Research Article

Investigation of the cytotoxicity, antioxidative and immune-modulatory effects of *Ligusticum porteri* (Osha) root extract on human peripheral blood lymphocytes

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ABSTRACT

**OBJECTIVE:** *Ligusticum porteri* is a traditional Native American herb. The roots of *L. porteri* are traditionally used in the treatment of many diseases, however, its cytotoxicity, antioxidative and immune-modulatory effects need to be investigated. In this study, we evaluated the effects of the root extract at different doses on human peripheral blood lymphocytes (PBLs).

**METHODS:** The lymphocytes were incubated with different concentrations of the root extracts (0, 50, 100, 200, and 400 μg/mL) and harvested every 6 h for 2 d (*P*<0.05). The protective effect of the herb against oxidative damage was determined by inducing oxidative stress with the administration of 50 μmol/L of hydrogen peroxide (H₂O₂).

**RESULTS:** Treatments with *L. porteri* at 200 and 400 μg/mL increased the viability of PBLs. The deleterious effect of H₂O₂ was ameliorated by 400 μg/mL *L. porteri* treatment. Addition of 400 μg/mL *L. porteri* reduced lipid peroxidation in stressed PBLs by 94% (*P*<0.05). Treatment with 400 μg/mL of *L. porteri* resulted in a 26.4% increase of reduced glutathione levels. Activities of superoxide dismutase and catalase increased by 17.5% and 55.2% respectively, when stressed PBLs were treated with 400 μg/mL *L. porteri* for 2 d (*P*<0.05). Treatment with 400 μg/mL *L. porteri* increased interferon-γ and interleukin-2 expressions in H₂O₂-challenged PBLs (*P*<0.05), however, the root extract did not cause a significant difference in interleukin-10 levels compared to the control (*P*<0.05).

**CONCLUSION:** The findings suggest that *L. porteri* might be a potential immune-modulating agent involving protective effects against oxidative damage.

**Keywords:** *Ligusticum porteri*; root extract; cytotoxicity, immunologic; oxidative stress; immune-modulatory; human peripheral blood lymphocytes

of medicinal ailments\cite{31}. Hispanics use the roots of \textit{L. porteri} to treat various respiratory ailments, including catarrh, colds, coughs, bronchial pneumonia, flu and other respiratory problems\cite{5,23,24}. Root preparations have been used externally to treat aches and pains, digestive problems, scorpion stings, wounds and skin infections\cite{23}.

Although \textit{L. porteri} has been used to treat a wide range of ailments, the knowledge pertaining to the pharmaceutical uses of \textit{L. porteri} is scarce due to the shortage of scientific literature in this area. Research conducted over the years has provided similar information on the traditional medicinal uses of \textit{L. porteri}, but little evidence of its efficacy and safety, leaving an area of potential study. As health care costs continue to rise, people are turning to natural sources of medicine such as herbs. \textit{L. porteri} has gained a reputation for its medicinal effects\cite{4}.

The development of anti-inflammatory drugs has recently focused on discovering plant-derived extracts that are potent and safe\cite{4}. Previous studies reported that oxidative stress, chronic inflammation, and cancers are closely linked\cite{4}. Under sustained stress, reactive oxygen species (ROS) is produced over a long period of time leading to chronic inflammation, which in turn mediates most chronic diseases and cancers\cite{6}. Pharmaceutical treatments are commonly marked by side effects, leading to the search for natural substances with less adverse side effects. Hence, this study investigated the effects of \textit{L. porteri} root extract on the oxidative and inflammatory indices that are often associated with the development of diseases. We evaluated the cytotoxicity, antioxidative, and immune-modulatory effects of \textit{L. porteri} root extract at different doses on human peripheral blood lymphocytes (PBLs).

