RESEARCH ARTICLE

Antioxidant and anti-inflammatory properties of ginger cultivar rhizome extract

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Ginger is known for its antioxidant and anti-inflammatory health benefits. However, few studies have examined how these properties vary among ginger cultivars. This study evaluated antioxidant potential and anti-inflammatory properties of fresh and freeze dried extracts from three different ginger cultivars of Chinese White, Kali Ma, Hawaii Yellow. Antioxidant capacity was measured using FRAP assay, while anti-inflammatory potentials of the extracts were determined using RAW 264.7 cells stimulated with lipopolysaccharide (LPS). Nitric oxide levels in the cell supernatant were measured by using Griess assay. Cytokine profiling of cell supernatants was identified using a cytokine array kit. IL-6 and IL-10 levels of treated cell supernatants were determined using enzyme-linked immunosorbent assay (ELISA). The results showed that no statistically significant differences were observed among the ginger cultivars in both antioxidant capacities and anti-inflammatory potentials. Distinct expression patterns of specific inflammatory related cytokines and chemokines in response to treatment with freeze-dried extracts from three different ginger cultivars were observed. There were no significant differences for IL-6 and IL-10 production on the treated cell supernatants among the three cultivars studied. However, the results indicated significant differences in the antioxidant and anti-inflammatory properties between the fresh and freeze-dried ginger samples with the latter showing the superior properties. No statistically significant differences in the antioxidant and anti-inflammatory properties among the cultivars studied suggested that cultivar identity did not substantially influence the medicinal benefits of ginger, which also indicated that the health benefits of ginger were largely conserved across cultivars, which had practical implications for farmers, clinical herbalists, and industries sourcing ginger for health-oriented applications.

Keywords: ginger; cultivar; anti-inflammatory; antioxidant; cytokines; RAW 264.7 macrophages.

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Introduction

Ginger is a spice that has been taken for over 2,000 years. This plant species is mostly cultivated in different parts of the world including Africa and Asia, making it one of the most famous food spices in the world [1]. Mainland USA does not grow ginger commercially due to undesirable climatic conditions; hence majority of the ginger consumed in the country comes from other

countries including China, India, and Peru. Notwithstanding the low level of commercial production, a few small-scale ginger producers in Florida and Hawaii produce ginger for local markets because of the warm, humid climates in these states [2]. Ginger is usually available in different forms including but not limited to fresh ginger, preserved ginger, dry ginger, ginger powder, ginger oil, ginger oleoresin, and ginger paste [3]. Drying ginger prolongs its shelf life while maintaining or boosting its bioactive compounds, making it a desirable post-harvest method [4].

Ginger has been adopted as a medicinal plant for centuries. Lately, there has been an increase in focus on its use to improve health and wellbeing, either as an alternative or complement to medicine. Several studies have indicated that ginger helps to treat several medical complications including arthritis, neurodegenerative diseases, nausea, cancer, diabetes, asthma, and allergies [5-9]. The reason why ginger can be used as a health-promoting plant is because of its rich phytochemicals. In ginger, there are some bioactive phytochemicals like 6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol, 8-shogaol, 10-shogaol, and paradols, which are known to inhibit the activation of pro-inflammatory cytokines including tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and interleukin (IL)-6 with 6-gingerol being the predominant bioactive constituent [10, 11]. Chronic inflammation is associated with many acute and chronic diseases in humans such as stroke, diabetes, and cancer [12]. In situations of elevated and unchecked acute inflammation, the intensity and duration of inflammatory reactions are connected to the overproduction of pro-inflammatory cytokines and mediators such as IL-6, TNF, and nitric oxide (NO) by activated macrophages. Large cellular components are frequently damaged by oxidative stress, which also modifies cell metabolism and physiology and ultimately destroys the body's immune system and inflammatory reactions [13].

Although several medicinal applications and effects of ginger have been studied, there are no reports currently related to the antioxidant and anti-inflammatory properties of different ginger cultivars that are grown in the USA. Ginger cultivars are selectively bred and propagated for specific desirable traits [14]. Comparing and identifying ginger cultivars with the most potent antioxidant and anti-inflammatory properties would help in the selection of ginger cultivars with excellent characteristics and provide theoretical basis for development and utilization of ginger for specific medicinal purposes. This study focused on evaluating the antioxidant and anti-inflammatory properties of fresh and freeze-dried extracts prepared from different ginger cultivars including Chinese White, Kali Ma, Hawaii Yellow to compare their antioxidant capacities and anti-inflammatory potentials using cell culture technology and explore their effects on cell cytokine profiling. The results of this study would provide practical implications for farmers, clinical herbalists, and industries sourcing ginger for health-oriented applications.

