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Investigation of the Bioactive Components of Ginger rhizome (*Zingiber officinale*) and Its Anti-Inflammatory Activity as Assessed by Solvent Fractions

Rabab J.H. Al Hasseny[1], Abbas K. Al-Mansoori[2], Safa Fadhil Rasool[3], Toqa M.A. Mhayyal[4]

[1]Medical Microbiology, Department of Health Food and Nutrition, College of Food Science, Al-Qasim Green University, Iraq.
[2]Department of Genetic Engineering, College of Biotechnology, Al-Qasim Green University, Iraq.
[3,4]AL-Qasim Green University, Faculty of Biotechnology, Department of Medical Biotechnology, Iraq.

Abstract

A popular spice known as ginger, or *Zingiber officinale*, has chemicals like shogaols and gingerols that have anti-inflammatory effects. These compounds lessen inflammation, discomfort, and swelling by blocking certain pathways like NF-κB and reducing inflammatory mediators like prostaglandins and leukotrienes. More large-scale trials are needed to confirm ginger's potential in controlling inflammatory disorders, but it shows promise in gastrointestinal difficulties and arthritis, and it has less adverse effects than some NSAIDs. With precise weighing, one gram of sample was mixed with one hundred milliliters of solvent. In the absence of a different instruction, it was centrifuged at 4000 rpm for 20 minutes after being shaken for 3 hours at room temperature. Whatman No. 1 filter paper was then used for the filtration process. New extracts were utilized in every trial. Alpha-Phellandrene, α-pinene, α-Terpineol, Gingerol, beta-Bisabolene, β-Elemene, 6-Shogaol, and Zingherone were the bioactive components of Zingiber officinale. The effects of Zingiber officinale ethyl acetate extract on the serum enzymes SGPT, SGOT, and ALP were studied in laboratory rats using in vitro experimental testing. The results for Zingiber officinale ethyl acetate extract were 93.00±4.40, 70.95±3.86, and 25.00±1.92, respectively. In contrast, the results for Di-(2- ethylhexyl) phthalate were 149.07±6.28, 114.00±4.37, and 35.06±2.08, respectively. The results for the Control group, which consisted of 0.5 ml/kg of corn oil, were 71.00±3.95, 62.04±2.09, and 16.05±1.08.

Keywords: Anti-inflammatory, *Zingiber officinale*, Bioactive components.

Corresponding Author: Rabab J.H. Al Hasseny, Medical Microbiology, Department of Health Food and Nutrition, College of Food Science, Al-Qasim Green University, Iraq.