2 Materials and methods

2.1 Preparation of \textit{L. porteri} root extract

The extract was prepared by following Beltran’s method\cite{7} with modifications. About 30 g of dry \textit{L. porteri} root (collected in 2008 from Crested Butte, Colorado, USA) was obtained from Ms. Susan Sparks, Chiron Holistic LLC, Corpus Christi, Texas, USA. It was pulverized and mixed with 300 mL of 40% ethanol. The mixture was sieved through a cheese-cloth, followed by a final filtration with 0.20 µm membrane. The filtrate was dried under vacuum, and stored at −20 °C for further use. The dried sample was weighed and then dissolved in dimethyl sulfoxide (DMSO) (Corning Cellgro, Virginia, USA) at a concentration of 400 µg/mL (stock solution). Final working concentrations of the root extract at 50, 100, 200, 400 µg/mL, and 0 µg/mL (the control containing only DMSO) were prepared by diluting the stock solution in DMSO just before the experiments. The range of concentrations of \textit{L. porteri} root extract prepared in this study was based on a previous study that used concentrations ranging from 12 to 400 µg/mL of \textit{L. porteri} crude extracts to study the cytotoxicity of the plant extract on the culture media of U-937 cell line\cite{7}. All assays were carried out in triplicate.

2.2 Preparation of cultured PBLs

Peripheral blood mononuclear cells (Sanguine Biosciences, California, USA) were cultured at the concentration of 10^5 cells/mL in RPMI 1460 medium containing 2 mmol/L of glutamine, supplemented with 1% (v/v) nonessential amino acids, 1% (v/v) sodium pyruvate, 50 U/mL penicillin, 50 mg/mL streptomycin, and 20% (v/v) fetal bovine serum (Corning Cellgro, Virginia, USA). Lipopolysaccharide at 10 µg/mL (obtained from \textit{Escherichia coli} serotype O55:B5, Sigma Aldrich, Missouri, USA) was added to activate lymphocyte differentiation. The cell suspension was incubated at 37 °C in a humidified atmosphere containing 5% CO₂. When cell growth reached 10^6 cells/mL, cells were harvested according to the manufacturer’s instructions (Sanguine Biosciences, California, USA). To prepare H₂O₂-induced-stress PBLs, an appropriate volume of 0.1 mol/L H₂O₂ was added to fresh cell culture medium so that the final concentration of H₂O₂ was 50 µmol/L\cite{8}. The cells were suspended in the prepared medium containing 50 µmol/L H₂O₂ and adjusted to the concentration of 10^5 cells/mL by using trypan blue (Corning Cellgro, Virginia, USA) method, followed by the addition of the root extract.

2.3 Determination of cell concentration

The number of viable cells was assessed by 0.4% trypan blue solution. A volume of 0.5 mL 0.4% trypan blue solution was transferred to a test tube, followed by adding 0.2 mL of cell suspension and 0.3 mL Hank’s balanced salt solution (American Type Culture Collection, Virginia, USA). The dilution factor used in this study was 5. The suspension was mixed thoroughly. With the cover slip in place, 10 µL mixture was pipetted to the chamber of the hemocytometer. The numbers of cells in the one-millimeter-center square and four one-millimeter-corner squares were counted. Non-viable cells stained blue while viable cells remained transparent. The total number of cells was determined using the following formula: cells/mL = average cells counted/square × dilution factor × 10^3.

2.4 Cytotoxicity assays

One hundred microliters of cell suspension (10^6 cells/mL) was seeded in each well in a 96-well plate. The plate was pre-incubated for 24 h in a humidified incubator at 37 °C with 5% CO₂. Subsequently, 10 µL of different concentrations of \textit{L. porteri} (50, 100, 200, 400 µg/mL, and the control) was added to test for cytotoxicity (ratio of root extract and cell suspension is 1:10 in volume), followed by incubation for 2 d for PBLs. Then, 10 µL of
cell counting kit-8 (CCK-8) solution was added to each well of the plate and the wells were incubated for 4 h at 37 °C. The absorbance of formazan was measured at 450 nm (Technical manual CCK-8, Dourindo, Maryland, USA).

2.5 Modulatory effects of *L. porteri* extract on oxidative stress in *H₂O₂*-challenged PBLs

Following treatment with 50, 100, 200, 400 μg/mL, and 0 μg/mL (control) of *L. porteri* root extract, cell pellets were harvested for the assays of lipid peroxidation, reduced glutathione levels, and superoxide dismutase activity and catalase activities.

