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The Prospective Impact of Some Edible Herbal Extracts on Cancer Cell Viability, Biochemical, and Cellular Immune Mediators *In vitro*

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NUMEROUS herbal plants have been examined for their significant therapeutic potential as antioxidants and anticancer agents, as well as their ability to support a non-specific immune system against infections and other related diseases. In the present study, DPPH scavenging assay and cytotoxicity were used to investigate the correlation between the antioxidant activity of herbal plants and MCF-7 breast cancer cells, with minimal effect on normal mammalian kidney cells. Using the MTT assay, the IC_{50} values for rosemary, sage, ginger, and thyme to inhibit cell proliferation were 37.05, 93.09, 75.64, and 92.38µg/mL, respectively. In contrast, the IC_{50} values for DPPH scavenging for ascorbic acid, sage, rosemary, ginger, and thyme were 9.51, 10.03, 12.01, 3.66, and 4.956µg/mL, respectively. The experimental animals demonstrated the ability of the ethanolic extracts to ameliorate hematological changes by increasing signs of the total count of white blood cells (WBCs) and lymphocytes compared with the cyclosporine group. This result is attributed to the treatment of rats with Cyclosporine-induced significant decrease in total counts and lymphocytes. Quantification of TNF-α and IFN-γ cytokines as a mediator of the immune system revealed an increase in their expression levels in groups of co-administrated sage and rosemary extracts. The present study aimed to evaluate the immunomodulatory and cytotoxicity activity of thyme, sage, ginger, and rosemary ethanolic by inducing oral administration of cyclosporin as an immunosuppressive agent *in vivo* and cell cytotoxicity on healthy and cancerous cells *in vitro*.

Keywords: Antioxidant, Cyclosporine, Ginger, Immunomodulation, Rosemary, Sage, Thyme.

Introduction

Archaeological evidence suggests that medicinal plants and culinary herbs were the primary sources of natural substances used to treat diseases and improve the quality of life (Afzal et al., 2017). Recent evidence demonstrates that these plants have antioxidant, anticarcinogenic, immunomodulatory, glucose-lowering, and cholesterol-lowering properties (Jiang, 2019; Mohamed & Sorour, 2020; Nazir et al., 2023). Several studies and relevant literature demonstrated the immunomodulatory effects of thyme, ginger, sage, and rosemary, which were positively able to stimulate or suppress the immune system. Since the Egyptian era, thyme has been cultivated in Southern Europe as a culinary and medicinal herb known for its antimicrobial, anti-inflammatory, and immunomodulatory properties (Patil et al., 2021). Natural products from medicinal plants are known to stimulate innate immunity (non-specific immune system) and its cell counts and mediators' levels, such as neutrophils, monocytes, macrophages, and cytokines. Numerous plants and their chemical composition have previously been investigated for the treatment of immune disorders (Alhazmi et al., 2021). The dietary supplement with thyme and rosemary extracts could modulate the cellular immune mediators

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by altering interleukins, cytokine production, and T-lymphocyte regulatory effects. Thyme (*Thymus vulgaris*) and thymol inhibited the production of Interleukin 6 (IL-6), Interleukin 1 beta (IL-1β), and tumor necrosis factor-alpha (TNF-α) (Khazdair et al., 2021). According to animal and human studies, the consumption of rosemary (*Rosmarinus officinalis*) extract exhibited immunomodulatory activity by protecting against the risk of chronic diseases, particularly inflammation and autoimmunity (Ahmed & Babakir-Mina, 2020). Furthermore, ginger (*Zingiber officinale*) and gingerol exhibited a concentration-dependent cytotoxic effect by protecting against host cell death through attenuation of nitric oxide production and amplification of Interferon-gamma (IFN-γ) (Amri & Touil-Boukoffa, 2016). Enhancing good microbiomes is one of the promising approaches to treating complicated cases, as infectious disease treatment involves the modulation of the immune system to fight against microorganisms in order to eradicate pathogenic microbes. Sage (*Salvia officinalis*) or Mediterranean sage (*Salvia fruticosa*) has demonstrated the potential effect to stimulate the immune system against Covid-19 (Alhazmi et al., 2021). In contrast, medicinal plants have garnered considerable interest as a source of anticancer agents. Currently, four classes of anticancer medications are derived from plants: vinca alkaloids, taxanes, camptothecin derivatives, and epipodophyllotoxins. A vast reservoir of natural chemicals, such as chemoprotective and novel cytotoxic drugs, exists in numerous plant species. Ginger rhizome extracts exhibited potent cytotoxic activity against MCF-7 breast cancer cells and HT29 colon cancer cells (Greenwell & Rahman, 2015). Ginger rhizome extracts displayed a potent cytotoxic effect against breast cancer cells MCF-7 and colon cancer HT29 (Ali et al., 2021). Moreover, Sage extracts demonstrated the ability to inhibit cell proliferation of breast, liver, and cervical cancer cells MCF-7, HeLA cells, and HepG-2 cells (Mohammed et al., 2021). The essential oil of thyme was nontoxic to normal mammalian cells and cytotoxic to liver and lung carcinoma (A549 and HepG-2) cells (Rodrigues et al., 2015).

