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Effect of Ethanol Extract on *Zingiber Officinale* on Serum Protein of Male Wistar Albino Rats Induced with Inflammation

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Abstract: Serum protein profiles, such as total protein, albumin, and globulin fractions, are frequently altered by inflammation and can be used as indicators for systemic immune response and hepatic function. Ginger, or *Zingiber officinale*, has long been used to treat a variety of inflammatory disorders due to its well-known anti-inflammatory and antioxidant qualities. This study examined the effects of *Zingiber officinale* extracts on serum proteins in male albino wistar rats that had been inflamed. Five (5) groups of rats were created: blank control, negative control, standard control, low-dose extract, and high-dose extract. With the exception of the blank control group, all groups were induced with inflammation. Serum protein activities including total protein, albumin, alpha-globulin, beta-globulin globulin and total globulin were evaluated following the 21-day experiment. With the exception of total protein, which had lower levels, the generated inflammation markedly raised serum protein activities, indicating toxicity. With the exception of total protein, where it boosted activity, *Zingiber officinale* ethanol extract dramatically lowered serum protein levels to normal levels, indicating protection. The albumin levels were shown to be reduced in a dose-dependent manner. When the high dose (6.44 ± 0.000) was compared to the blank control (5.51 ± 0.001), the negative control (17.57 ± 0.00) showed the greatest reduction ($p<0.05$), followed by the standard control (14.69 ± 0.011). These results suggested that *Zingiber officinale* ethanol extracts could be used as a medicinal treatment since they could reduce inflammation-induced toxicity.

Keywords: Zingiber officinale, Total protein, Albumin, Alpha-globulin, Beta-globulin globulin and Total globulin.

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Introduction

The body's natural defense mechanism, inflammation, is brought on by damaging stimuli such as infections or tissue injury and results in a localized reaction in living tissues (Kumar *et al*., 2019). Vasodilation, increased vascular permeability, leukocyte infiltration, and the release of inflammatory mediators such histamine, prostaglandins, cytokines, and chemokines are all characteristics of the inflammatory response. These reactions are intended to remove the initial source of damage, remove necrotic tissue, and start the healing process. Usually, there are two phases to inflammatory reactions: acute and chronic, each with unique characteristics. Acute inflammation is typically protective, but if it persists, it can result in chronic inflammation, which can damage tissue and is associated with a number of illnesses, including cardiovascular disease, arthritis, and liver dysfunction (Medzhitov, 2008). The change in serum protein profiles, particularly with regard to albumin and globulin fractions, which are important markers of the acute-phase response, is one of the major systemic impacts of inflammation. Plasma protein production is changed during inflammation, particularly for serum proteins like albumin and globulins, which are essential for the acute-phase response.

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are frequently used in traditional medicine to treat pain and inflammation. Long-term usage, however, may result in adverse effects, increasing the need for safer, natural substitutes and raising interest in medicinal plants with established pharmacological qualities.

Zingiber officinale, also known as ginger, is a flowering plant belonging to the Zingiberaceae family (Mashhadi *et al*., 2013). Because of its medicinal qualities in treating a variety of illnesses, it is extensively used in traditional medicine (Singh *et al*., 2018). A plant with a long history of traditional use that is prized for its many therapeutic advantages, such as lowering inflammation, preventing oxidative stress, and shielding the liver.

Gingerols, shogaols, and paradols are the bioactive substances found in ginger. These substances have been shown to fight oxidative stress and prevent the synthesis of pro-inflammatory cytokines. According to studies, ginger's bioactive compounds can reduce the production of prostaglandins by inhibiting cyclooxygenase enzymes (COX-1 and COX-2), suppressing the activation of nuclear factor-kappa B (NF-κB), and inhibiting pro-inflammatory cytokines.