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Introduction

Zingiber officinale, a member of the Zingiberaceae family, is a spice that has a long history of culinary and beverage use around the globe. For a long time, ginger has been a traditional remedy in Southeast Asia for a wide variety of gastrointestinal issues, coughs, fevers, sore throats, and more. The volatile and non-volatile chemicals found in ginger are responsible for its biological activity. Essential oils, such as sesquiterpenes and monoterpenoids, are ginger's volatile components and give the spice its signature scent. Gingerol, shogaol, zingerone, and paradol are examples of the non-volatile components that contribute to ginger's spicy, pungent flavor. Gingerol is the primary component of fresh ginger; however, in ginger-based goods, it is transformed into shogaol, zingerone, and paradol[1]. When it comes to immunology and general bodily function, the immune system is crucial. Thus, a wide range of diseases can be brought about by any disruption to the immune system. As the body's initial line of defense against a broad variety of environmental threats, the immune system is an intricate network mainly composed of white blood cells and other immune components like antibodies[2,3], proteins, and cytokines. It is easier to develop an appropriate immune response because of the interaction of these distinct immunological components. While causing inflammation, cell or tissue damage, cell death, and wound healing, the immune system finds and kills infections in order to prevent numerous illnesses. Failure to maintain homeostasis can lead to infectious diseases, metabolic disorders, inflammatory diseases, immune-mediated diseases, and metabolic disorders. There is a significant death rate due to degenerative diseases like diabetes mellitus and hypertension. The severity of infectious diseases is amplified by degenerative disorders as well[4,5,6]. The immune system can be compromised by oxidative stress caused by environmental free radicals, which persists even during a pandemic. Cleaning up one's living conditions, limiting one's social circle, and strengthening one's immune system are, thus, the simplest and most direct means of reducing the spread of infectious diseases. When it comes to warding off infectious and chronic diseases, as well as recovering from them, the immune system plays a pivotal role. A healthy diet rich in vitamins, minerals, and bioactive substances can help the body fight against illness[7]. The immune system can benefit from the bioactive components found in herbal plants. Herbal plants not only have medicinal uses, but they may also possess antioxidant properties that help the body deal with free radicals, oxidative stress, and inflammation. Substances that aid in the regulation of the immune system are known as immunomodulators. Endogenous and exogenous immunomodulators are also accessible; the antioxidant qualities of the former are typically the basis of their mode of action. Alkaloids, flavonoids, flavones, flavonols, isoflavones, quinones, and terpenoids are some of the natural plant antioxidants that have immunomodulatory characteristics. By controlling the immune response and the balance between oxidants and antioxidants, immunomodulators made from antioxidants aid in health maintenance[8,9]. The elimination of reactive oxygen-nitrogen species and an increase in the body's antioxidant equilibrium are two of the main ways in which antioxidants function. Researchers have looked at the bioactive components of several plants because of their possible use as immunomodulators. Many investigations have looked into the biological effects of ginger, its active ingredients, and how they work. Researchers have shown that ginger crude extract contains biological activities including antioxidant, anti-inflammatory, antibacterial, antiviral, and anticancer effects in both in vitro and in vivo studies. A number of recent articles highlighted ginger's many uses, including its hepatoprotective and antiallergic properties, among its other bioactivities[10].

Materials and Methods

Gathering vegetation and extracting a specimen

Using distilled water for washing, the fresh ginger root (*Zingiber officinale*) was dried in an

oven set at 40°C. After grinding, it was placed in an airtight container and kept in the fridge. With precise weighing, one gram of sample was mixed with one hundred milliliters of solvent. In the absence of a different instruction, it was centrifuged at 4000 rpm for 20 minutes after being shaken for 3 hours at room temperature. Whatman No. 1 filter paper was then used for the filtration process. New extracts were utilized in every trial.

Analysis Mass spectrometry and gas chromatography.

The American company Agilent Technologies manufactured the gas chromatograph/mass selective detector (an acronym) that was utilized to conduct the experiment. The dimensions of the HP-5MS-constructed column were 30 m by 0.25 mm, with a layer thickness of 0.25 mm. The injector was fed half a milliliter of 99.999 percent pure helium gas at a rate of one milliliter per minute. The temperature within the evaporator detector was 300 degrees Celsius. Both the injection temperature and the ion-source were kept at 250 o C and 280 Co, respectively. After 5 minutes at 50 C, the oven was increased to 220 C at 4 C/min, and subsequently to 300 C at 10 C/min. After 10 minutes of isothermal heating at 300 Co, the run was completed. Parts cataloguing It took around 50 minutes and considered over 62,000 patterns to identify components by interpreting GC-MS mass spectra against the NIST database. As part of our regular process, we checked the final product's component concentrations. For this experiment, we used just the components that, by pure chance, had a probability higher than 90%. Three times each, they were computed independently.

Experiments Using Live Animals

Healthy male albino rats ranging in weight from 200 to 250g were utilized as volunteers in the research. The animals were housed in polypropylene cages and given regular laboratory food while being kept in a controlled environment with a temperature of 23±2°C. Before the experiments, the rats were given at least eight hours to get used to the lab environment. Albino rats ranging in weight from 200 to 250 grams (or around 5 ounces) were used in the experiments. The animals originally originated from the animal breeding facility, where they originated. Their cages were designed to provide adequate ventilation, and they were maintained in a controlled environment with consistent temperature and humidity levels (23±3 degrees Celsius, 55-70% relative humidity, and 12 hours of darkness and light cycle). They were also provided with standard rat food. There were a total of twelve rats, and they were divided into three groups of four. Animals in group I were administered maize oil to serve as a vehicle control and a normal control. As a positive control, 100 mg/kg of Di-(2- ethylhexyl) phthalate was given in all trials involving Apium graveolens methanol fraction. The first group received 100 mg/kg of Di-(2-ethylhexyl) phthalate, while the second and third groups received 0.50 and 0.75 mL/kg of ginger rhizome fractions, respectively.