2.5.1 Lipid peroxidation

The pellets (10⁶ cells/mL) were lysed in ice-cold physiological saline by sonication, followed by centrifugation at 28 000 × g for 5 min at 4 °C. The cellular supernatants were used immediately for the measurement of the levels of malondialdehyde[9]. The formation of malondialdehyde, an end product of fatty acid peroxidation, was measured at 532 nm by using a thiobarbituric acid (TBA) reactive substance as described by Genet et al.[10]. Briefly, the final reaction mixture of 3 mL contained the following: 1.5 mL of 10 mmol/L potassium phosphate buffer (pH 7.4), 0.5 mL of the cellular supernatant, 0.5 mL of 30% trichloroacetic acid (TCA) and 0.5 mL of TBA (0.53%). The mixture was heated for 1 h at 80 °C, cooled and centrifuged for 5 min at 2 700 × g. The absorbance of the clear supernatant was measured at 532 nm against a blank. Total protein in the cellular supernatant was determined by using a Stanbio kit (Instruction manual, Stanbio Laboratory, Texas, USA).

2.5.2 Reduced glutathione

Reduced glutathione levels were measured following Ellman’s method[11]. The harvested pellets were lysed in hypotonic solution for 45 min at 37 °C and then processed for the assay[12]. One hundred microliters of the lysate was mixed with 10% TCA and centrifuged at 2 000 × g for 15 min. One milliliter of supernatant was treated with 0.5 mL of Ellman’s reagent (19.8 mg of 5,5′-dithiobisnitrobenzoic acid (DTNB) in 100 mL of 0.2 mol/L phosphate buffer (pH 8.0)). The absorbance was read at 412 nm.

2.5.3 Superoxide dismutase

The cell pellets (10⁶ cells/mL) were lysed by sonication in buffer (cold 20 mmol/L HEPES buffer (pH 7.2) containing 1 mmol/L EGTA, 210 mmol/L mannitol, and 70 mmol/L sucrose) (Technical manual for superoxide dismutase assay, Cayman Chemical, Michigan, USA). After sonication, the lysate was centrifuged at 1 500 × g for 5 min at 4 °C. The cellular supernatant was used for the measurement of superoxide dismutase activity. One milliliter of reaction mixture contained 500 μL of 0.1 mol/L sodium phosphate buffer, 32 μL of 3.3 mmol/L EDTA, 60 μL of 8.1 mmol/L pyrogallol, and an appropriate amount of cellular supernatant containing 7–10 μg protein. The change in absorbance at 420 nm of the mixture was monitored for 2 min at 25 °C against the blank that contains all ingredients except the supernatant. One unit of enzyme is defined as the amount of enzyme that causes half maximal inhibition of pyrogallol autooxidation[10].

2.5.4 Catalase

The cell pellets (10⁶ cells/mL) were lysed by sonication in buffer containing 50 mmol/L potassium phosphate (pH 7.0) and 1 mmol/L EDTA. The lysate was centrifuged at 10 000 × g for 15 min at 4 °C. The activity of catalase was measured in the supernatant[13]. Reaction mixture was prepared by adding 500 μL of 0.1 mol/L sodium phosphate buffer pH 7.0 (50 mmol/L), 100 μL of H₂O₂ (10 mmol/L), and 100 μL cellular supernatant treated with 30 μL of 1% Triton X-100. The decrease in absorbance was measured at 240 nm for 5 min at 25 °C against a blank containing all the ingredients without the supernatant[10].

2.6 Modulatory effects of *L. porteri* extract on inflammatory cytokines in *H₂O₂*-challenged PBLs

Cultures of *H₂O₂*-challenged PBLs (10⁶ cells/mL) were treated with 400 or 0 μg/mL (control) *L. porteri* extract. After 2 days of incubation, the supernatants were removed for analyses of cytokines. Cytokine levels of interferon (IFN)-γ, interleukin (IL)-2, and IL-10 were determined by using commercial enzyme-linked immunosorbent assays (ELISA) obtained from Thermo Scientific (Instructions for human IFN-γ, IL-2, and IL-10 ELISA kits, Illinois, USA).

2.7 Statistical analysis

Data are presented as mean ± standard error of the mean. Values were obtained from three separate experiments and each experiment included the control and four different concentrations of *L. porteri* extract performed in triplicate. One-way analysis of variance was used to test for differences among concentrations. Post hoc analysis was carried out using Duncan’s multiple range test to test for significant difference among the means (P<0.05).