Animal models are crucial for assessing the anti-inflammatory qualities of plant extracts in biomedical research. The egg albumin-induced paw edema paradigm in rats is a well-established technique for researching acute inflammation. Egg albumin is injected into the rat's paw in this model to cause inflammation, which results in localized edema and an increase in inflammatory mediators. Histamine and serotonin are released

during this reaction, which causes edema development that usually peaks two seconds after injection. An active inflammatory paw process is commonly indicated by histological investigations that show leukocyte infiltration in the inflamed paw tissues (Barung *et al*., 2021).

By effectively isolating these active chemicals, ethanol extraction increases the therapeutic value of the plant. The ability to extract a wide range of bioactive chemicals, including both polar and non-polar elements, makes the ethanol extract of ginger stand out among the many solvent extracts of ginger. According to Barung *et al*. (2021), ginger's ethanol extract may lessen inflammation by preventing the synthesis of chemicals that cause pain and swelling. Serum proteins are crucial indicators of inflammation, especially albumin and globulin. Globulin levels may increase, but albumin levels frequently decrease. Monitoring these alterations aids in determining the effects of inflammation and the efficacy of therapies.

Inflammation, while essential for tissue repair and immune defense, becomes problematic when persistent or improperly current anti-inflammatory drugs, although effective, are known for their serious side effects, especially when used over extended period. These include gastrointestinal ulcers, renal dysfunction and immunosuppression. Additionally, there is a growing incidence of resistance to conventional drugs and a corresponding increase in public interest in alternative medicine. Despite the popularity of herbal remedies, many of them lack rigorous scientific evaluation and standardization. Ginger (*Zingiber officinale*) has shown promising anti-inflammatory potential in various *in-vitro and in-vivo* studies, yet there is limited information on its impact on serum protein dynamics during inflammation.

The increasing concerns over the side effects associated with orthodox anti-inflammatory medications have spurred interest in natural, effective and safer alternatives. *Zingiber officinale*, renowned for its anti-inflammatory properties, stands out as a promising candidate. Research indicates that ginger and its active constituents, such as gingerols and shogaols, possess significant anti-inflammatory and antioxidant effects, making it a viable option for managing inflammation-related conditions. Exploring its impact on serum protein profiles could enhance scientific insight into its systemic actions during inflammatory conditions and support the development of plant-derived therapeutic agents. This study may contribute to advancing herbal remedies for managing inflammation related disease.

Aim of study

The study's main objective was to assess how *Zingiber officinale* ethanol extract affected the serum protein levels of male wistar albino rats that were inflamed.

Specific Objectives of the Study

The specific objectives of the study were to:

- Determine the effect of ethanol extract of *Zingiber officinale* on serum total protein of male wister albino rats.
- Determine the effect of ethanol extract of *Zingiber officinale* on albumin of male wister albino rats.
- Determine the effect of Ethanol Extract of *Zingiber officinale* on α-globulin of male Wister Albino Rats.
- Determine the effect of Ethanol Extract of *Zingiber officinale* on β- globulin of male wister albino rats.
- Determine the effect of ethanol extract of *Zingiber officinale* on total globulin.

Materials and Methods

Plant collection and Identification

Fresh rhizomes of *Zingiber officinale* were procured from Nkpokiti market, independent layout, Enugu. The plant was validated by Prof. C. S. Eze of the Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Enugu, Nigeria.

Preparation of Plant Extracts

The 76.4g of ginger rhizomes were cleaned, cut into slices, and dried at 105 degrees Celsius in an analytical oven. A mechanical grinder was used to grind the dry slices into a fine powder. 300 milliliters of pure ethanol were used as the extraction solvent after the powdered material was put in a Soxhlet apparatus. Five hours were spent on the Soxhlet extraction procedure. Whatman No. 1 filter paper was used to filter the mixture. To get semi-solid crude ethanol extract, the filtrate was concentrated using a rotary evaporator under decreased pressure at 40–45 degrees Celsius and then dried in a water bath (Harbone, 1998).

Animal Collection

The Department of Physiology at the University Nigeria Enugu Campus provided thirty (30) male wistar albino rats weighing between 150 and 200 grams. The rats were divided into five groups, with six albino rats in each group. After being labeled, the group was put in wire-gauzed cages. Before the experiment started, they were acclimated for three weeks in the Animal House of Power Tech Analytical and Scientific Research Laboratory.

