

Membrane Potential and Immune Stability effect on albino rats administered with *Spinacia oleracea* leaf extract

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Abstract

This study was carried out to determine the effect of membrane potential (MP) and immune stability (CD4 + T cell and CD8 + T cell counts) of albino rats fed with *Spinacia oleracea* leaf extract. Materials and Methods: Fourteen albino rats were randomly divided into two groups with seven rats in each group (n=7); Control (C) which is group 1 received normal feed only. Group 2 was administered with *Spinacia oleracea* leaf extract 150 mg/kg, this lasted for 28 days. The level of Membrane potential was determined by Nernst equation, while the levels of CD4 + T cells (µl/count) and CD8 + T cells (µl/count) were determined using the flow cytometric method. The levels of Membrane potential, CD4+ T cells, and CD8+ T cells were significantly increase in albino rats fed with 150 mg/kg leaf extract of *Spinacia oleracea* compared to the control group (P < 0.05). Conclusion: The increased Membrane potential, CD4 +, and CD8 + T cell counts in albino rats fed with *Spinacia oleracea* leaf extract could probably imply stability of Membrane potential and immune parameters in albino rats

Keywords: membrane potential, immune stability, effect, albino rats, *spinacia oleracea* leaf extract

Introduction

Spinach, or *Spinacia oleracea*, is a leafy, green plant that comes in a wide variety. Nutrient-dense and widely consumed as food. Magnesium, nitrates, lutein, zeaxanthin, beta-carotene, folic acid, vitamin C, vitamin E, and vitamin K are all abundant in spinach [1,2]. Like quinoa and beets, it is a member of the amaranth family. Efo Iyana-Ipaja is the Yoruba term for spinach, while Ugu Oyibo is the Igbo term. Nigerian cuisine frequently uses spinach, a lush green vegetable [3].

Furthermore, because it is rich in antioxidants and nutrients, it is regarded as being quite healthful. A seed stalk emerges from the rosette-shaped arrangement of the edible leaves [4]

The simple leaves might be flat or puckered, and they are oval or somewhat triangular. Small, dry fruits are produced by the unobtrusive blooms. For optimal leaf area and rapid growth, spinach needs deep, rich, well-limed soil and cool temperatures [5]. Consuming spinach may help diabetics better control their blood sugar, reduce their risk of developing cancer, and strengthen their bones. A nutrient-dense, leafy green vegetable, spinach may help maintain healthy bones, skin, and hair. This vegetable also contains vitamins and minerals that provide a variety of health benefits [6]. Iron, potassium, magnesium, and vitamins C and E are all abundant in spinach. It can improve the digestive system, boost the immune system, and possibly even have anticancer effects when included in a healthy diet [7]. The extract of *Spinacia oleracea* includes a number of components that may combine to improve other food items, in addition to a significant quantity of a specific

mineral that aids in the catalysis of specific enzymes, the preservation of homeostasis, and immunological function. It is essential to investigate the biochemical effects of these plant compounds in order to ascertain their anticipated effects on their subjects [8]. Hence, it is necessary to look into how *Spinacia oleracea* extracts affect the immunological stability (CD4 + T cell, CD8 + T cell counts, and the CD4/CD8 ratio) and membrane potential (MP) of male albino rats. Recent studies have explored the health benefits of spinach (*Spinacia oleracea*), particularly its impact on immune function and related parameters [9]. While direct evidence linking spinach consumption to CD4+ T-cell counts is limited, spinach's rich nutrient profile suggests potential supportive effects on the immune system. *Spinacia oleracea* is frequently used as a spice in nutrition. It is necessary to look into their effects in this study because of their extensive use in traditional medicine and nutrition [10]. Therefore, the present study was conducted in order to determine effects of *Spinacia oleracea* extract on immunological stability (CD4 + T cell and CD8 + T cell counts) and membrane potential (MP) of male albino rats

Material and Methods:

Plant material and extraction

The *Spinacia oleracea* leaves were bought from Ekenuwa market. It was identified and confirmed in the Department of Plant Biology and Biotechnology of Imo State University Owerri. They were washed, sundried

and ground into powder for use. The dried *Spinacia oleracea leaves* were milled to get a coarse powder used for the extraction. The powder was macerated in a 400 g percolator with 250 mL of distilled water. The mixture was allowed to stand for 48 hours after it was filtered. The filtrate was then placed in an oven to evaporate and the solid residue was referred to as extract. The appropriate concentrations of the extract were made in distilled water for the experiment. Hence, the 150 mg concentrations was prepared

Experimental design

The albino rats weighing (180-300 g) obtained from the Animal House of Imo State University were used in this investigation. The animals were kept in cages in a room and maintained at room temperature with a 12-hours light dark cycle for one week to acclimatize. The animals were randomly assigned to two experimental groups with seven rats in each group.