Materials and methods

Ginger cultivation

Ginger rhizomes of three cultivars, Chinese White (CW), Hawaii Yellow (HY), and Kali Ma (KM), were cultivated and harvested in the greenhouse at the farm of North Carolina Agricultural and Technical State University, Greensboro, North Carolina, USA. Rhizomes originally sourced from Hawaii were cut into 20 – 25 g pieces, each with one or two growing points, and used as seeds. The cut pieces were placed in seed trays with coconut coir, spaced one inch apart, and maintained moist *via* routine watering. After three months of sprouting in the trays, the ginger seedlings were transplanted into 10-gallon plastic pots filled with soil formulated with metro mix and compost. Ginger plants were grown and maintained following established production and research protocols, and the rhizomes were harvested in January 2023, eight months after transplanting. The stems and adventitious roots

were removed. The rhizomes were rinsed and cleaned with water, and then stored in a cool, dry place.

Ginger extract preparation

Ginger extracts from freeze-dried and fresh ginger rhizomes were used in this study. To prepare fresh ginger extracts, freshly picked ginger rhizomes were minced in a blender. Freeze-dried ginger extracts were prepared according to the protocol of Ho *et al*. [4]. Ginger rhizomes were first sliced into thin pieces and frozen in a -80°C freezer for 48 hours followed by lyophilization in a Labconco 8-Liter Benchtop freeze-dryer (Labconco, Kansas City, MO, USA) for 48 hours. Each of the completely dried ginger rhizome samples were pulverized separately. The powdered ginger rhizome samples were packed in air-tight tubes and stored at -80°C until use to make extracts. To prepare extracts, ten (10) grams of freeze-dried ginger rhizome powder or fresh minced ginger from each of the three ginger cultivars studied were placed into a 250 mL Erlenmeyer glass flask with 100 mL of 90% ethanol. The glass flasks were tightly closed/capped and shaken for 24 hours at 150 rpm. The suspension was allowed to settle for 24 hours before the supernatant was collected. The supernatants were filtered through 0.22 µm polyvinylidene fluoride (PVDF) membranes using a vacuum filter under sterilized conditions and stored at –20°C.

Assessment of antioxidant activity

The antioxidant potential of the different ginger extracts was evaluated using OxiSelect™ Ferric Reducing Antioxidant Power (FRAP) Assay Kit (Cell Biolabs, San Diego, CA, USA) according to the manufacturer's protocol. Briefly, 100 µL ginger rhizome extracts from each ginger cultivar were added to 100 µL of FRAP reagent in a 96-well plate in triplicate and mixed thoroughly by pipetting. The 96-well plate was incubated for 10 minutes at room temperature and the absorbances were read at 540 nm using a SpectraMax M5 spectrophotometer (Molecular Devices, San Jose, CA, USA). The net OD was determined by subtracting the zero standard value from the values of each extract and standard of Iron (II) oxide (Fe^{2+}) from 0 – 500 µM, and the FRAP values were quantified and expressed as µM Fe^{2+}.

Cell culture

RAW 264.7-TIB-71 macrophages (ATCC, Manassas, VA, USA) were maintained using Corning T-75 flasks in complete DMEM with 10% FBS (Corning, Corning, NY, USA) and 1% penicillin at 37°C, 5% CO_2. Cells were collected and seeded in a 12-well plate at 3×10^5 cells/well, pre-treated with ginger extracts for 2 hours and treated with 1 µg/mL lipopolysaccharide (LPS) for 24 hours. Ginger rhizome extracts at 1.0%, 0.8%, 0.6%, 0.4%, 0.2% from the freeze dried CW cultivar was tested to determine the best dose to be used in dose subsequent experiments. The anti-inflammatory capacities of the different ginger cultivars were tested by seeding cells in a 12-well plate at 3×10^5 cells/well, pre-treated with the 0.8% different ginger extracts or 10 µM dexamethasone for 2 hours and treated with 1 µg/mL LPS for 24 hours. Cell supernatants were collected after 24 hours and stored at -80°C.

