	1.51 ± 0.19	1.83 ± 0.05	1.78 ± 0.13	1.73 ± 0.09	1.88 ± 0.05	1.65 ± 0.07
			Blend 10%					Blend 10% + LPS 1 μg/mL				
	Control	LPS 1 μg/mL	0.05 μg/mL	0.5 μg/mL	5 μg/mL	50 μg/mL	500 μg/mL	0.05 μg/mL	0.5 μg/mL	5 μg/mL	50 μg/mL	500 μg/mL
Carbonyl groups (nmol/mg prot)	24.85 ± 0.20	35.04 ± 0.74 *	23.13 ± 0.56	21.07 ± 0.18	22.19 ± 1.94	28.60 ± 0.43	25.94 ± 2.58	36.16 ± 2.97 *	32.57 ± 0.48 *	33.26 ± 1.32 *	32.57 ± 0.39 *	34.53 ± 0.54 *
Thiol groups (μmol/mg prot)	103.90 ± 6.25	61.85 ± 2.53 *	82.28 ± 3.75	100.15 ± 6.86	107.56 ± 3.07	97.61 ± 4.41	111.15 ± 4.54	76.16 ± 3.11 *	105.96 ± 2.00	108.05 ± 3.19	104.96 ± 5.32	75.62 ± 3.38
SOD (U/mg prot)	17.96 ± 1.09	27.24 ± 2.44 *	22.63 ± 1.03	22.74 ± 1.20	20.15 ± 0.90	23.90 ± 2.01	23.87 ± 1.72	26.85 ± 2.53 *	26.65 ± 1.93 *	24.91 ± 2.03 *	32.77 ± 3.08 *	30.65 ± 2.82 *
CAT (U/mg prot)	2.48 ± 0.04	3.61 ± 0.05 **	1.48 ± 0.05 *	1.26 ± 0.18 *	1.63 ± 0.09 *	1.68 ± 0.10 *	1.56 ± 0.19 *	1.84 ± 0.18 *	1.98 ± 0.21 *	2.14 ± 0.19	1.98 ± 0.14 *	1.73 ± 0.17 *
			Blend 15%					Blend 15% + LPS 1 μg/mL				
	Control	LPS 1 μg/mL	0.05 μg/mL	0.5 μg/mL	5 μg/mL	50 μg/mL	500 μg/mL	0.05 μg/mL	0.5 μg/mL	5 μg/mL	50 μg/mL	500 μg/mL
Carbonyl groups (nmol/mg prot)	29.04 ± 3.83	38.58 ± 1.26 *	24.13 ± 0.97	20.90 ± 2.38	33.82 ± 0.70	31.42 ± 2.86	24.34 ± 1.46	32.72 ± 1.42	37.62 ± 1.08 *	36.93 ± 0.65 *	37.55 ± 0.80 *	34.95 ± 0.30 *
Thiol groups (μmol/mg prot)	101.93 ± 3.34	61.05 ± 0.67 *	86.92 ± 5.42	81.54 ± 2.88	92.29 ± 2.31	100.83 ± 4.77	85.61 ± 6.05	75.45 ± 2.85 *	88.96 ± 0.89	84.75 ± 1.71	100.11 ± 1.92	89.01 ± 2.88
SOD (U/mg prot)	18.92 ± 1.34	38.31 ± 3.78 *	25.40 ± 1.04	22.87 ± 1.07	29.64 ± 1.45 *	29.38 ± 2.09	25.26 ± 1.61	29.19 ± 2.05 *	35.21 ± 2.88 *	37.66 ± 2.57 *	38.97 ± 2.91 *	31.57 ± 2.35 *
CAT (U/mg prot)	2.61 ± 0.13	3.65 ± 0.09 **	1.29 ± 0.14 *	1.19 ± 0.18 *	1.81 ± 0.36 *	1.51 ± 0.08*	1.53 ± 0.14	1.77 ± 0.25*	1.55 ± 0.11*	1.58 ± 0.15*	1.65 ± 0.08 *	1.64 ± 0.09 *

*Different from Control,Tukey test for $p < 0.05$.