Drugs and Animals

Research employed albino rats ranging in weight from 200 to 250 grams, with a 5 gram standard deviation. A facility that specializes in animal breeding provided the animals. The rats were kept in cages with good ventilation and kept in a typical environment with a temperature range of 23±3°C, relative humidity of 55-70%, and a 12-hour light/dark cycle. They were also given regular rat food. Twelve rats were distributed among three groups of four. The first set of animals served as the control group and got the vehicle control (corn oil). All studies that included the ginger rhizome (*Zingiber officinale*) methanol fraction used 100 mg/kg of di-(2-ethylhexyl) phthalate as the positive control. Two groups used fractions of ginger rhizome at doses of 0.50 mL/kg and 0.75 mL/kg, respectively, while Group 1 included 100 mg/kg of di-(2-ethylhexyl) phthalate.

Evaluation Using Statistical Methods

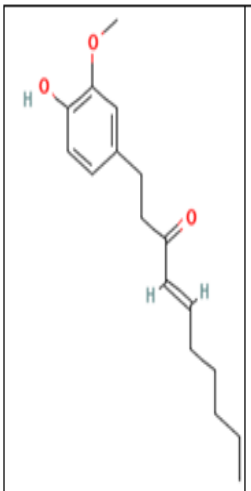
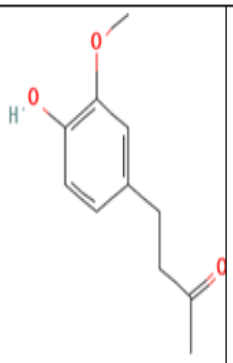
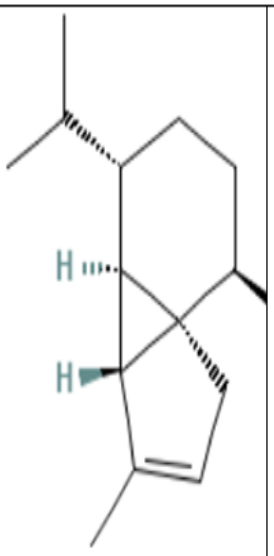
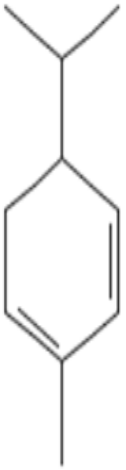
We used the GraphPad Prism 5 Statistical Package, developed by GraphPad Software in the USA, to conduct our statistical study. Bonferroni correction and one-way analysis of variance (ANOVA) were applied to the data. We presented