Cytotoxicity assay of ginger rhizome extracts

The cytotoxic effects of the ethanolic extracts of ginger rhizomes (fresh and freeze dried) on RAW 264.7 cells were evaluated by lactate dehydrogenase (LDH) cytotoxicity detection kit (Roche, Indianapolis, IN, USA) following manufacturer's protocol. Briefly, RAW 264.7 cells were seeded into a 96 well plate at 5.0×10^4 cells/well and allowed to adhere for 24 hours. Cells were treated with ginger extract from the CW cultivar at concentrations of 0.2%, 0.4%, 0.6%, 0.8%, 1.0% or 10 µM dexamethasone as positive control and 90% ethanol as vehicle control for 2 hours followed by another 2 hours incubation. The potential cytotoxic effects of different ginger extracts were determined by treating cells with ginger extracts using the pre-determined optimum dose. Following the 2 h incubation period, the medium was discarded, cell surfaces were washed and replaced with 1 µg/mL lipopolysaccharide solution and incubated for another 24 hours. After 24 hours of

incubation, 100 µL of cell-free supernatants were removed and transferred into a corresponding well in a new plate. The 100 µL of freshly prepared reaction mixture was then mixed with supernatants in each well and incubated at room temperature for 30 minutes. The OD of the mixture was measured at a wavelength of 490 nm by using a SpectraMax M5 spectrophotometer. The cytotoxicity level of the ginger extracts was determined relative to the control group. Cytotoxicity tests were conducted in triplicate.

Nitric oxide (NO) production

Nitric oxide (NO) levels in the supernatants of RAW 264.7 cells pretreated with ginger extracts and stimulated with LPS were measured with the Griess assay (Promega, Madison, WI, USA) per manufacturer's protocol. Briefly, 50 µL of cell-free supernatants from treated cells were transferred into a new 96-well plate in triplicates. 50 µL of Griess reagent (1.0% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 2.5% phosphoric acid) was added into each well. The assay reaction mixture was incubated for 10 minutes at room temperature in a dark environment followed by measuring the absorbance at 535 nm. The quantity of nitrite (stable form of NO) was determined from a sodium nitrite standard curve. The experiment was repeated three times.

Cytokine array

Quantification of 40 cytokines and chemokines in supernatants was performed using the Proteome Profiler mouse cytokine array kit (R&D Systems, Minneapolis, MN, USA) following manufacturer's instructions. Briefly, supernatants from three independent cell culture experiments were pooled by pipetting 200 µL from each experiment to generate a representative sample for each treatment. The mixture comprising 1.5 mL of the pooled sample representing each group, buffer, and detection antibody was applied to the prepared membrane and incubated overnight at 4°C. After incubating with HRP-streptavidin for 30 minutes, the image was developed with the Chemi Reagent Mix and subjected to chemiluminescence detection. The pixel density of each spot in the array was generated by using Bio-Rad Laboratories 2020, Version 6.1 (Bio-Rad Laboratories, Hercules, CA, USA). The fold change of each cytokine was determined by calculating spot densities on the membranes. Fold change results were presented as the fold-difference between treatment groups and the control group (unstimulated). For each membrane, the values were normalized by subtracting the average background from the average adjusted volumes of each cytokine. The relative expression of a cytokine was determined by dividing the positive control signal density of the reference array by the signal density of another array membrane multiplied by the average spot density of the particular cytokine.

Interleukin 6 and interleukin 10 Enzyme-Linked Immunosorbent Assays (ELISA)

The levels of IL-6 and IL-10 in the assay supernatants were evaluated using ELISA assays (BD Biosciences, Newark, NJ, USA) following manufacturer's instructions. Supernatants collected from the RAW 264.7 cell cultures were diluted at a ratio of 1:100 for IL-6 and 1:10 for IL-10 before being added to the wells with 100 µL of each. The murine IL-10 (0 – 2,000 pg/mL) and murine standard IL-6 (0 – 1,000 pg/mL) were employed for the calibration curves. The relative absorbance was measured between 450 and 570 nm and was repeated three times.

Statistical analysis

All data were expressed as means ± standard error mean (SEM). Raw data from the microplate reader were exported into an excel file and inserted into the GraphPad Prism 10.2.0 software (https://www.graphpad.com/) for analysis. The effects of ginger cultivars and ginger rhizome samples forms (whether freeze-dried or fresh) prior to extract preparation were evaluated using two-way ANOVA with Tukey post-hoc test. Two-tailed significance testing with probability value of alpha < 0.05 was used to determine significance.

Results

Antioxidant activity of ginger

The antioxidant activities of freeze-dried and fresh ginger ethanolic extracts from the CW, HY, and KM cultivars demonstrated that there was a significant difference between the antioxidant activity of extracts prepared from fresh ginger rhizome and of their corresponding freeze-dried extracts for all ginger cultivars tested (Figure 1). However, the antioxidant activity between the different freeze-dried or fresh rhizomes of ginger cultivars (CW, HY, KM) were not significantly different.