Experimental design

After being sampled, the animals were divided into five groups, each of which had six rats. A full randomized experimental design was used for the study. Group A: (Blank control) comprised five rats that were given food and water but were not induced nor treated with the extract. Group B: (Negative control) The rats in this group were not given any medication, but they were stimulated with concentrated egg albumin. Group C: (Standard control) The rats in this group were given a standard inflammatory medication after being stimulated with egg albumin+. Group D: (Low-dose ginger extract) The rats in this group were given 50 mg/kg body weight of Zingiber officinale extract after being stimulated with egg albumin. Group E: (High-dose ginger extract) The rats in this group were given 200 mg/kg body weight of Zingiber officinale extract after being stimulated with egg albumin. After egg albumin induction, treatment was given via intubation once a day for three weeks in a row.

Collection of serum sample

Chloroform was used to put the rats to sleep at the conclusion of the therapy. Capillary tubes were used to penetrate the eyes in order to gather the data. Plain bottles were filled with the blood. After allowing the blood to coagulate at room temperature, the serum was separated from the clot by centrifuging it for ten minutes at 3000 rpm. Using a micropipette, the serum was

carefully collected and placed in tubes for later examination. Until it was time for examination, the sample was kept in a refrigerator.

Biochemical analysis

Serum total protein

The Biuret procedure is a colorimetric procedure for quantitating total protein concentrations based on the formation of a violet compound from the reaction of peptide bonds in proteins with copper (II) ions in an alkaline environment. The intensity of this color which can be spectrophotometrically measured at approximately 540 nm is directly related to the number of peptide bonds and thereby the protein concentration of the sample. The maximum quantity of protein is determined by measuring the absorbance of the test sample and comparing the value to an equal volume of a standard protein solution such as bovine serum albumin (Gornall *et al.*, 1949).

Serum Albumin

The Bromocresol Green (BCG) method is a dye-binding assay commonly used to determine the concentration of albumin in biological samples such as serum or plasma. In this method, the pH of the sample is adjusted to an acidic range, typically around pH 4.2, at which albumin carries a positive charge. Bromocresol Green, an anionic dye, binds specifically to albumin under these conditions to form a green-colored complex. The intensity of this color, measured spectrophotometrically at approximately 628 nm, is directly proportional to the albumin concentration in the sample. A calibration curve prepared using standard albumin solutions allows for the quantification of unknown samples (Doumas *et al.*, 1971).

Globulin fraction

Serum globulin levels were obtained indirectly by subtracting the measured albumin concentration from the total protein values, which were determined using the Biuret method and the Bromocresol green (BCG) dye-binding technique (Gornall *et al*., 1949; Doumas *et al.*, 1971). Further classification of the globulin fraction into alpha, beta, and gama globulins was achieved through serum protein electrophoresis, a method that separates proteins according to their electrical charge and molecular size (Putnam, 1975). However, the total globulin fraction can be estimated by subtracting the albumin level from the total protein level in a blood sample.

Statistical analysis

The Statistical Package of Social Science (SPSS) for Windows (version 16) was used for all statistical analyses. ±SEM was used to express the measured parameter values. The effects of Zingiber officinale ethanal extract on the parameters under investigation were assessed using one-way analysis of variance (1-way ANOVA), and Duncan's multiple range tests were employed to differentiate the differences between means. The significance test was conducted at the 0.05 probability level.