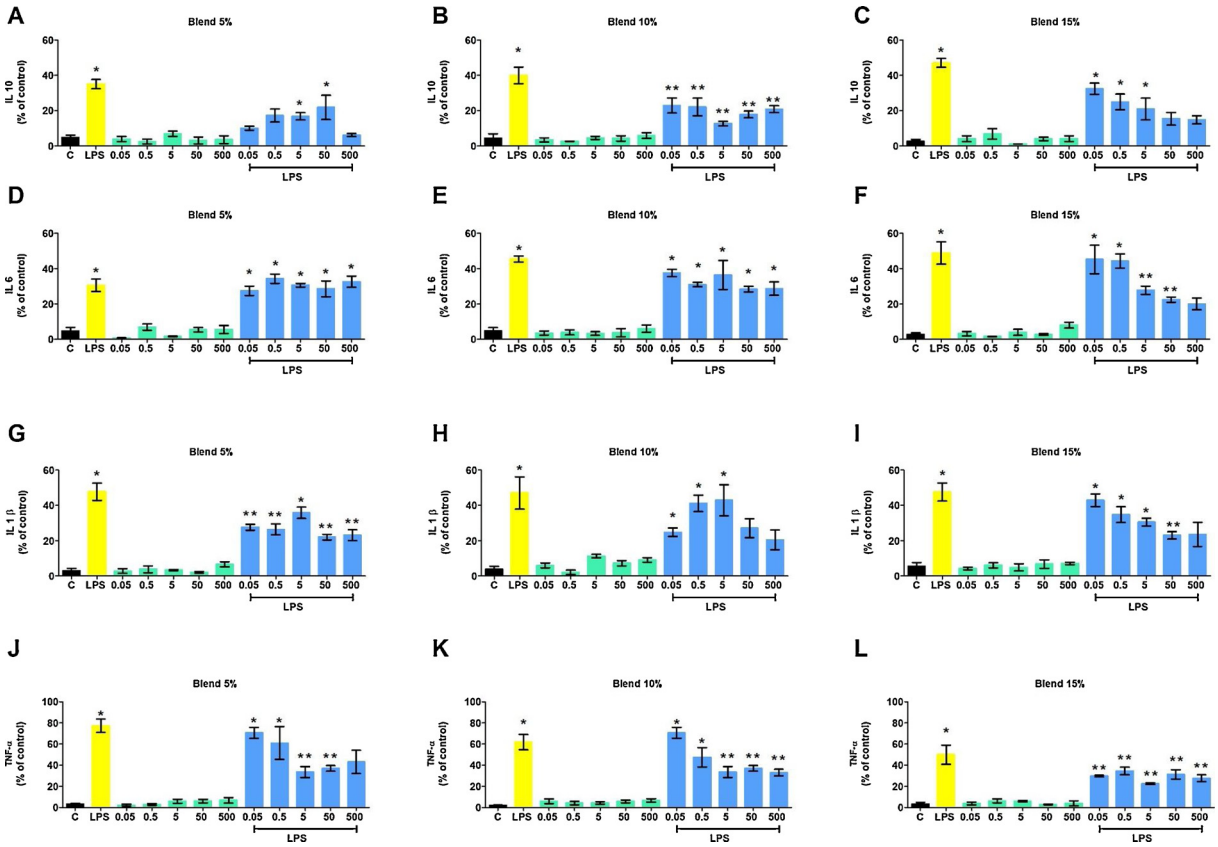

Fig. 3. Effect of blends (0.05 μg/mL, 0.5 μg/mL, 5 μg/mL, 50 μg/mL and 500 μg/mL) on the inflammatory cytokine release. (**A, B, C)** IL-10 ; **(D,E, F)** IL-6;, **(G,H, I)** IL-1β ; (*J*,**K, L)** TNF-α content release in RAW 264.7 cells and RAW 264.7 LPS-stimulated after 24 h. Release of cytokines was performed by ELISA assays. Data represent mean ± S.E.M. from three independent experiments (n = 6 per group to ELISA assays). One-way ANOVA followed by the post hoc Tukey's test, $*p < 0.05$ *vs* the control group; $**p < 0.05$ *vs* the LPS-stimulated.

pathways [66]. In terms of IL-6, our results reveal primarily that blends did not alter the IL-production. Also, was possible evaluate that co-treatment with blend did not mediate the inhibition of IL-6 secretion in macrophages LPS-stimulated. IL-6 is a known cytokine involved in pleiotropic actions. Other cytokine involved in the inflammatory process is IL-10. The role of IL-10 is regulate through different ways the proinflammatory cytokines during the inflammatory process [67]. Here, the blends treatments did not alter the levels of IL-10 in the RAW 264.7 macrophage cells. Interestingly, the co-treatment with blends were able to prevent the increase of IL-10 levels observed in macrophages LPS-stimulated. These results corroborate with anti-inflammatory effects attributes to both *Camellia sinensis* L and *Malpighia emarginata* D.C., and that probably here were potentialized through the blend formulated. TNF-α secretion also contribute with immunological system for induces inflammation in pathological conditions however is yet unclear the ROS/RNS mechanisms involved in TNF-α pathway signaling [68]. Here, interestingly only the co-treatment with blend formulated with 10% of maltodextrin at different doses were able to mediate the inhibition of TNF-α secretion in macrophage LPS-stimulated cells. Taken together, our cytokines results reveal that the blends produced and test in our study present a potential effect anti-inflammatory in a cellular model of *in vitro* inflammation. Moreover, the results also suggest that antioxidant properties of the molecules present in the blends corroborate to enhanced anti-inflammatory response presented for the blends when used in co-treatment of RAW 264.7 macrophage cells LPS-stimulated.

In conclusion, the results presented here demonstrate for the first time that blends produced from *Camellia sinensis* L and *Malpighia emarginata* D.C. exhibit potentialized antioxidant and anti-inflammatory properties in a biological system. Moreover, our data suggesting that these effects are potentialized for synergistic composition of the blends formulated and reinforce the importance of herbal blends consumption in her potential to health benefits promoted. Our findings also contribute for improve the comprehension of the properties and mechanism of action mediated by synergistic composition of blends in biological systems.

Authors contributions

Conceptualization and methodology: N.C.S., H.M.L.O., E.N.O.N., E.G.P.S., R.J.S.D., and M.A.B.P.; formal analysis, writing—original draft preparation, N.C.S., H.M.L.O., I.B.O., M.E.R.M.C.M., D.P.G., J.C.F.M, R.J.S.D., and M.A.B.P.. All authors have read and agreed to the published version of the manuscript.

Data availability

The data set of this manuscript can be request directly for Natalia Cabral Souza – at nataliacamocim@hotmail.com.

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Declaration of Competing Interest

The authors do not have any conflict of interest.

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