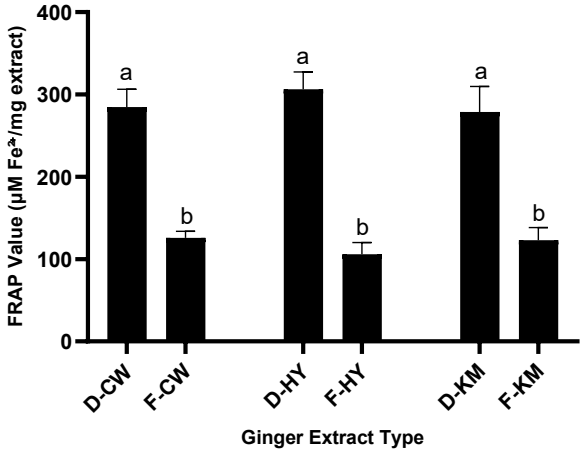


Figure 1. Antioxidant activities of different ginger cultivars. Data represented as mean ± SEM. The different lower case letters indicated statistically significant ($P < 0.05$). D represented freeze-dried ginger, while F represented fresh ginger.

Cytotoxicity of ginger rhizome extract

The safe concentration of ginger rhizome extracts to RAW 264.7 macrophage murine cell line showed that LDH activity did not significantly differ between the control group (media + LPS), pre-treated with varying concentrations of ginger rhizome extracts, or LPS-stimulated cells, indicating no detectable cytotoxicity of ginger rhizome extracts at concentrations up to 1.0% (Figure 2A). There was no significant difference in LDH activity among control group, cells pre-treated with different ginger rhizome extracts at the optimal dose, or LPS-stimulated cells, confirming that none of the tested ginger rhizome extracts exhibited cytotoxic effects at the 0.8% concentration (Figure 2B).

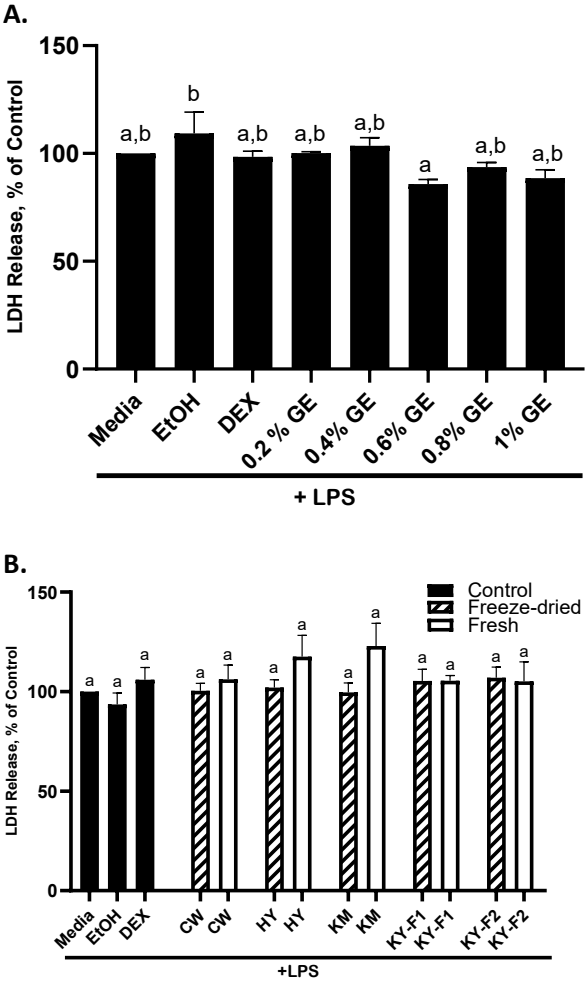


Figure 2. Cytotoxic effect of freeze-dried Chinese White ginger extracts at different concentrations (**A**) and extracts from different ginger cultivars (freeze-dried, fresh) at optimum concentration (**B**) on LPS-stimulated RAW 264.7 cells. Values were represented as mean ± SEM.

Determining optimal dose *via* nitric oxide levels

To determine the optimum dose of ginger extract that inhibited nitric oxide production, increasing concentrations of extracts from the freeze-dried CW cultivar (0.2%, 0.4%, 0.6%, 0.8%, 1.0%) were tested. The results showed that cells treated with only LPS released significantly higher levels of nitric oxide of 10.49 ± 1.062 μM than the non-treated cells of 0.5320 ± 0.1832 μM ($P < 0.05$),

while there was no significant difference between the vehicle control group of 9.265 ± 1.101 µM and the LPS only treated cells. Significant difference was also observed between cells treated with Dexamethasone and cells pre-treated with 0.8% and 1% ginger extract (P < 0.05). Significant decreases in NO production were observed in cells pre-treated with 0.4%, 0.6%, 0.8%, and 1.0% ginger extracts in a dose-dependent manner. The pre-treatment concentration of 0.8 % showed the greatest inhibition of NO production as 2.367 ± 0.3848 µM compared to other concentrations of extracts at 0.2%, 0.4%, 0.6%, and 1.0% (Figure 3). Based on the NO reduction results and the cytotoxic analysis, the dose of 8 µg/mL (0.8%) was subsequently used for the comparative anti-inflammatory test.