Results

When compared to the controls (19.32±0.001, 16.57±0.000), the experimental groups (standard dosage, low-dose, and high-dose) had significantly different mean total protein levels ($p<0.05$) (18.61±0.011, 18.51±0.004, and 18.44±0.00) (Table 1). There was a significant difference ($p<0.05$) between the negative control (16.57±0.000) and blank control (19.32±0.001) after 21 days of produced inflammation and treatment with ethanol extract of *Zinigber officinale*, indicating that the induced inflammation decreased the rat's total protein. There was a substantial increase when treatment groups (18.61±0.011, 18.51±0.004, and 18.44±0.00) were compared to the negative control (16.57±0.000). This suggests that *Zinigber officinale* was able to lessen the inflammatory effects. When compared to the controls (17.57±0.00, 5.51±0.001), the albumin levels of the rats that were inflamed revealed a significant difference ($p<0.05$) between the experimental groups (standard dosage, low-dose, and high-dose) (14.61±0.011, 13.51±0.004, 6.44±0.000) (Table 1). Following 21 days of produced inflammation, there was a significant difference ($p<0.05$) between the blank control (5.51±0.001) and negative control (17.57±0.00), indicating that the inflammation raised the rat's albumin levels. There was a notable increase when treatment groups (14.61±0.011, 13.51±0.00, 6.44±0.000) were compared to the negative control (17.57±0.00). This suggests that *Zinigber officinale*, particularly at high doses, was able to lessen the symptoms of inflammation. This suggests that inflammation raised albumin levels, and *Zingiber officinale* dose-dependently reduced them. When compared to the controls (1.45±0.001, 6.57±0.000), the experimental groups (standard dosage, low-dose, and high-dose) had significantly different levels of alpha-globulin ($p<0.05$) (3.61±0.011, 4.41±0.004, and 2.44±0.000) (Table 1).

There was a significant difference ($p<0.05$) between the negative control (17.57±0.00) and blank control (1.45±0.001) after 21 days of generated inflammation, suggesting that the rat's alpha-globulin levels were raised by the inflammation. Alpha-globulin levels significantly decreased when treatment groups (3.61±0.011, 4.41±0.004, 2.44±0.000) were compared to the negative control (6.57±0.000). This suggests that *Zinigber officinale*, particularly at high doses, was able to lessen the symptoms of inflammation. This suggests that inflammation raised alpha-globulin levels, and *Zinigber officinale* dose-dependently decreased them. When comparing the experimental groups (standard dosage, low-dose) (3.21±0.011, 3.51±0.004, 1.44±0.000) to the blank control (1.51±0.001, 3.57±0.000), the beta-globulin levels of the inflammation-induced rats exhibited a significant difference ($p<0.05$) (Table 1). Following 21 days of produced inflammation, the negative control (3.57±0.000) and blank control (1.51±0.001) showed a significant difference ($p<0.05$), suggesting that the inflammation raised the rat's beta-globulin levels. There was no significant difference ($p>0.05$) between the treatment groups (standard dosage, low-dose) (3.21±0.011, 3.51±0.004) and the negative control (3.57±0.000). Nevertheless, there was no significant difference ($p>0.05$) between the high-dose (1.44±0.000a) group and the blank control. This suggests that *Zingiber officinale*, particularly at high doses, was able to lessen the symptoms of inflammation. This suggests that inflammation raised beta-globulin levels, and *Zingiber officinale* dose-dependently decreased them. When comparing the experimental groups to the blank control, the total-globulin levels of the inflammatory-induced rats revealed a significant difference ($p<0.05$) (Table 1). Following 21 days of produced inflammation, there was a significant difference ($p<0.05$) between the blank control (1.51±0.001) and negative control (3.57±0.000), suggesting that the inflammation raised the rat's total-globulin levels. There was a significant difference ($p<0.05$) between the treatment groups and the negative control. Nevertheless, there was no significant difference ($p>0.05$) between the high-dose group and the blank

control. This suggests that *Zingiber officinale*, particularly at high doses, was able to lessen the symptoms of inflammation. This suggests that inflammation raised total-globulin levels, and *Zingiber officinale* dose-dependently decreased them.