Materials and Methods

Plant materials and extraction

Aerial parts of thyme, sage, rosemary, and ginger rhizome were purchased from a local market in Cairo, Egypt. The dust was removed by an automated sieve. Leaves and rhizomes were homogenized with a blender. In a dark bottle, the powder was soaked in 70% ethanol in a weight-to-volume ratio of 1/3 for three days. The remaining extracts were concentrated by rotary evaporation and filtered with a 3mm filter paper Whatman no. 4 and stored at -4°C.

DPPH radical acavenging activity

A freshly prepared (0.004%w/v) methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was stored in the dark at 10°C. A methanolic solution of the test compound was prepared. An aliquot of the methanol solution (40µL) was added to 3mL DPPH solution. Absorbance measurements were immediately recorded with a UV-visible spectrophotometer (Milton Roy, Spectronic 1201). The decrease in absorbance at 515nm was continuously measured, with data being recorded at 1min intervals until the absorbance stabilized (16min). The absorbance of the DPPH radical without antioxidant (control) and the reference compound ascorbic acid were also measured. All the measures were performed in three triplicates and then averaged. The percentage inhibition (PI) of the DPPH radical was calculated according to the formula (Yen & Duh, 1994):

$$PI = [\{(AC- AT)/ AC\} \times 100]$$

where, AC= absorbance of the control at t = 0min and AT= absorbance of the sample + DPPH at t= 16min

The 50% inhibitory concentration (IC50) required for 50% DPPH radical scavenging activity was estimated based on graphic plots of the dose-response curve using GraphPad Prism software (San Diego, CA., USA).

Experimental assays

The study utilized 36 male Wistar albino rats weighing between 80 and 120g obtained from the Food Technology Research Institute's Animal House. Animals were kept in ventilated cages. The animals were divided into six groups (each containing six rats). All animals were housed in an animal house under normal room conditions. The animals were fed a commercial pellet diet and water, as well as libitum.

Treatment regimen

The first group (G1) served as a negative control and received gavage oral water administration.

The second group (G2), the positive control, was given cyclosporine (25mg/kg boy weight) via gavage for seven days, after which the rats were given water until the end of the experiment on day 30. The other groups, G3, G4, G5, and G6, were given cyclosporine at a dose of 25mg/kg boy weight per day for seven days, after which the animals in group G3 received 100mg/kg of ginger extract per day. In G 4, animals received 100mg/kg of sage extract daily. G5 animals were also administered 100 mg/kg of Thyme extract orally daily. During the duration of the experiment, daily animal behavior observations and weekly animal weight measurements were conducted (Lekhooa et al., 2017; Elsayed et al., 2018). The animal experiment was ethically approved by the animal subject committee affiliated with Food Technology Research Institute according to statements on compliance with ethical standards.

Biochemical and hematological analysis

Blood specimens

Blood collection was carried out at the end of the experimental period by sacrificing the animals under Ketamine anesthesia, "intramuscular administration of Ketamine 35mg/kg," to avoid pain distress. The blood samples were collected through retro-orbital sinus by a capillary tube for collecting a large volume of blood. A capillary tube was inserted through the retro-orbital sinus to collect a large volume of blood samples. Blood samples were collected on bottles containing anticoagulant lithium heparin or Potassium EDTA and centrifuged at 450 RCF for 10min to separate blood cells from serum. The serum was placed in sterile tubes and refrigerated at -20°C until the following day for analysis.

Hematological analysis

The blood samples were thoroughly mixed and then analyzed using a Medonic analyzer and Diagon D-cell 60 Blood analyzer for counting red blood cells (RBC), hematocrit, hemoglobin, platelets, WBC, RBC parameters, mean cell volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

Manual quantification of differential leukocytes

Whole blood specimens were collected in 0.5M sodium EDTA. Blood smear film on a microscopic glass slide was obtained by quickly and smoothly spreading one blood drop (30µL) close to the end of the slide. The blood film was then stained with May-Grünwald's eosin methylene blue and Giemsa's azure eosin methylene blue at a pH range of 6.4–7. Following the staining of differential leukocyte populations, 100 or 200 cells were manually counted using a light microscope (Abdel-Salam et al., 2021).

Quantification of cytokines in serum

A sandwich ELISA, 100µL of serum, was used to measure cytokines by using murine antibodies DuoSet ELISA kit to detect antigen-containing samples to the plate. According to the manufacturer's protocol, a capture murine monoclonal antibody of the TNF-α, INF-ɣ, was added into 96-well polyvinylchloride microtiter plates and overnight incubated at room temperature. The following day, the plates were washed three times with a washing buffer. 150µL of 2% bovine serum albumin (BSA) dissolved in PBS was added for 2h at room temperature to block any non-specific binding sites on the surface. Plates were twice rinsed with 0.1% BSA in Phosphate Buffered Saline. The wells were filled with 50µL of the antigen solution (samples or standard) and incubated for at least 2h at room temperature. Subsequently, 25µL from the detected antibody was added and incubated 2h, and then plates were rinsed three times and 50µL streptavidin conjugated to horseradish-peroxidase solution was added to each well, and the plates were incubated for 20min at room temperature. Plates were washed, and the substrate solution, ophenylenediamine was diluted in 0.03M citrate buffer (pH 5.0) containing 0.02% 30 v/v H_2O_2 was added to each well and then incubated in a dark place at room temperature for 30min. The reaction was stopped by adding 25µL/ well from 2N sulfuric acid, and the optical density (OD) of the color was immediately measured at 490nm using an ELISA reader (Spectramax-Molecular Devices®), A standard curve of 15 to 2000pg/mL^{-1} dilutions of recombinant murine was prepared. The results were expressed as picograms per milliliter.