3 Results

3.1 Cytotoxicity assays

Figure 1 shows that addition of *L. porteri* at 200 and 400 μg/mL enhanced the PBLs’ survival. Treatments with 200 and 400 μg/mL *L. porteri* resulted in 2- and 2.5-fold increases respectively in cell viability compared to the control after 2 days of incubation.

Figure 2 shows that oxidative stress induced by H₂O₂ reduced the viability of PBLs. Due to the adverse effect of H₂O₂, the survival of PBLs was reduced after 2 days of incubation in the presence of 50 μmol/L H₂O₂.

Figure 3 shows that treatment with 400 μg/mL *L. porteri* significantly ameliorated the adverse effects of H₂O₂ on PBLs. Treatment with 400 μg/mL of *L. porteri* extract...
was most effective in boosting the cell viability when compared to other treatment groups. Treatment with 50 μg/mL of the extract did not ameliorate the effect of H2O2. The cell viability in this group was reduced as much as 47% after 2 days of incubation.

3.2 Modulatory effects of *L. porteri* extract on oxidative stress in H2O2-challenged PBLs

### 3.2.1 Lipid peroxidation

Table 1 shows that two-day incubation of human lymphocytes with 50 μmol/L H2O2 caused a significant formation of malondialdehyde. This high level of malondialdehyde was significantly inhibited by the supplementation of *L. porteri* extract. The inhibitory effect of *L. porteri* on lipid peroxidation was greater with increasing *L. porteri* concentrations. Treatment with 400 μg/mL *L. porteri* significantly decreased lipid peroxidation by 94.3%, when compared to oxidative-stressed PBLs untreated with the root extract.

### 3.2.2 Reduced glutathione

Table 2 shows that 50 μmol/L of H2O2 decreased reduced glutathione levels in PBLs as compared to other treatments with the root extract. The oxidation of reduced glutathione was ameliorated by treatments with increasing concentrations of *L. porteri*. Oxidation of reduced glutathione in stressed PBLs was significantly inhibited when the cells were treated with 400 μg/mL *L. porteri*. This effect was marked by an elevation of 26.4% in reduced glutathione levels as compared to the control. Root extract concentrations lower than 200 μg/mL reduced the glutathione (reduced) levels but their ameliorating effects were less as that induced by the addition of 400 μg/mL *L. porteri*.

### 3.2.3 Superoxide dismutase and catalase activities

Tables 3–4 show the effects of *L. porteri* on superoxide dismutase and catalase activities in PBLs after inducing oxidative stress with 50 μmol/L H2O2. Fifty μmol/L H2O2 caused a significant decrease in superoxide dismutase and catalase activities compared to cell cultures treated with *L. porteri*. Treatment with the root extract significantly increased superoxide dismutase and catalase activities in PBLs (*P*<0.05). The activities of superoxide dismutase and catalase were increased by 17.5% and 55.2% respectively when stressed PBLs were incubated with 400 μg/mL *L. porteri* for 2 d.

### 3.3 Modulatory effects of *L. porteri* on inflammatory cytokines in H2O2-challenged PBLs

Figure 4 shows the modulatory effects of *L. porteri*
Effects of *Ligusticum porteri* on lipid peroxidation in H2O2-induced-stress PBLs after 2 days of treatment

<table>
<thead>
<tr>
<th><em>L. porteri</em> concentration (μg/mL)</th>
<th>Malondialdehyde (μmol/mg protein)</th>
<th>Inhibition (%) by <em>L. porteri</em> treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>421.69 ± 23.36</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>337.28 ± 11.63</td>
<td>20.0</td>
</tr>
<tr>
<td>100</td>
<td>326.32 ± 14.84</td>
<td>22.6</td>
</tr>
<tr>
<td>200</td>
<td>296.73 ± 20.56</td>
<td>29.6</td>
</tr>
<tr>
<td>400</td>
<td>23.83 ± 1.91 <em>Â</em> **</td>
<td>94.3</td>
</tr>
</tbody>
</table>

One-way ANOVA and Duncan’s multiple range test were used to statistically compare the difference in malondialdehyde levels among the treatment groups. *P*<0.05, vs control group; *Â* *P*<0.05, vs 50 μg/mL; *P*<0.05, vs 100 μg/mL; *P*<0.05, vs 200 μg/mL.