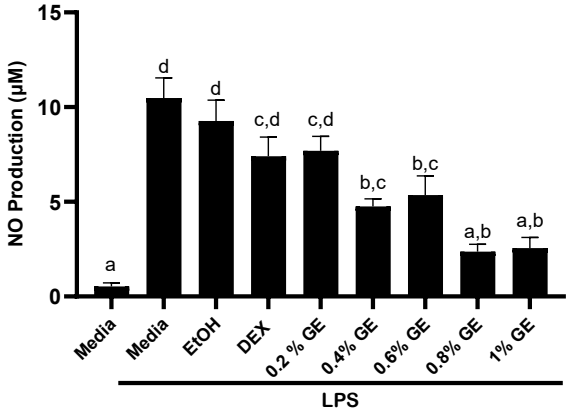


Figure 3. Effect of freeze-dried Chinese White ginger extracts at different concentrations on nitric oxide production. Values were represented as mean ± SEM. Different lower case letters indicated significantly different (P < 0.05).

Ginger rhizome extracts on LPS-induced nitric oxide production

A significant difference in NO production was observed between the LPS-only group and all other treatments (P < 0.05). Additionally, a significant difference was found between the vehicle control group (90% ethanol) and RAW 264.7 cells pre-treated ginger extract from freeze-dried ginger rhizome samples (P < 0.05). However, no difference was noted between the vehicle control group (90% ethanol) and ginger rhizome extracts obtained from fresh ginger rhizome samples. The results showed that fresh ginger rhizome extracts were less effective at reducing NO release compared to their corresponding freeze-dried ginger rhizome extract. There was no difference among the three ginger cultivars from freeze-dried or fresh ginger rhizome samples (Figure 4). The results indicated that, among the ginger cultivars, there was no difference in their NO inhibitory activity. However, the ginger rhizome sample state (freeze-dried or fresh) prior to extract preparation significantly influenced this activity.

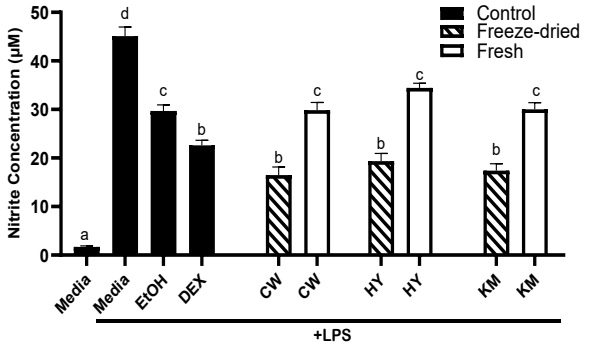


Figure 4. Comparative analysis of nitric oxide production in RAW 264.7 cells by different ginger cultivars. Values were represented as mean ± SEM. Different lower case letters indicated the significantly different (P < 0.05).

Inhibition of LPS-induced cytokine secretion by ginger extracts in RAW 264.7 cells

The results demonstrated a significant elevation of multiple cytokines and chemokines in response to LPS including IL-6, IL-1, IL-1β, RANTES, and GM-CSF. The different ginger rhizome extracts showed a remarkable inhibitory effect on most cytokines including IL-6 with 16.30 - 10.16 fold change for CW and IL-1β with 5.5 - 3.26 fold change for KM. In contrast, dexamethasone and ginger rhizome extracts from CW ginger cultivar showed high levels of the anti-inflammatory cytokine IL-10 with fold change values of 5.17 and 4.96, respectively. High level of chemokines, macrophage inflammatory protein-1 alpha (MIP-1α) and beta (MIP-1β), were observed in the unstimulated cells compared to the ginger rhizome extract treated

Table 1. Fold changes in the upregulation and downregulation of cytokines and chemokines in LPS stimulated and ginger extract treated RAW 264.7 cells.