Biochemical analysis

According to the manufacturer's instructions, liver, kidney functions, and lipid profile tests were carried out using commercially available kits from Diamond Diagnostics Company (Egypt). Blood urea nitrogen (BUN), Creatinine, Total protein (TP), albumin (AL), Alanine Transferase (ALT) and Aspartate Transferase (AST), Cholesterol, Triglycerides, HDL, LDL. Assays were also estimated according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using the GraphPad Prism 7 software (San Diego, CA. USA). The variability of results was expressed as mean + SD. The significance of differences between mean values was determined using a one-way analysis of variance (ANOVA-one-way) test, followed by the Tukey multiple comparison test.

Results and Discussion

Due to their medical applications and commercial potential as flavor enhancers, cosmetics, and pharmaceuticals, the significance of medicinal and culinary plants is currently being considered. There is a paucity of data on medicinal plants and their biological effects. These plants contain bioactive secondary metabolites such as Phenolic compounds, flavonoids, and anthocyanins, which play a vital role in the pharmaceutical sciences due to their biological effects like antioxidant and anticancer properties (Csepregi et al., 2020). Antioxidant substances found in medicinal plants may prevent the oxidation of macromolecules in the cell and neutralize free radicals such as ROS and NOS, thereby inhibiting the formation of toxic products that may cause cancer and other chronic diseases (Yashin et al., 2017). Several studies on phenolic and flavonoid content investigated the connection between antioxidant and cytotoxic activity (Hassan et al., 2014). The correlation between antioxidant activity measured by the DPPH scavenging assay and cytotoxicity against breast cancer cells, MCF-7, with minimal effect on normal kidney mammalian cells, Vero cells. IC_{50} of DPPH of medicinal plants displayed in the current study was calculated in the range from 0 to 250µg/mL compared to the standard antioxidant substance, ascorbic acid. The IC_{50} values were 9.51±0.77, 10.03, 12.01, and 3.66µg/mL for Ascorbic acid, sage, rosemary, ginger, and thyme, respectively, as demonstrated in Fig. 1. The percentage of DPPH scavenging inhibition at the higher concentration was 94.25, 94. 62, 92.91, 86.17, and 89.67% for Ascorbic acid, sage, rosemary, ginger, and thyme, respectively. Sage and rosemary extracts demonstrated potent antioxidant activity in vitro, resulting in the ability to reduce DPPH better than ginger and thyme. Our data are compatible with previous studies' findings (Mohammed et al., 2019; Embuscado, 2015).

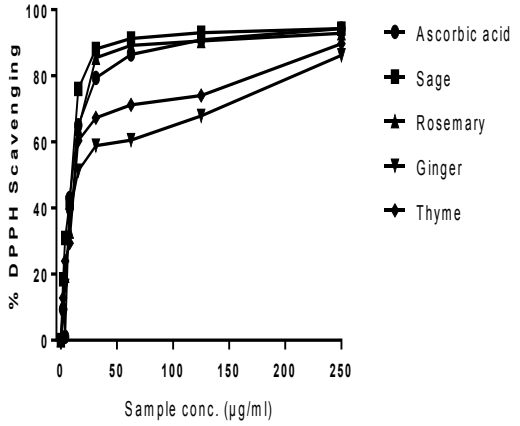

Fig. 1. The antioxidant activity of ascorbic acid, sage, rosemary, thyme and ginger methanolic extracts [All the determinations were performed in three replicates and averaged. The percentage inhibition and IC_{50} of the DPPH radical were calculated]

Prior studies demonstrated a significant correlation between free radical scavenging activity and cytotoxicity against cancer cells or antiproliferative effect in 57 medicinal and culinary plant extracts, including sage, thyme, ginger, and rosemary (Sammar et al., 2019). Our findings revealed that ethanolic extracts dependent concentration range from 1.5 to 250µg/mL. In addition, all plant extracts exhibited a potential to suppress proliferation and metabolism of breast cancer cells MCF-7 *in vitro* carried by MTT assay with IC_{50} values of inhibition of cell proliferation were 37.05, 93.09, 75.64 and 92.38µg/mL for rosemary, sage, ginger, and thyme respectively. Consequently, rosemary and ginger were more likely to kill breast cancer cells than sage and thyme at the same concentration range. Figure 2 displays data and a representative dose-response curve. This experiment aimed to determine whether plant extracts could affect cancer cell line survival compared to the healthy or normal cell line. Therefore, the toxicity of ethanolic extracts in the range of 1.5 to 250µg/mL for each one was evaluated against representative models of normal kidney cells (Vero).

Regarding the results of a dose-response curve that showed higher values of 50 % cell cytotoxic concentration (CC50 >100µg/mL), different from that observed in cancer cell MCF-7. CC_{50} values of rosemary, sage, ginger, and thyme extracts were 37.05, 91.09, 75.64, & 92.38µg/mL, respectively, whereas CC50 against Vero cells were 113.4, 157, 121.1, and 131.5µg/mL, respectively as

shown in Fig. 3. We also have tested plant extracts cytotoxicity on Vero cells as a healthy cell line from African green kidneys as a model to compare the ability of plant and herbal extracts to induce toxicity in both conditions-disease and health. Our data demonstrated that plant extracts were less toxic to the Vero cell line than MCF-7 cell line, thereby improving cytotoxicity toward cancer cells more than normal cells (Artun et al., 2017).