Fourteen albino rats were randomly divided into two groups (n=7); Control (C) received normal feed only. Group 2 was administered with *Spinacia oleracea leaf* 100 mg/kg BW. In all groups, the extract was administered through oral route. This treatment was performed by oral compulsion. All animal were allowed free access to food and water throughout the experiment. This lasted for 28 days. The blood samples were collected and

the level of immunological stability (CD4 + T cell, and CD8 + T cell counts) and membrane potential (MP) were then measured

Blood collection

The animals were anaesthetized with chloroform vapour, quickly brought out of the jar and sacrificed. Whole blood was collected by cardiac puncture from each animal into clean dry test tubes. The blood in the clean dry test tubes was allowed to stand for about 15minutes to clot and further spun in a Westerfuge centrifuge (Model 1384) at 10000 g for 5 minutes, serum was separated from the clot

Biochemical Assay

The MP was determined by calculation using the Nernst Equation. The CD4 + and CD8 + T cells were determined within 2 h of collection using the flow cytometric method.

Statistical analysis

The results were expressed as mean+ standard deviation. The statistical evaluation of data was performed by using student T- test

Results:

Parameters	Group 2	Group 1 Control	P value
Membrane Potential(J)	132.02±9.64	211.92±18.9	0.000*
CD4+ T cells (µl/count)	304 ± 65	692± 121.	0. 000*
CD8 +T cells (µl/count)	339± 39	612 ±71	0.000*

Table 1: The level of membrane potential and immune status in albino rats fed with *Spinacia oleracea* leaf and control.

Discussion

Antioxidants such as vitamins C and E, carotenoids (such as lutein and beta-carotene), and phenolic compounds, which fight oxidative stress by scavenging free radicals in the body and may lower the risk of chronic diseases like cancer and heart disease, are the main way that spinach leaves provide health benefits. Additionally, spinach contains vital nutrients like folate, iron, and fiber, which support general health and wellness [11]. In this study, rats fed *Spinacia oleracea* had a considerably higher level of MP than the control group. This is most likely related to *Spinacia oleracea*'s higher nutritional value [12]. Reduced free radicals may be connected to this rise in MP. Numerous health benefits of this vegetable have been demonstrated [13]. Spinach may help prevent cancer and heart disease, reduce oxidative stress, and enhance eye health. Alpha-lipoic acid (ALA) is an antioxidant found in spinach. According to research on animals and in the lab, ALA may improve insulin sensitivity, decrease blood sugar, and stop oxidative stress-related alterations. The membrane potential may be more affected by this [14]. The antioxidative, antiproliferative, and anti-inflammatory qualities of spinach leaves, which contain flavonoids among other active ingredients, improve the stability of membrane potentials in biological systems [15]. Additionally, the possible immunomodulatory benefits of spinach (*Spinacia oleracea*), particularly its influence on the CD4+ T-cell count, a crucial indicator of immunological function, have been investigated [16].

In this study, albino rats given 150 mg/kg body weight had considerably higher levels of CD4 + and CD8 + T cells than the control group. This is consistent with previous reports. The substantial rise in CD8 + T cells in group 2 rats suggests that there may be a chance for improved survival. Spinach can be included in a healthy, immune-boosting diet, but it is not a direct treatment for raising the CD4 level [17,18,19]. The nutrient-dense leafy green vegetable spinach (*Spinacia oleracea*) is well-known for its positive immune-boosting properties. It includes vital vitamins, minerals, and bioactive substances that have a variety of effects on immunological parameters. The immunological stability qualities of spinach are responsible for the many health advantages that come with eating it [20]. Spinach offers several vital nutrients, which together promote immunological health, and can be included in a balanced diet. Although spinach by itself might not directly raise CD4+ T-cell numbers, its nutritional makeup supports immune

system upkeep and function. Numerous biological processes are impacted by spinach, such as detoxification, immunological regulation, gut microbiota balance, anti-inflammatory actions, and antioxidant protection [21,22]. Together, these systems strengthen the immune system, guard against long-term illnesses, and improve cellular performance [203,24]. This shows how important spinach is for immunological and membrane stability.

Conclusion

Albino rats given spinach leaf extract showed markedly higher levels of Membrane Potential, CD4 +, and CD8 + T cell counts. This may indicate that the membrane potential is stable and that the immune system is strengthened.

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