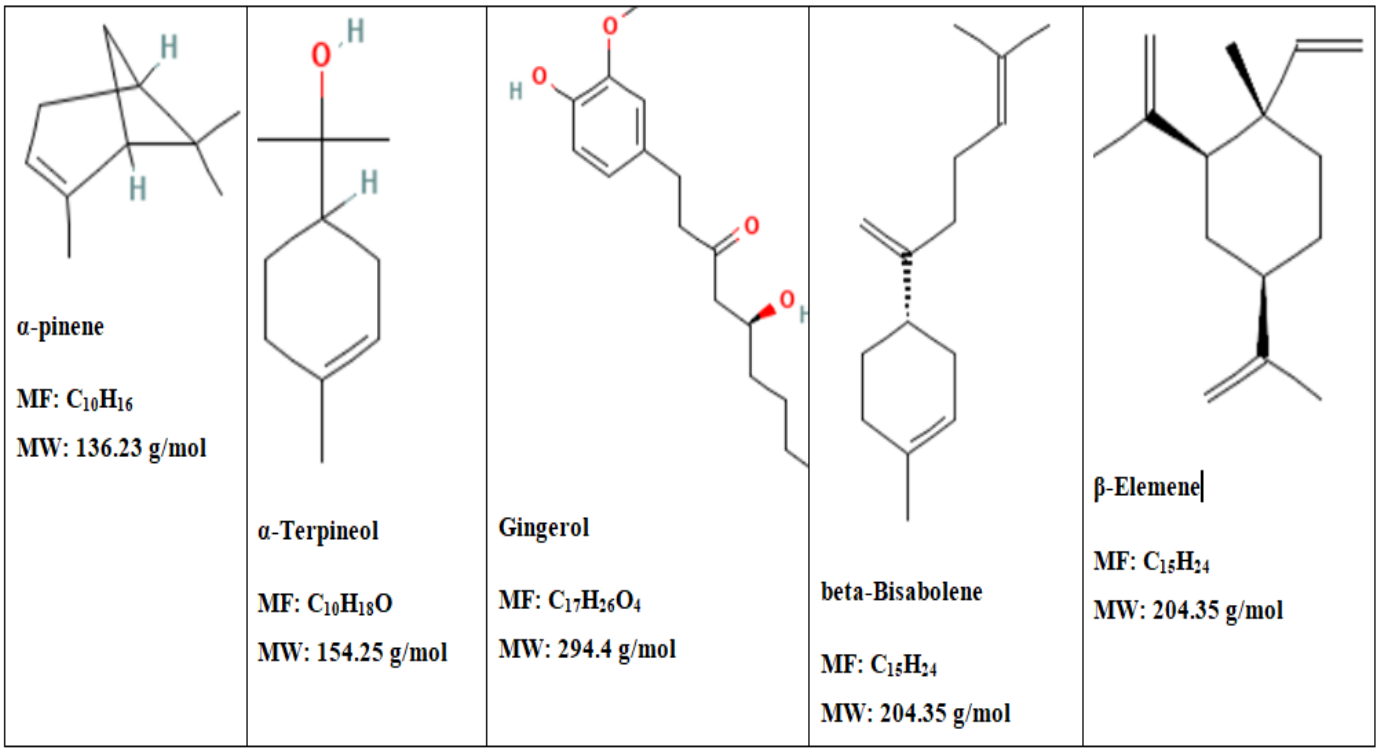
the results of the in vitro IC50 as the mean plus or minus the standard error of the mean for the three separate tests. The quantification of phytochemicals was shown as the mean ± standard deviation, while the free radical scavenging capabilities were expressed as a percentage. $P<0.05$ was used to determine statistical significance.

Results and Discussion

Nutrition, alternative medicine, and the utilization of plants for therapeutic purposes continue to see annual increases in interest. *Zingiber officinale* Roscoe is a very significant plant because of its many culinary and medicinal uses. Reports of ginger's usage in traditional medicine date back to at least 3500 BC. Worldwide, ginger is grown as a spice. It is a member of the Zingiberaceae family. Worldwide, ginger output is estimated at 100,000 tons per annum, making it one of the most important and popular spices in the world. The flavorful properties of ginger rhizome make it a popular spice, flavoring agent[11,12,13], and ingredient in cooking all around the globe. Pills, syrups, and teas that contain it are dietary supplements. The broad range of biological activities validated by several in vitro models and clinical experiments has led to the widespread use of ginger rhizome or preparations in medicine. Bioactive components of *Zingiber officinale* were 6-Shogaol, Zingherone, beta-Cedrene, α-Cubebene, Alpha-Phellandrene, α-pinene, α-Terpineol, Gingerol, beta-Bisabolene and β-Elemene. The plant's potent antiemetic properties have led to its widespread usage in the treatment of nausea caused by motion sickness, morning sickness, and chemotherapy. In addition, the plant's pronounced anti-inflammatory characteristics have led to its use in pharmaco-therapeutic techniques for the treatment of osteoarthritis, thanks to its analgesic and painkilling impacts.