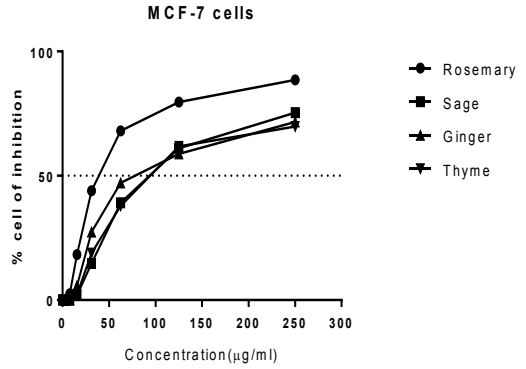

Fig. 2. Representative dose-response curves of the effect of four ethanolic extracts of rosemary, sage, ginger, and thyme on the viability of the MCF-7 cell line [Cells were treated with extracts at various concentrations for 48h. Cell viability was determined by MTT assay. IC_{50} is the concentration of compound that inhibits cell growth by 50%. Values represent ± SD of independent experiments in triplicates]

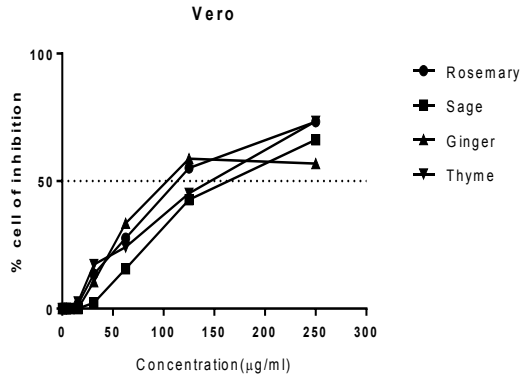

Fig. 3. Representative dose-response curves of the effect of four ethanolic extracts of rosemary, sage, ginger, and thyme on the viability of the Vero cell line [The Cytotoxic activity against Mammalian cells from African Green Monkey Kidney (Vero) cells was detected using MTT assay under these experimental conditions with 50 % cell cytotoxic concentration, CC_{50} ±SD]

Many phytochemicals consumed by humans have been associated with a low risk of cancer incidence and inflammation (Aswad et al., 2018). Plant bioactive components were extensively studied for a long time to have pharmacologic activity. Several reports proved to be safe with exposure for the long term. Some of these plant-extracted are currently available in the pharmaceutical market as antioxidants, triggering many types of cancers and thus could prevent the propagation and development of cancer cells *in vivo* (Ali et al., 2015; Aswad et al., 2018). The results of this study demonstrated that differences in cytotoxic effects among the extracts are not solely attributable to the levels of antioxidants. However, they could also be correlated with the inhibitory effects via other signaling pathways due to the abundance of certain compounds in these plant extracts. For example, rosemary extract contains high concentrations of essential polyphenols such as carnosic acid and rosmarinic acid, which have a potent anti-cancer effect against leukemia, breast, colon, stomach, and lung cancers (Moore et al., 2016). Ginger and its constituent gingerol could inhibit cell proliferation and induce the apoptosis cell death mechanism in a variety of cancer cell types in vitro, mainly through ROS radicals which mediated autosis cell death (Akimoto et al., 2015). Dried thyme extracts reduced tumor volume and weight by 53% in the 4T1 murine breast cancer model with high potency to kill MCF-7 and MDA-MB-231 human breast cancer cells (Kubatka et al., 2019). The aqueous extract of sage possessed antioxidants, cytotoxicity, and treatment of rheumatism, inflammation, and hyperglycemia (Ghorbani & Esmaeilizadeh, 2017).

In contrast, medicinal plants not only contain an abundance of antioxidant, anticancer, and antimicrobial agents but can also modulate the immune system to control and combat the undesirable activities of pathogens such as viruses, bacteria, and protozoa by enhancing the body's immune responses. Numerous medicinal plants have long been used to treat immune system disorders such as autoimmune diseases (Patil et al., 2021; Ogunrinola et al., 2022). Generally, immune stimulants are used as a safeguard from the invasion of pathogens that compromise the immune system in some illness cases like malnourished children and chronic diseases patients. In the domain of nutrition, particularly functional foods and nutraceuticals

that induce augmentation of immunity are a significant concern of dietary regimens, and an array of medicinal and culinary plants hold immunomodulatory activities like ginger, black cumin, purple coneflower, and St. John's wort as natural immune boosters (Sultan et al., 2014). Immune boosters might stimulate specific components of the immune system or promote collateral mechanisms such as antioxidants that make the immune system's functions efficient (Lekhooa et al., 2017). The main concern of this study is identifying the potential effect of rosemary, sage, ginger, and thyme ethanolic extracts on the immune system in rats that received cyclosporin drug, which suppresses immune systems. Cyclosporin is an inhibitor of Calcineurin, and it is used to treat autoimmune diseases like psoriasis, rheumatoid arthritis, and rejection of transplanted organs (Tedesco & Haragsim, 2012). The findings demonstrated that the ethanolic extracts ameliorated hematological changes by significantly increasing the total count of WBCs and lymphocytes compared with the Cyclosporine group because treating rats with cyclosporine induced a significant decrease in total counts and lymphocytes.Furthermore, cyclosporine treatment enhanced the production of total neutrophils compared with the normal feeding control group.