Inflammatory Mediators		Unstimulated	LPS	DEX	CW	HY	KM
Pro-inflammatory cytokines	IL-1 alpha	1	6.34	4.33	4.26	4.76	4.20
	IL-1 beta	1	5.54	3.73	3.65	3.30	3.26
	IL-6	1	16.30	12.29	10.16	14.37	15.04
	TNF- alpha	1	1.57	1.35	1.27	1.44	1.37
Anti-inflammatory cytokines	IL-10	1	3.29	5.17	4.96	1.11	3.78
	IL-1ra	1	1.45	1.25	1.41	0.90	0.84
Chemokines	JE	1	0.37	0.37	0.56	0.40	0.28
	MIP-1 alpha	1	0.93	0.78	0.89	0.75	0.61
	MIP-1 beta	1	0.58	0.49	0.66	0.46	0.37
	RANTES	1	4.62	3.71	3.58	3.31	3.26
	MIP-2	1	1.02	0.81	1.26	0.75	0.58
	IP-10	1	1.07	0.64	0.76	0.66	0.75
	I-TAC	1	1.33	0.24	0.15	0.52	0.29
	SDF-1	1	1.15	0.35	0.36	0.07	0.05
Acute-phase proteins	G-CSF	1	1.81	1.72	1.25	1.53	1.11
	GM-CSF	1	16.79	14.03	1.67	17.92	6.02
	M-CSF	1	9.91	0.97	0.30	1.14	0.30
	ICAM-1	1	1.39	1.11	1.18	0.80	1.10
	TIMP-1	1	5.36	2.48	1.18	1.17	2.81

and LPS stimulated cells. The CW ginger cultivar generally exhibited stronger inhibitory effects on cytokines and inflammatory mediators compared to the HY and KM ginger cultivars except for IL-6 and TNF-α (Table 1).

Ginger Rhizome Extracts on IL-6 and IL-10 Production

The concentrations of IL-6 and IL-10 were measured to assess the impact of the ginger rhizome extracts on inflammatory responses. The levels of IL-6 and IL-10 production in LPS-stimulated RAW 264.7 cells showed that the LPS-treated only RAW 264.7 cells demonstrated the highest concentration of IL-6, which indicated that LPS successfully induced inflammation, prompting the production of pro-inflammatory cytokines in RAW 264.7 cells compared to cells without LPS treatment. Pre-treatment of LPS-activated RAW 264.7 cells with freeze-dried ginger extracts significantly reduced level of IL-6. Freeze-dried extracts from the CW ginger cultivar appeared to have greater effects in inhibiting IL-6 level compared to the other ginger cultivars, but this difference did not reach statistical significance (Figure 5). There was no significant difference in IL-6 production between cells treated with dexamethasone and different ginger extracts except fresh HY ginger extracts. Notably, there was significant difference in the IL-6 levels between the extracts from freeze-dried and fresh ginger rhizome samples among ginger cultivars ($P < 0.05$).

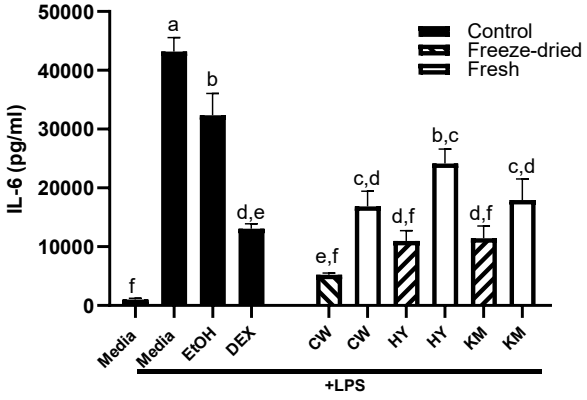


Figure 5. Variation in IL-6 levels in RAW 264.7 cells exposed to different ginger cultivars. Values were represented as mean ± SEM. The different lower case letters indicated significant difference ($P < 0.05$).

Ethanolic extracts from the freeze-dried ginger rhizome samples resulted in a reduction in IL-10 release compared to LPS only stimulation, while ethanol (vehicle control) resulted in a statistically significant induction of IL-10 release (Figure 6). However, pretreatment with fresh ginger rhizome extracts produced comparably a slight reduction in IL-10 release in the RAW 264.7 cultures. The secretion of IL-10 in LPS stimulated cells pre-treated with ginger rhizome extracts from the three ginger cultivars were not statistically significant.