Table 1: Ginger rhizome (*Zingiber officinale*) phytochemical study of bioactive components.

 6-Shogaol, MF: $C_{17}H_{24}O_3$ MW: 276.4 g/mol	 Zingherone MF: $C_{11}H_{14}O_3$ MW: 194.23 g/mol	 beta-Cedrene MF: $C_{15}H_{24}$ MW: 204.35 g/mol	 α-Cubebene MF: $C_{15}H_{24}$ MW: 204.35 g/mol	 Alpha-Phellandrene MF: $C_{10}H_{16}$ MW: 136.23 g/mol



The main flavor component of ginger, gingerone, is responsible for the sweet flavor that ginger takes on when cooked. Cooking or drying ginger produces gingerols, which create an inverse aldol reaction to gingerols without a particular amount, while fresh ginger does not contain zingerone. Zingerone inhibited enzymes involved in the generation of reactive oxygen species (RONS), demonstrating its great efficiency as a free radical scavenger. Research conducted in living organisms has demonstrated that 100mg/kg dosages of zingerone can reduce IL-1β levels and inhibit NF-KB activity in mice. Furthermore, the in vitro investigations on MLE12 cell cultures using 50mg/kg doses of zingerone have shown that it can enhance the activity of SOD, GSH, CAT, and IFN-γ while decreasing the levels of MDA, IL-1β, IL-4, IL-5, IL-13, TNF-, NF-ΓB p65 expression, and p-IκB. By inhibiting NF-œB and activating the Nrf2/HO-1 signaling pathway through AMPK, Zingerone likewise aids in the treatment of asthma[14,15]. One of the components of ginger oleoresin is gingerol, an unstable molecule with a pale-yellow hue. Gingerol, a phenolic chemical, is responsible for the spicy flavor of fresh ginger. Gingerol breaks down quickly when exposed to heat. Rumor has it that the chemical 6-gingerol can reduce inflammation and acts as an antioxidant. Research on adults with solid tumors has demonstrated that gingerols, particularly 6-gingerol[16,17,18,19], can raise levels of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and reducing levels of malondialdehyde (MDA) and nitric oxide (NOx). Compared to the placebo group, patients undergoing chemotherapy who take ginger extract (20 mg/day) as a daily supplement have higher antioxidant levels. Patients may see an improvement in their oxidative stress and antioxidant activity after taking ginger supplements. In vivo investigations on rats have shown that the 6-gingerol-rich fraction (50 mg/kg) can decrease H2O2 and MDA levels, while simultaneously increasing antioxidant enzyme activity and GSH levels. It has been observed that the fraction rich in 6-gingerol can lower MDA levels and protect mice from catalase activity and GSH depletion when administered a dose of 100 mg/kg. Research on mouse macrophages in vitro confirmed that 6-gingerol might suppress the generation of NO and PGE2. One of ginger's components, shogaol, gets released into the food chain when drying or cooking[20]. The dehydration reaction of gingerol can produce shogaol, which is responsible for ginger's loss of pungency when cooked. 6-shogaol, at doses of 20 uM, can reduce inflammation caused by UVB, enhance iNOS, COX-2, and TNF-α, and boost antioxidant

enzymes in HaCaT cell culture. Additionally, it can act as a healing agent in cells going through oxidation, thereby preventing free radical-induced aging. Additionally, 6-shogaol (100 uM) has the ability to suppress the NF-κB and PI3k/Akt signaling pathways in human intestinal epithelial cells[21]. It is also known that 6-dehydroshogaol, in doses of 2.5μM, 5μM, and 10μM, can decrease the production of NO and PGE2 in mouse macrophage cells. Laboratory rats were subjected to in vitro experimental testing to determine the impact of *Zingiber officinale* extract oral administration of extract on the serum enzymes SGPT, SGOT, and ALP recorded (93.00±4.40, 70.95±3.86 and 25.00±1.92) respectively for Zingiber officinale ethyl acetate extract while (149.07±6.28, 114.00±4.37 and 35.06±2.08) were recorded respectively for using Di-(2- ethylhexyl) phthalate and (71.00±3.95, 62.04±2.09 and 16.05±1.08) were recorded to Control (vehicle) (0.5 ml/kg Corn oil) (Figure 1, 2, 3).