Conversely, significant reductions in neutrophil counts were observed in groups treated with thyme, ginger, rosemary, and sage extracts. Moreover, no significant difference in monocyte count was detected, as demonstrated in Fig. 4. Other hematological parameters include Red Blood Cell counts, hemoglobin, and others. No remarkable or significant differences are observed among groups, as shown in Table 1. These results are compatible with data obtained from previous studies. Cyclosporine induced a reduction in total counts and increased the percentage of neutrophils (Elsayed et al., 2018). Consistent with our findings, a previous study revealed that Wistar rats treated with cyclosporine and echinacea extract displayed the oral administration of echinacea extract with cyclosporine inducing a significant elevation in WBCs and reduction as compared with the cyclosporine group, as well as a positive effect on lymphocytes count (Khattab, 2019).

Immunological tests help determine the immunomodulatory properties of medicinal plants. To determine if these plant extracts could stimulate and regulate the immune system via the secretion of cytokines such as TNF-α and IFN-γ. The obtained data from the quantification of TNF-α and IFN-γ in blood samples of male Wistar rats treated with cyclosporin for 7 days and then a daily intake of plant extracts (100mg/kg) for 21 days. Tumor necrosis factor alpha (TNF-α) and Interferon-γ (IFN-γ) were augmented significantly in the group that received cyclosporin for 7 days. Conversely, groups treated with rosemary and sage demonstrated significant expression of TNF-α and IFN-γ. Stimulatory effects have shown clearly in Fig. 5. These data reinforce the results of differential leucocytes. Cytokines, including TNF-α and IFN-γ, play a crucial role in the immune response against infection by stimulating the elimination of pathogens, inflammation, cancer, and immune disorders (Jorgovanovic et al., 2020). Sage, *Salvia officinalis* has a potent antioxidant and antibacterial activity, and its extract enhances the production of Nitric oxide and TNF-α in LPS-induced inflammation in rats (Kolac et al., 2017). Further, rosemary extract induced a more significant release of TNF-α than ginger extract in macrophages. Additionally, ginger and rosemary might be associated with a reduction of inflammatory mediators (Justo et al., 2015). The immunomodulation potential of medicinal and culinary plants, including oregano, thyme, basil, and rosemary, is derived from their bioactive chemical constituents like terpenoids, alkaloids, and flavonoids, which can stimulate non-specific immune responses by elevating the expression of IL-6, IL-8, TNF-α, and INF-γ (Alhazmi et al., 2021).

To emphasize whether ethanolic extracts of these edible plants are safe to utilize To determine whether ethanolic extracts of these edible plants are safe for daily use, parameters of the liver, kidney, and lipid profiles were determined. As shown in Fig. 6 and Table 2, daily consumption of these extracts may result in mild liver damage. Despite the fact that thyme syrup exhibited antioxidant activity, co-administration of thyme syrup unexpectedly increased ALT and AST activity in the blood serum of rats (Rašković et al., 2015). On the contrary, previous studies have reported that aqueous and alcoholic extracts of thyme, rosemary, and ginger have hepatoprotective effects accompanied by decreasing activity of liver enzymes (Hegazy et al., 2018; Alshathly, 2019). Despite the widespread perception that consuming natural goods is safe, herbs may

injure several organs, especially the liver, which is responsible for their processing, leading to herb-induced liver injury (Ballotin et al., 2021). Furthermore, Rosemary and thyme supplements were shown to elevate serum ALT and AST significantly in the serum of intoxicated rats by aflatoxins (Esmail, 2018).

A lipid profile is a panel of blood tests used to find lipids abnormalities, including low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides, and total cholesterol. Our data showed that cyclosporine and ethanolic extracts could not alter lipid profile except the current observation about triglyceride levels, which significantly reduced in all treated groups except the ginger group compared with the normal feeding control. The data is shown in Table 3. Previous studies indicated that thyme, ginger, sage, and rosemary and their extract fractions possess a hypolipidemic and antioxidant potential (Hegazy et al., 2018; Ghorbani & Esmaeilizadeh, 2017).