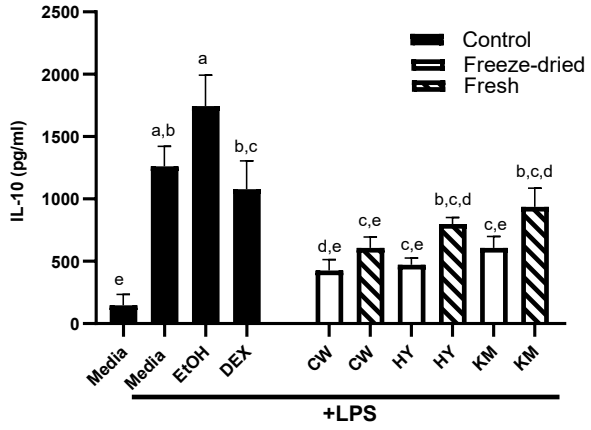


Figure 6. Effect of extracts from different ginger cultivars on IL-10 concentration in LPS- stimulated RAW 264.7 cells. Values were represented as mean ± SEM. Different lower case letters indicated significantly difference ($P < 0.05$).

Discussion

The generation of reactive oxygen species (ROS) or free radicals during metabolism and other activities that surpass a biological system antioxidant capacity result in oxidative stress, which is associated with many diseases including heart disease, cancer, neurological illnesses, and even the aging process [15]. The regulation of enzymatic antioxidants is linked to defense mechanisms against oxidative stress in living organisms [16]. Antioxidants prevent cell damage by delaying oxidation of major biomolecules within the cell by chelating metals or scavenging free radicals and regulating gene expression, which makes antioxidants a necessity to reduce oxidative cell damage. Oxidants and antioxidants must be balanced to minimize oxidative stress-related molecular and cellular tissue damage. The Fe (III) reducing power of ginger rhizome extracts was evaluated by measuring the FRAP. Ferrous ions serve as key catalysts in oxidative processes, facilitating the formation of hydroxyl radicals and the decomposition of hydroperoxides through the Fenton reaction [17]. The findings of this research were consistent with the report of previous study that relatively higher FRAP values were observed in extracts from freeze-dried ginger compared to fresh ginger [18]. Similarly, Mustafa *et al*. reported that freeze-dried ginger ethanolic extracts exhibited greater FRAP activity than those from fresh ginger [19]. Overall, the three ginger cultivars tested in this study exhibited comparable antioxidant activity, which might be attributed to comparable levels of phenolic compounds present in different ginger cultivars. Ghosh *et al*. reported that there was a strong positive correlation between the antioxidant activity of ginger and its total phenolic content, suggesting that ginger phenolic compounds were major contributors to its free radical scavenging properties [20]. Pawar *et al*. also evaluated antioxidant and phenolic compounds of different ginger cultivars in India and concluded that ginger cultivars with high levels of 6-gingerol had the highest antioxidant activity [21]. The production of nitric oxide (NO) by activated immune cells controls the activities of other immune cells and may serve as a secondary mediator of various proinflammatory cytokine actions. Individual phenolic compounds in ginger have also been associated with the inhibition of inflammatory markers and cytokines in stimulated RAW 264.7 cells [22]. Present study evaluated the effects of ginger rhizome extracts from different ginger cultivars on LPS-induced production of NO, IL-6, and IL-10. The results demonstrated that pretreatment with ginger rhizome extracts from different ginger cultivars significantly reduced the inflammatory response of LPS-stimulated RAW 264.7 cells. Ethanolic ginger rhizome extracts from three ginger cultivars showed similar nitric oxide inhibitory

activity, which might be attributable to similar levels of bioactive compounds, especially gingerols and shogaols in the ginger cultivars. The anti-inflammatory properties of ginger have been reported to be influenced by the bioactive compounds [23].