Table 1: Effect of Zingiber officinale ethanol extract on the key protein markers of male wistar albino rats induced with inflammation

Groups	Total Protein	Albumin	Alpha-Globulin	Beta Globulin	Total Globulin
A	19.32±0.001[a]	5.51±0.001[a]	1.45 ± 0.001[a]	1.51 ± 0.001[a]	2.97 ± 0.001[a]
B	16.57±0.000[b]	17.57±0.00[b]	6.57 ± 0.000[b]	3.57 ± 0.000[b]	10.14 ± 0.00[b]
C	18.61±0.011[c]	14.61±0.011[c]	3.61 ± 0.011[c]	3.21 ± 0.011[b]	6.3 ± 0.011[c]
D	18.51±0.004[c]	13.51±0.004[c]	4.41 ± 0.004[c]	3.51 ± 0.004[b]	6.3 ± 0.011[c]
E	18.44±0.000[c]	6.44±0.000[a]	2.44 ± 0.000[c]	1.44 ± 0.000[a]	3.72 ± 0.000[a]

The values are expressed as (mean ± SEM). *Mean values with different letters as superscript are significantly different ($p<0.05$)*

Discussion

According to the current study, inflammation significantly reduced the content of total protein. On the other hand, there was a dose-dependent rise in total protein following the addition of *Zingiber officinale* ethanol extract. This result is in line with earlier research by Iwalokun *et al*. (2011), who investigated *Zingiber officinale*'s anti-inflammatory and immunomodulatory properties in wistar rats. In a similar vein, the study supported the findings of Ajibade *et al*. (2016), who reported an increase in total protein levels following *Zingiber officinale* treatment. Additionally, it backs up Javid *et al*. (2017), who claimed that *Zingber officinale* improved blood profiles.

The current investigation demonstrated that induced inflammation resulted in a notable rise in albumin content. Additionally, there was a dose-dependent drop in albumin following the introduction of *Zinigber officinale* ethanol extract. This finding was consistent with earlier studies conducted by Javid *et al*. (2017). When rats subjected to oxidative stress were given ethanolic ginger extract, their albumin levels significantly returned to normal when compared to controls. Additionally, it corroborated the findings of Javid *et al*. (2017), who reported an increase in albumin levels following *Zingiber officinale* treatment.

The current investigation shown that following generated inflammation, the concentration of alpha-globulin significantly increased. Additionally, there was a dose-dependent rise in alpha-globulin following the ethanol extract of *Zingiber officinale*. The results of Oluwole *et al*. (2013), who examined the effects of *Zingiber officinale* (ginger) on blood parameters in lead-exposed Wistar rats and found notable improvements in total protein, albumin, and globulin levels, are in line with the current study. Additionally, it corroborated the findings of Almasi *et al*. (2013), who reported that *Zingiber officinale* ethanol extract improved blood profiles.

The current investigation demonstrated that induced inflammation resulted in a notable rise in beta-globulin content. Additionally, there was a dose-dependent drop in beta-globulin following the administration of *Zingiber officinale* extract. This study is in line with earlier research by Oluwole *et al*. (2013), who examined how *Zingiber officinale*, or ginger, affected blood parameters in lead-exposed Wistar rats and found that globulin levels significantly improved. It is also consistent with the findings of Almasi *et al.* (2013), who reported that *Zingiber officinale* ethanol extract normalized blood profiles.

According to the study, induced inflammation led to a notable rise in the concentration of total globulin count. After *Zinigber officinale* ethanol extract was added, there was a dose-dependent decrease in total globulin levels. This study differs from that of Bardi *et al*. (2013), who reported notable increases in his cirrhotic control group following the administration of *Zinigber officinale* ethanolic extract. He recorded a decrease in increased total globulin levels that brought them closer to normal. Additionally, it concurred with Almasi *et al*. (2013), who reported that *Zingiber officinale* ethanol extract normalized blood profiles.

Conclusion

The study's findings demonstrated that *Zinigber officinale* has defensive qualities that can offset the altered protein profile brought on by inflammation.

Recommendations

Based on the findings of this study, it is recommended that *Zinigber officinale* be further investigated for its potential in pharmaceutical applications. Its natural, cost-effective properties make it a promising candidate for the development of drugs aimed at treating inflammation related conditions.

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