Effects of *Ligusticum porteri* on reduced glutathione content in H2O2-induced-stress PBLs after 2 days of treatment

<table>
<thead>
<tr>
<th><em>L. porteri</em> concentration (μg/mL)</th>
<th>Reduced glutathione (μmol/L/mg protein)</th>
<th>Elevation (%) by <em>L. porteri</em> treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>47.80 ± 1.02</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>48.48 ± 1.56</td>
<td>1.4</td>
</tr>
<tr>
<td>100</td>
<td>48.57 ± 50.17</td>
<td>1.6</td>
</tr>
<tr>
<td>200</td>
<td>50.17 ± 2.45</td>
<td>5.0</td>
</tr>
<tr>
<td>400</td>
<td>60.46 ± 2.84</td>
<td>26.4</td>
</tr>
</tbody>
</table>

One-way ANOVA and Duncan’s multiple range test were used to statistically compare the difference in reduced glutathione levels among the treatment groups. *P*<0.05, vs 400 μg/mL. PBLs: peripheral blood lymphocytes.

Effects of *Ligusticum porteri* on superoxide dismutase activity in H2O2-induced PBLs after 2 days of treatment

<table>
<thead>
<tr>
<th><em>L. porteri</em> concentration (μg/mL)</th>
<th>Superoxide dismutase (mU/(mg protein - min))</th>
<th>Increase of superoxide dismutase activity by <em>L. porteri</em> treatment (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>38.05 ± 1.05</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>38.05 ± 1.64</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>44.39 ± 2.09 <em>Â</em></td>
<td>16.7</td>
</tr>
<tr>
<td>200</td>
<td>44.39 ± 1.92 <em>Â</em></td>
<td>16.7</td>
</tr>
<tr>
<td>400</td>
<td>44.71 ± 1.83 <em>Â</em></td>
<td>17.5</td>
</tr>
</tbody>
</table>

One-way ANOVA and Duncan’s multiple range test were used to statistically compare the difference in superoxide dismutase activity among the treatment groups. *P*<0.05, vs control group; *Â* *P*<0.05, vs 50 μg/mL. PBLs: peripheral blood lymphocytes.

extract on inflammatory cytokines (IFN-γ, IL-2, and IL-10) in cell cultures that were challenged with 50 μmol/L of H2O2 and treated with 400 μg/mL of *L. porteri* extract after 2 days of treatment. There was no detection of IFN-γ in untreated cell cultures and the group of cell culture treated with only 50 μmol/L of H2O2. Treatment with 400 μg/mL of *L. porteri* extract induced IFN-γ production in H2O2-induced-stress PBLs. IL-2 was significantly up-regulated after a 2-day incubation with 400 μg/mL *L. porteri* extract. Hydrogen peroxide at 50 μmol/L caused secretion of IL-2 in stressed PBLs; however, there was a significant increase of IL-2 levels after these stressed cells were treated with 400 μg/mL *L. porteri* extract. Treatment of 50 μmol/L H2O2 significantly reduced IL-10 levels. However, treatment with 400 μg/mL *L. porteri* extract did not significantly change IL-10 levels.

4 Discussion

This study is the first to report the medicinal effects of *L. porteri* on PBLs. The herb has been used in traditional medicine for years but its acclaimed effects remain...
Effects of *Ligusticum porteri* on catalase activity in H$_2$O$_2$-induced PBLs after 2 days of treatment

<table>
<thead>
<tr>
<th><em>L. porteri</em> concentration (μg/mL)</th>
<th>Catalase (U/(mg protein • min))</th>
<th>Increase of catalase activity by <em>L. porteri</em> treatment (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9.33 ± 0.36</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>11.14 ± 2.34</td>
<td>19.4</td>
</tr>
<tr>
<td>100</td>
<td>11.02 ± 1.59</td>
<td>18.1</td>
</tr>
<tr>
<td>200</td>
<td>11.48 ± 1.23</td>
<td>23.0</td>
</tr>
<tr>
<td>400</td>
<td>14.48 ± 1.41*</td>
<td>55.2</td>
</tr>
</tbody>
</table>

One-way ANOVA and Duncan’s multiple range test were used to statistically compare the difference in catalase activity among the treatment groups. *P*<0.05, vs control group; $\dagger$ *P*<0.05, vs 50 μg/mL; $\ddagger$ *P*<0.05, vs 100 μg/mL; $\star$ *P*<0.05, vs 200 μg/mL. PBLs: peripheral blood lymphocytes.