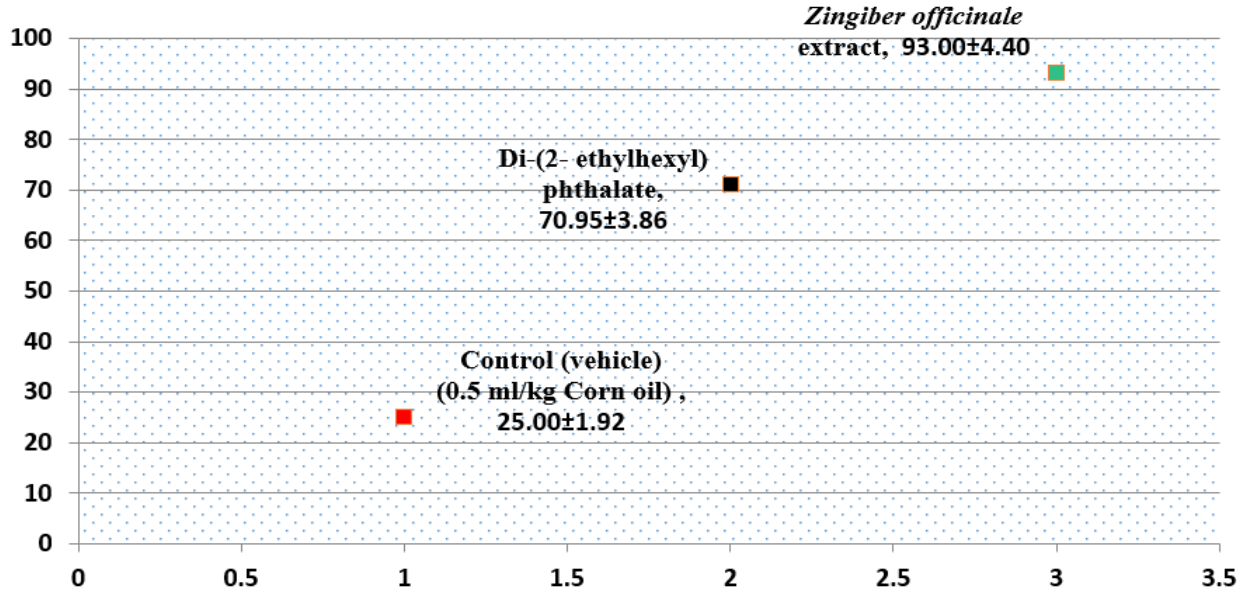


Figure 1. Effect of oral administration of sample extract compare with control (vehicle) on the blood enzyme SGPT.