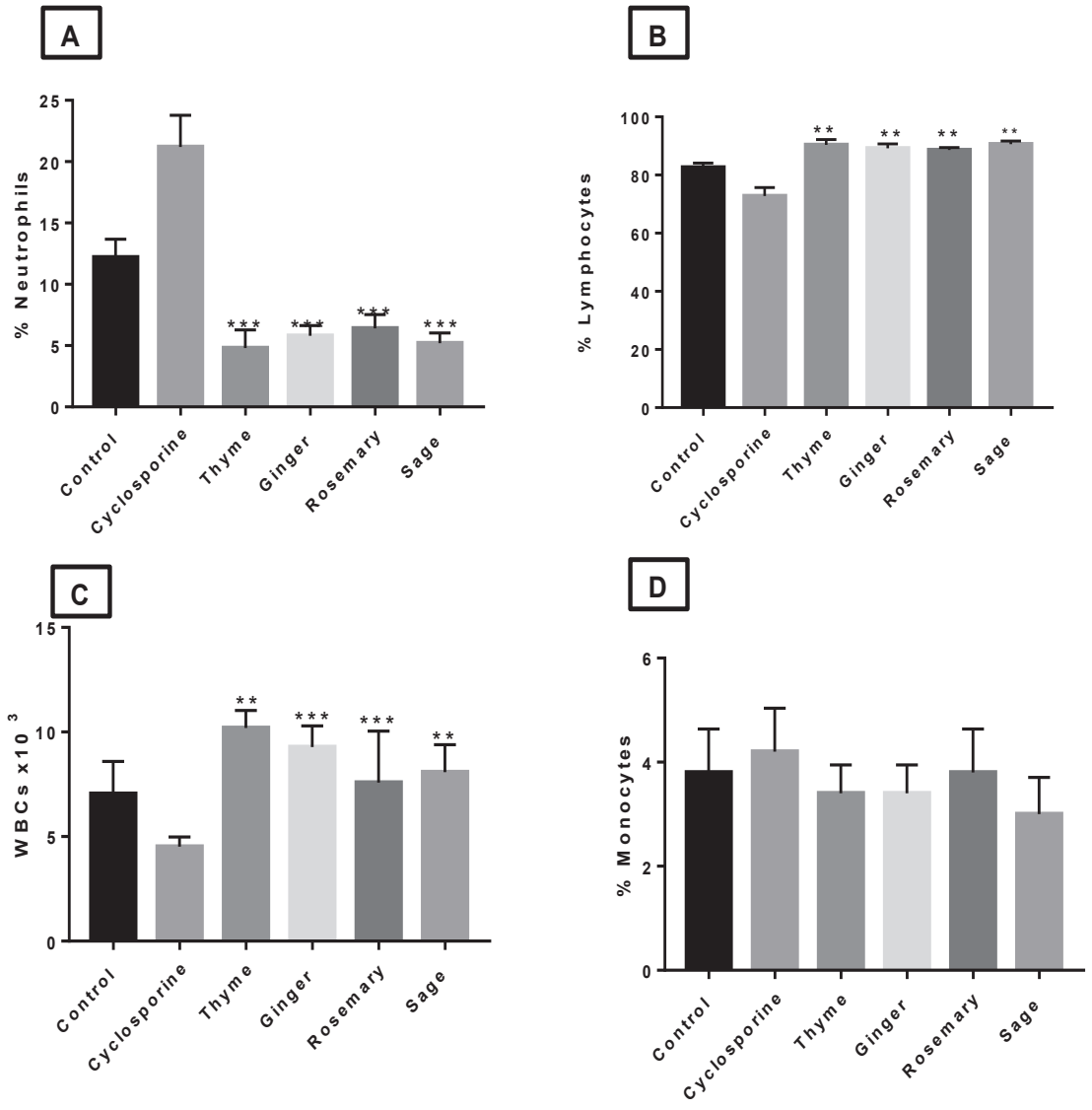

Fig. 4. Leukogram of peripheral blood counts in male Wistar rats. The control received normal feeding only. The Cyclosporin group received 25mg/kg for 7 days [Other groups received thyme, ginger, rosemary, and sage extract 100mg/kg for 21 days after receiving Cyclosporin 25mg/kg for 7 days. (A) Total leukocytes count × 103, (B) % lymphocytes, (C) % neutrophils, (D) % monocytes. Data are presented as mean ± SD. * P< 0.05 compared to the Cyclosporine group, n= 6 animals]

TABLE 1. Impact of thyme, ginger, rosemary, and sage extract (100 mg/kg) on some hematological parameters

Groups	RBCs (10^6/mm^3)	Hemoglobin (g/dl)	MCV (fl)	PCV (%)	MHC (pg)	MHCH (%)
Control group	7.85±0.1	14.8±0.2	48.5±1.8	63±2	18.2±1.1	29±0.9
Cyclosporine group	8.5±0.2	15±0.4	52.05±1.8	62.2±1.4	18.2±0.2	29.3±0.4
Thyme group	8.42±0.2	15.7±0.6	53.25±1.5	62±1.1	18.6±0.3	29.9±0.5
Ginger group	8.31±0.3	15.4±0.2	54±1.6	65±1.6	18.2±0.5	28.5±0.6
Rosemary group	8.5±0.2	16.1±0.9	57.5±5.6	63.8±3.1	18.7±0.5	29±0.6
Sage group	8.4±0.2	15.1±0.6	53.85±2.5	63.4±2.5	17.7±0.5	28.9±9.3

RBC: erythrocytes, Hb: hemoglobin, MCV: mean cell volume, packed cell volume (PCV), MCH: mean corpuscular hemoglobin and MCHC: mean corpuscular hemoglobin concentration. The results were expressed as mean ± SD. P< 0.05 compared to the control group, n= 6 animals. Non-significant results were detected among groups.