One-way ANOVA and Duncan’s multiple range test were used to statistically compare the difference in catalase activity among the treatment groups. *P*<0.05, vs control group; $\dagger$ *P*<0.05, vs 50 μg/mL; $\ddagger$ *P*<0.05, vs 100 μg/mL; $\star$ *P*<0.05, vs 200 μg/mL. PBLs: peripheral blood lymphocytes.

Figure 4 Change in the levels of IFN-γ, IL-2, and IL-10 induced by PBLs after two days of treatment with 400 μg/mL *Ligusticum porteri*

One-way ANOVA and Duncan’s multiple range test were used to statistically compare the difference in the production of inflammatory cytokines (IFN-γ, IL-2, IL-10) among the groups (Groups 1–3). Group 1 = untreated; Group 2 = 50 μmol/L of hydrogen peroxide; Group 3 = 50 μmol/L of hydrogen peroxide and 400 μg/mL of *L. porteri*. $^*$ *P*<0.05, vs group 1; treatment with 400 μg/mL of *L. porteri* extract induced IFN-γ production in group 3 was not detected in groups 1 and 2; $\dagger$ *P*<0.05, vs group 3. IFN-γ: interferon-γ, IL-2: interleukin-2, IL-10: interleukin-10.

unknown. Today, due to the rising cost of pharmaceuticals, it is essential to investigate herbal remedies that are affordable and efficient in treating diseases. This study identified three properties of *L. porteri*: the cytotoxicity, the antioxidative and the immune-modulatory effects on human lymphocytes.

Data from the cytotoxicity assays suggest that the applications of ethanolic root extract of *L. porteri* at concentrations as high as 400 μg/mL increased the viability of human lymphocytes by 1.5 times. The enhancement of the viability of normal PBLs by *L. porteri* root extract may be beneficial to boost the immune system. Hydrogen peroxide is a highly toxic agent that is capable of exerting a strong adverse effect on cell viability. Saito et al.[14] reported that cells treated with 50 μmol/L H$_2$O$_2$ activated apoptotic caspase-3 and caspase-9 with subsequent cell death. A low-dose of intracellular H$_2$O$_2$ signals the activation of lymphocytes and fights against invading pathogens[15]. Excessive induction of H$_2$O$_2$ caused oxidative stress and impaired cell activity. A common consequence of oxidative stress is cell apoptosis or programmed cell death. It was previously reported that 50 μmol/L of H$_2$O$_2$ caused cellular apoptosis while the use of H$_2$O$_2$ at 500 μmol/L induced necrosis in human T-lymphoma Jurkat cells[16]. The stress induced by 50 μmol/L H$_2$O$_2$ resulted in about 4-fold reduction of the growth of PBLs after 2 d. We then investigated other biochemical indices like oxidative and inflammatory responses after 2 days of treatment with different concentrations of *L. porteri* extracts. Findings from this study demonstrated for the first time that treatments with *L. porteri* might protect PBLs from oxidative stress caused by H$_2$O$_2$. Previous studies proposed that a mechanism to attenuate the deleterious effect of H$_2$O$_2$ is through the inhibitory effects on the levels of malondialdehyde[17]. This study revealed that the exposure of PBLs to 50 μmol/L H$_2$O$_2$ led to a significant increase in malondialdehyde content. The significant reduction after *L. porteri* treatment suggests a protective influence of the root extract against oxidative stress. Reduced glutathione is an important cellular antioxidant, involved in the protection against free radicals and other cytotoxic compounds. The cellular reduced glutathione content is an important determinant to regulate the redox status in cells. When the level of reduced glutathione is elevated, the oxidative stress is reduced and the level of lipid peroxidation, represented by malondialdehyde content, is low. In contrast, low level of reduced glutathione is an indicator for the rising amount of malondialdehyde, which consequently leads to impaired cellular oxidative status[18]. Findings from this study indicate that the reduced glutathione levels in H$_2$O$_2$-induced-stress PBLs after treatment with *L. porteri* were significantly higher than those observed in the untreated cell cultures. Previous studies suggested that oxidative stress and the deficiency
of thiol compounds might be the primary cause for the development of immune deficiencies. Moreover, the shortage of intracellular reduced glutathione may interrupt T-cell function. Depletion of reduced glutathione levels was seen in cells exposed to 50 μmol/L H2O2 alone, which indicated an impaired oxidative status in these cells. However, supplementation with L. porteri at high doses significantly increased the reduced glutathione content as compared to the control. These results suggest that the L. porteri root extracts may be a potential antioxidant that possesses a protective effect against oxidation of reduced glutathione. The activities of superoxide dismutase and catalase were increased in oxidative-stressed PBLs treated with the herbal extract as compared to the untreated cell cultures. In the cellular defense system against oxidative stress, these two enzymes are responsible for catalyzing the reactions to convert toxic reactive oxygen species into non-toxic compounds. The role of superoxide dismutase is to convert superoxide radicals to H2O2 that is further degraded to water and oxygen. Thus, the activities of superoxide dismutase and catalase are known to play a key role in modulating the cellular redox status. The results of the present study indicate that L. porteri may be effective in preventing oxidative damage by increasing the activities of antioxidant enzymes (superoxide dismutase and catalase).