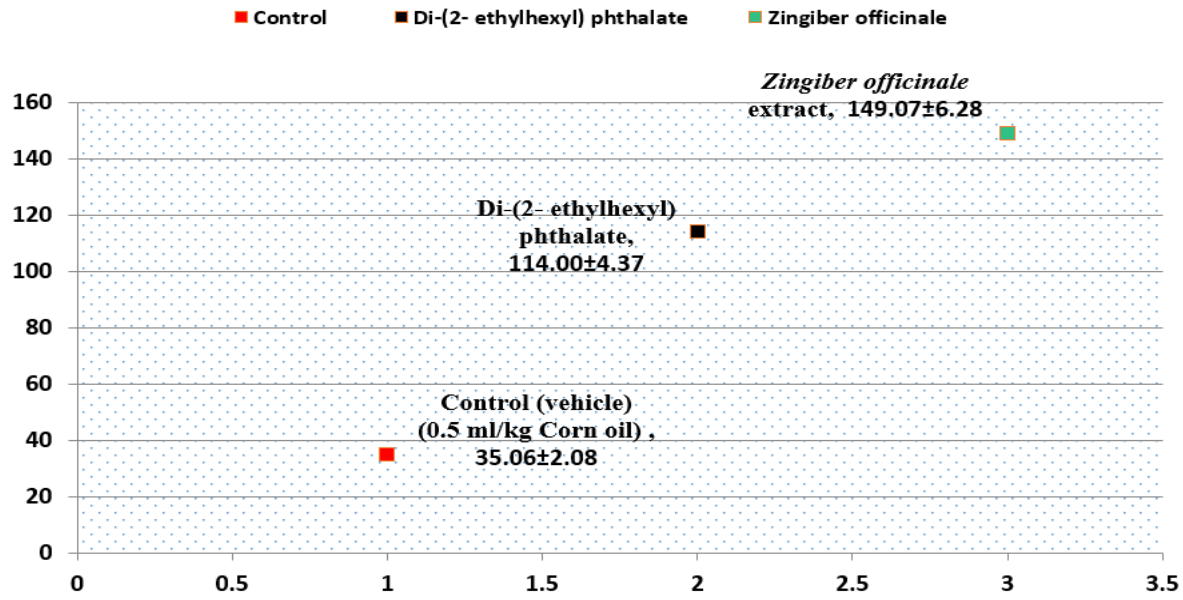


Figure 2. Effect of oral administration of sample extract compare with control (vehicle) on the blood enzyme SGOT.

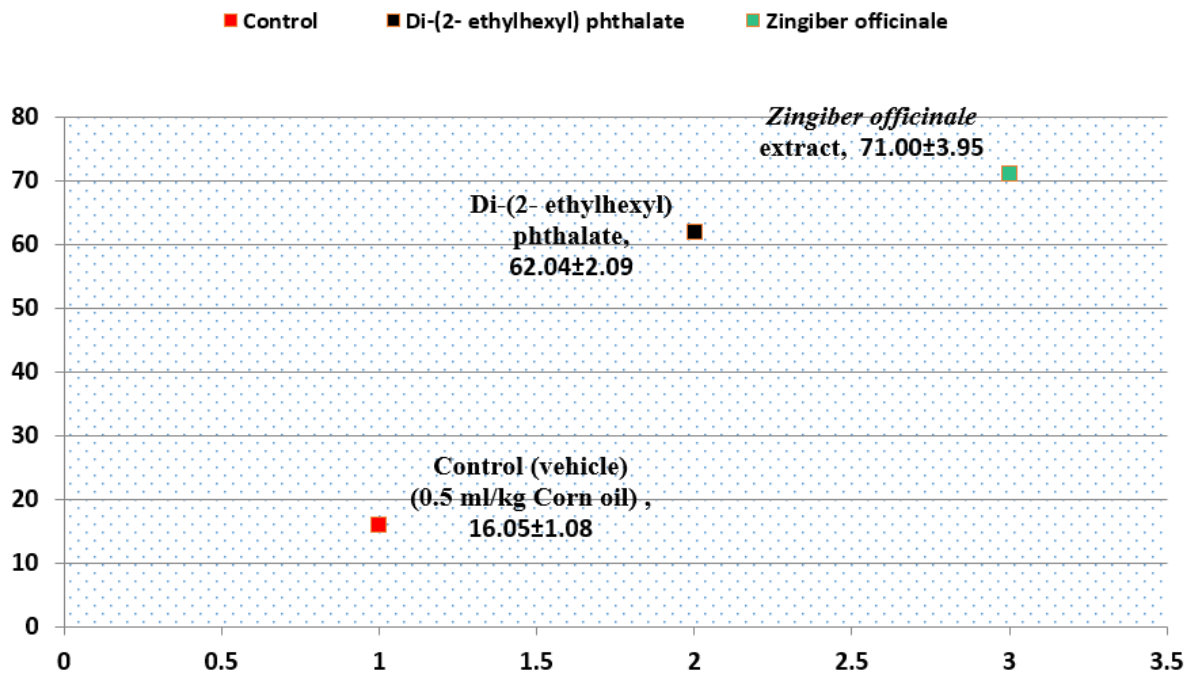


Figure 3. Effect of oral administration of sample extract compare with control (vehicle) on the blood enzyme ALP.