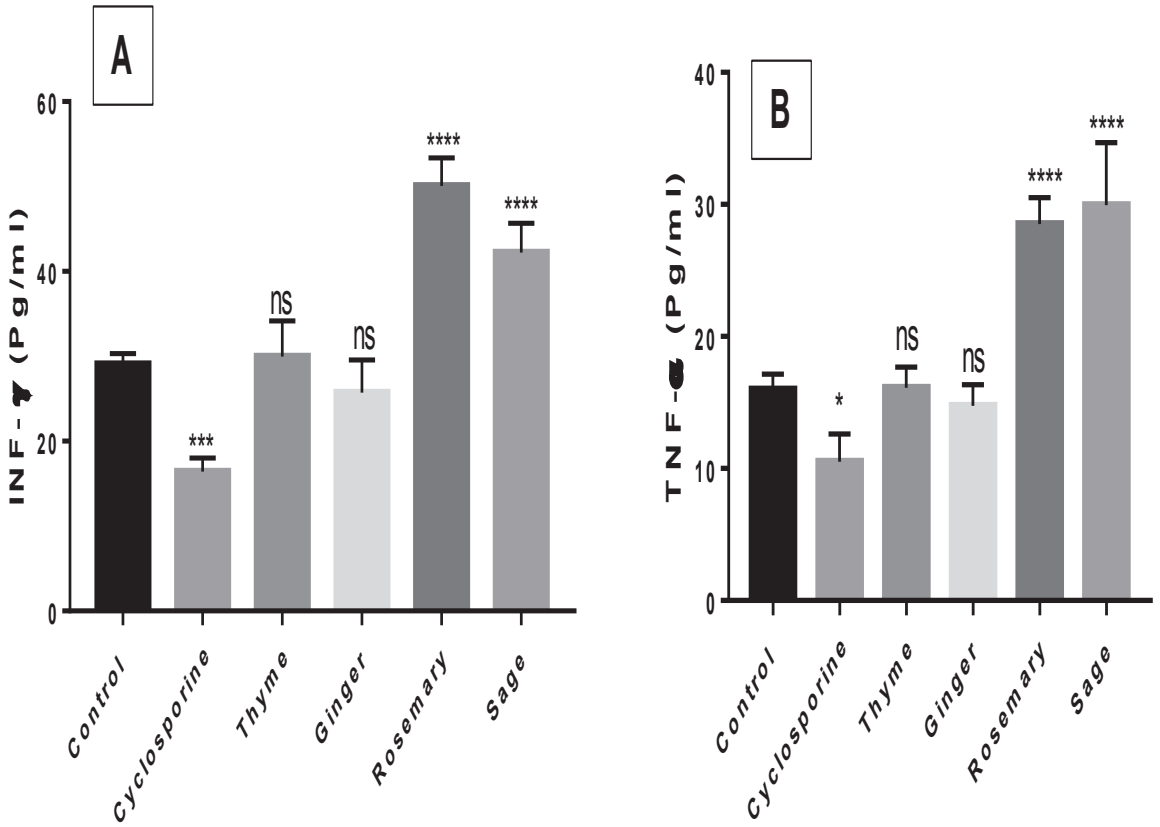

Fig. 5. IFN-γ and TNF-α levels in the serum of male wistar rats after treating with cyclosporin and thyme, ginger, rosemary and sage ethanolic extracts. Individual values of mice (pg/mL) are presented with an average of each group [Statistical variation among groups was carried out in 5 animals of each group in duplicate using One-way ANOVA, P< 0.05. ns: not significant]

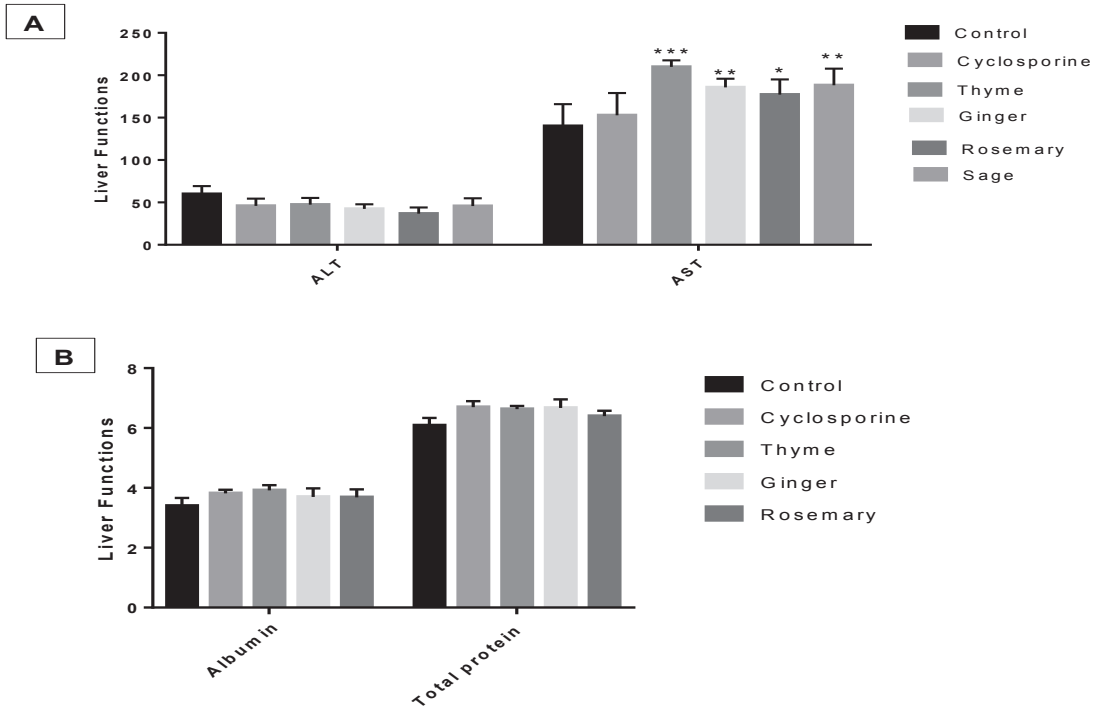

Fig. 6. Comparison of liver function tests in different studied groups versus normal control and Cyclosporine positive control [(A) ALT and AST enzyme activity (U/L). (B) Albumin and Total protein (g/dl). Data are presented as mean ± SD. * P< 0.05 compared to the Cyclosporine group, n= 6 animals]