The results of this study demonstrated that extracts from freeze-dried ginger rhizomes had higher anti-inflammation capacity compared to extracts from fresh ginger rhizomes. Previous research reported that dried ginger, whether oven-dried, freeze-dried, or sun-dried, exhibited greater inhibitory effects on nitric oxide production compared to fresh ginger [24]. The drying process could also cause the breakdown of several cells. Ginger drying procedure makes the ginger tissue samples more brittle, which leads to the breakdown of its cell walls during milling and then enhances the extraction process compared to fresh ginger [25]. Dried samples are also higher in porosity, which enhances the diffusion rate of solutes and solvents during extraction, resulting in higher extract yields compared to fresh samples [26]. The antioxidant activity in dried ginger has been reported to be higher than that of fresh ginger, which has been attributed to gingerols undergoing dehydration conversions into shogaols during drying that reportedly possess stronger antioxidant and anti-inflammatory abilities than gingerols and other bioactive compounds [18, 22]. Jolad *et al*. reported that dry ginger contained higher contents of 6-shogaol than fresh ginger [27]. Therefore, the higher anti-inflammatory activity observed in the freeze-dried ginger rhizome samples could be associated with increased concentration of shogaols during the drying process. Pro-inflammatory cytokines including IL-6, IL-1β, TNF, and others are well-established as key mediators of cytokine storms and have been widely studied for their critical roles in various inflammatory diseases [28]. This cytokine storm has been attributed to many severe and serious inflammatory diseases and ailments [29]. This study evaluated the effects of the freeze-dried ginger rhizome samples on different cytokines and mediators because they showed a superior NO inhibitory capacity compared to their corresponding fresh ginger rhizome samples. The ginger rhizome extracts, especially KM cultivar, effectively reduced RANTES that linked closely to autoimmune diseases like Addison disease, systemic lupus erythematosus, and rheumatoid arthritis [30-32]. Ginger rhizome extracts from ginger cultivars CW and KM significantly reduced the levels of multiple chemokines including granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF). GM-CSF and G-CSF have been strongly implicated in the pathogenesis of autoimmune diseases such as rheumatoid arthritis and in chronic inflammatory conditions [33]. Reduction in the level of tissue inhibitors of metalloproteinases (TIMPs) was observed with cells treated with the different ginger rhizome extracts, especially from CW and HY ginger cultivars, compared to the LPS only treated cells with fold values changing from 5.39 to 1.18 and 1.17, respectively. TIMPs play a vital role in controlling the activity of matrix metalloproteinases (MMPs), thereby affecting cell structure, cell growth, and apoptosis [34]. Elevated levels of MMP-3 along with its corresponding TIMPs have been recorded in Crohn's disease and ulcerative colitis [35]. Elevated levels of TIMPs have also been found in neurodegenerative diseases including multiple sclerosis, Alzheimer's disease, and Parkinson's disease [36]. This phenomenon, which was found whereby the ginger rhizome extracts from all the cultivars reduced most of the elevated levels of cytokines, demonstrated the positive abilities of ginger as an anti-inflammatory remedy. Ginger has been shown to reduce inflammatory bowel disease symptoms by blocking damaging factors like IL-17 and TNF-α, while also promoting remedial factors like IL-10 and TGF-β [37]. An inappropriate response to inflammation can trigger various cellular reactions, thereby affect the body's physiological functions and potentially even endanger life [38]. These findings confirmed the impact of ginger in lowering the levels of pro-inflammatory cytokines and some chemokines as evidenced by their reduced concentrations compared to cells stimulated with LPS.

Due to the semi-quantitative nature of the mouse cytokine analysis with Proteome Profiler, ELISA method was employed to validate and precisely quantify the effects of different ginger rhizome extracts, particularly on IL-6 and IL-10. IL-6 is a pro-inflammatory cytokine that contributes in the inflammatory process, and inhibiting molecule production is thought to be very vital in controlling cellular inflammation [39]. LPS stimulation produces mediators that support the natural defense mechanisms. However, an overabundance of these mediators can lead to acute inflammation, which can result in tissue damage and organ dysfunction [40]. This study confirmed that ginger rhizome extracts inhibited the production levels of LPS-induced IL-6, which were significantly elevated in LPS-induced inflammation. Similar studies have shown that ginger and its key bioactive compounds are potent inhibitors of pro-inflammatory cytokines IL-1β, TNF-α, and IL-6 [41]. The relatively high inhibitory activity of extracts from freeze-dried ginger compared to fresh ginger can be associated with the relatively high levels of shogaols in dried ginger [22]. IL-10 is a multifunctional cytokine that regulates the activity of various adaptive immune cells. While it is primarily recognized for its immunosuppressive and anti-inflammatory effects, IL-10 also exhibits immunostimulatory properties [42]. The activation of these immune-related cells may be linked to the inflammatory response.

Conclusion

This study assessed the antioxidant and anti-inflammatory properties of three different ginger cultivars grown in the USA. The results demonstrated that all three ginger cultivars (CW, HY, KM) expressed varying degrees of antioxidant and anti-inflammatory activities although the differences were not statistically significant, suggesting that cultivar might not be a critical factor to consider when cultivating ginger for its health benefits. For farmers and producers targeting health-oriented markets, these findings implied that cultivar selection decisions might be more appropriately guided by agronomic performance and yield rather than by expected differences in antioxidant and anti-inflammatory output. Notably, freeze-dried ginger rhizome extracts showed a significantly high antioxidant and anti-inflammatory activities compared to fresh ginger rhizome extracts, suggesting that the drying process increased the level or availability of some bioactive compounds vital to the antioxidant and anti-inflammatory properties in ginger. The findings of this research provided scientific evidence that the antioxidant and anti-inflammatory properties of ginger were relatively consistent across different cultivars and reduced the reliance on specific cultivars for these health benefits.

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