The body's reaction to an invader or disruption is incomplete without inflammation. Immune cells and cells surrounding the infection site release mediators that control the inflammatory response. Diseases associated with inflammation can be indicated by mediators such as cytokines, proteins, and inflammatory enzymes. Different cytokines contribute to inflammation in different ways and come from different places. A cytokine storm occurs when the body's cytokine production is out of control, which can happen in cases of severe infectious disease. Cell and tissue damage, organ failure, and death can result from an overabundance of immune cells at the site of infection. Furthermore, oxidative stress is bidirectional and has strong ties to inflammation. While oxidative stress results from inflammation, RONS production is a two-way street that starts with inflammation. Inflammation, oxidative stress, and cell tissue damage can all be prevented or at least mitigated with the help of antioxidants. Some proinflammatory cytokines, such interleukin-6 (IL-6) and tumor necrosis factor (TNF-α), can have their production reduced by antioxidants. White blood cells play an important role in immunity and illness prevention, and antioxidants make them even more effective. Superoxide dismutase (SOD) converts O2− into H2O2, a byproduct of respiration and metabolism. The Fenton/Harber Weiss Reaction can transform it into OH−. These processes are all part of the body's natural RONS production system. Oxidative stress may be caused by a combination of exogenous RONS (such as pollution, smoke, metals, medicines, etc.) and endogenous RONS. Inflammation can be induced by illnesses and infections through the increase of proinflammatory cytokines[22]. When the body is exposed to harmful substances, the immune system reacts by identifying and eliminating them. The capacity to ward off dangerous invaders is dictated by the two branches of the immune system, the innate and the adaptive. The body's natural defences against infections are known as innate immunity. These defences include epithelium protection, antimicrobial proteins and peptides, humoral components, and cells. In order to eliminate the ingested pathogen, leukocytes established ROS. When foreign antigens are detected, the adaptive immune system is activated by the innate immune system. Regulatory T cells (T-regs), T lymphocytes, and B lymphocytes carrying antigens make up the adaptive immune responses. When it comes to cytokines, T-

lymphocytes are king[23]. These cells are able to identify harmful invaders because they have antigen receptors on the surface of their cells.

Conclusion

Gingerol, shogaol, zingerone, and paradol are some of the bioactive components found in ginger. These compounds are recognized for their antioxidant and anti-inflammatory properties. Additionally, research has demonstrated that ginger oleoresin, ginger juice, ginger tea, and ginger extract may have immunomodulatory effects via anti-inflammatory and antioxidant mechanisms. The anti-inflammatory pathway is enhanced by ginger's bioactive components, which also reduce pro-inflammatory responses and raise levels of anti-inflammatory cytokines. Eliminating reactive oxygen species (ROS), decreasing oxidative stress parameters, enhancing antioxidant enzymes, and boosting antioxidant capacity are all ways in which ginger's bioactive ingredients might increase oxidative stress tolerance. As a herbal product, ginger could be commercialized at any time. There is a recent uptick in the number of people selling herbal remedies through internet marketplaces. A high-quality product assortment that follows trends, flows well, and sells at the correct moment will also contribute to a thriving herbal industry. Beyond the ability to mix or make things, the entrepreneurial attitude and thinking should extend to everything. In Iraq, ginger-based herbal products have found their way into processed foods like candies and traditional cakes, in addition to traditional medicinal uses and herbal beverages. When designing a product with ginger, it's important to think about things like production process, packaging, component stability, and herbal product shelf life, as well as to have a thorough and thorough knowledge in these areas. Thus, the items could be eaten, and the processed food containing herbal ingredients still has the ginger's benefits. There is a lot of room for innovation in the functional food product market thanks to ginger's anti-inflammatory and oxidative stress-fighting properties, which might be enhanced through research and development. The toxicity of ginger and the recommended daily intake for humans also requires additional research.

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