TABLE 2. Effect of cyclosporin and ethanolic extracts of thyme, ginger, rosemary, and sage on serum levels of liver and kidney functions

	Control group	Cyclosporine group	Thyme group	Ginger group	Rosemary group	Sage group
Blood urea nitrogen (mg/dl)	26±2.1	22±0.7	22±1.3	22±1.3	21±0.8	22±0.4
Creatinine (mg/dl)	0.3±0.0	0.3±02	0.3±11	0.3±0	0.3±0	0.3±0
Albumin (g/dl)	3.4±0.2	3.7±0.1	3.8±0.1	3.8±0.2	3.9±0.3	3.6±0.1
Total protein (g/dl)	6±0.2	6.6±0.2	6.7±0.1	6.5±0.1	6.8±0.2	6.3±0.2
AST (U/L)	140±25.8	156±26.2	208±7.8	189±10.1	172±17.9	196±19.5
ALT (U/L)	62±9.4	43±8.4	46±7.8	44±5.4	35±7.3	48±9

The results were expressed as mean ± SD. P< 0.05 compared to the control and Cyclosporine groups , n= 6 animals.

TABLE 3. lipid profile distribution among studied male wistar rat groups

	Control group	Cyclosporine group	Thyme group	Ginger group	Rosemary group	Sage group
Total cholesterol (mg/dl)	78±8.9	72±9.6	96±10.5	88±6.9	76±8.4	76±5.7
Triglycerides(mg/dl)	115±25.9	50±6.6**	82±13.8*	99±25	52±20.3**	85±28.8*
HDL(mg/dl)	45±5.7	50±6.6	49±4.3	52±4.7	48±3.0	47±1.9
LDL(mg/dl)	15±6.1	18±8.6	31±9.8	17±7.5	23±9.2	12±1.9
Risk ratio	1.76±0.0	1.67±0	1.91±0.1	1.71±0.1	1.58±0.1	1.6±0.0

The results were expressed as mean ± SD. P< 0.05 compared to the control and Cyclosporine groups , n = 6 animals.

Conclusion

The present study revealed that ethanolic extracts of thyme, rosemary, ginger, and sage might ameliorate the immune system in immunosuppressed rats induced by the co-administration of cyclosporine followed by plant extracts. The data exhibited immunomodulatory effect through increasing counts of lymphocytes and TNF-α and IFN-γ expression levels without a notable impact on hematological and biochemical parameters, as well as the potent antioxidant activity and potency to kill breast cancer cells with minimal effect on normal cells.

Competing interests The authors report no conflicts of interest regarding this work.

Authors' contributions: The authors made contributions to all aspects of this review

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التأثير المحتمل لتأثير بعض مستخلصات الأعشاب الصالحة للأكل على حيوية الخلايا السرطانية وبعض الصفات الكيميائية الحيوية ووسطاء المناعة الخلوية في المختبر

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تم تقييم عدد من النباتات العشبية لمعرفة إمكاناتها الهامة كمضادات للأكسدة وعوامل مضادة للسرطان، فضلاً عن قدرتها على دعم الجهاز المناعي ضد الالتهابات والأمراض الأخرى ذات الصلة. في هذه الدراسة، تم استخدام تقدير DPPH والسمية الخلوية لتقدير العلاقة بين نشاط مضادات الأكسدة للنباتات العشبية وخلايا سرطان الثدي MCF-7، مع تأثير ضئيل على خلايا الكلى الطبيعية في الثدييات. باستخدام اختبار MTT، كانت قيم K50 لإكليل الجبل والمريمية والزنجبيل والزعتر لمنع تكاثر الخلايا هي 37.05، 93.09، 75.64، و92.38 ميكروجرام/مل، على التوالي. في المقابل، كانت قيم K50 لكسح DPPH لحمض الأسكوربيك والمريمية وإكليل الجبل والزنجبيل والزعتر 9.51، 10.03، 12.01، 3.66، و4.956 ميكروجرام/مل، على التوالي. أظهرت حيوانات التجارب قدرة المستخلصات الإيثانولية على تحسين التغير في اختبارات الدم عن طريق زيادة العدد الإجمالي لخلايا الدم البيضاء والخلايا الليمفاوية مقارنة مع مجموعة السيكلوسبورين. كشف القياس الكمي للسيتوكينات TNF-α وIFN-γ كوسيط للجهاز المناعي عن زيادة في مستويات التعبير عنها في بعض مستخلصات المريمية وإكليل الجبل المستخدمة. تهدف الدراسة الحالية إلى تقييم النشاط المناعي والسمية الخلوية للزعتر والمريمية والزنجبيل وإكليل الجبل الإيثانولي عن طريق تحفيز تناول السيكلوسبورين عن طريق الفم كعامل مثبط للمناعة في الجسم الحي والسمية الخلوية على الخلايا السليمة والسرطانية في المختبر.