Data from this study shows that the ethanolic root extract of L. porteri enhanced cell viability in PBLs. The underlying mechanism might be through the herb’s ability to regulate the expression of IFN-γ, IL-2, and IL-10 in these cells. Secretion of IFN-γ, IL-2, and IL-10 plays an important role in the activation of immune cells. It has been known that IL-2 is a key cytokine in stimulating T-cell proliferation, cytokine production, and functions of B cells, macrophages, and natural killer cells. The IFN-γ is known as a major pro-inflammatory cytokine and it is exclusively produced by activated lymphocytes and natural killer cells in the adaptive immune response. Upon secretion, IFN-γ could up-regulate a number of lymphoid cell functions; as well as exert strong regulatory influences on the proliferation, differentiation, and responses of B cell and T cell subsets. The production of IFN-γ and IL-2 in stressed PBLs was increased by the treatment with 400 μg/mL L. porteri. Zhou et al. reported that IL-10 generates T-cell tolerance. This cytokine (IL-10) is released in order to balance the dramatic increase in pro-inflammatory cytokines in stressful situations, and therefore it could control the intensity and duration of the inflammatory response. The induction of IL-10 is believed to suppress the activity of other pro-inflammatory cytokines and down-regulate eosinophil activity. It was shown that due to the treatment of 50 μmol/L H2O2, the cellular balance between pro- and anti-inflammation was impaired. The imbalance was marked by low amounts of induced IL-10 and high amounts of IL-2 and IFN-γ secreted after exposure to the stress. The root extract of L. porteri used at 400 μg/mL rendered a mild anti-inflammatory response toward the H2O2-induced stress. The observed immune-modulating effects correlate with the report of Del-Angel et al. that showed semi-synthetic phthalides and the natural phthalides extracted from L. porteri had anti-inflammatory activities in mice with carrageenan-induced paw edema.

5 Conclusion

This study showed that the ethanolic root extract of L. porteri improved the viability of PBLs, exerted an antioxidative effect by inhibiting lipid peroxidation, enhanced reduced glutathione and activities of superoxide dismutase and catalase. The expressions of IFN-γ and IL-2 were up-regulated significantly as compared to stressed cells that were not treated with the root extract. Data from this study also showed that L. porteri may be a potential immune-modulating agent that may accrue some health benefits. However, further studies are needed to determine the active compounds that may be responsible for the therapeutic applications of the root extract in the treatment of diseases.

6 Acknowledgements

We thank Ms. Susan Sparks of Chiron Holistic LLC for the supply of L. porteri root.

This study was supported by a grant from Texas A&M University-Corpus Christi.

7 Competing interests

The authors declare that they have no competing interests.

REFERENCES


