

Original Article



Effects of Methanolic Extract of *Aloe vera* on C-Reactive Protein and White Blood Cell Count of Wistar Rats With Inflammation

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Abstract

Background: It has been known for centuries that plants and plant-derived chemicals are useful in treating diseases, with *Aloe vera* being one of the biologically active plants. A significant role is played by the C-reactive protein (CRP) in many inflammatory conditions, while leukocytes play an integral role in the response of the immune system to infectious diseases.

Objectives: This study explored the potential anti-inflammatory effects of *Aloe vera* methanolic extract on the total white blood cell (TWBC) count and CRP levels in Wistar rats received formalin.

Methods: A total of 25 Wistar rats were divided into five groups (A to E), with group A serving as the control and the other groups receiving formalin to induce inflammation, followed by increasing doses of *Aloe vera* extract. The CRP level was measured using an enzyme-linked immunosorbent assay (ELISA) kit, and the TWBC count was determined using a Sysmex haematology auto-analyzer.

Results: Research findings showed that 200 mg of *Aloe vera* methanolic extract effectively reduced the CRP level (0.056 ± 0.0027) ($P=0.04$), while 600 mg of the extract significantly increased the TWBC count (13.920 ± 4.757) ($P=0.03$).

Conclusion: These findings suggest that *Aloe vera* extract may be an effective natural supplement for improving the immune system function and reducing inflammation in the body. However, further research is needed to fully understand the underlying mechanisms for these effects and to determine the optimal doses and potential side effects.

Keywords: *Aloe vera*, C-reactive protein, Leukocytes, Immune system, Inflammation, ELISA



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Background

Plant extracts represent an ongoing attempt to discover novel anti-pathogen compounds. Approximately 20% of all plants on the planet have undergone biological or pharmacological testing, and a significant percentage of novel antibiotics introduced to the market are derived from natural or semi-synthetic resources (1). *Aloe vera*, also known as *Aloe barbadensis* Miller, belongs to the Alliaceae family. *Aloe vera* is a plant with fleshy leaves, which

grows up to a height of 8-10 m, matures in 4-6 years, and survives for around 50 years in excellent circumstances. Among about 400 known species, *Aloe vera* has the highest biological activity (2). According to the World Health Organization, plants with medicinal properties are among the best options for getting a wide range of medications (3). The *Aloe vera* plant is indigenous to the southern and eastern parts of Africa, especially around the upper Nile in Sudan. It was subsequently introduced into northern



Africa. It grows naturally in the Mediterranean region and other countries across the globe (4). *Aloe vera* is a very popular plant with a wide range of medicinal applications (5,6). It was used as a traditional treatment for its immunostimulant, antiseptic, burn and wound healing, antitumoral and laxative effects (7). C-reactive protein (CRP) refers to an acute pro-inflammatory polypeptide that can increase as much as 1000-fold in sites of inflammation or infection (8). Tillett and Francis identified CRP in 1930 (8). CRP was named after a material found in the serum of patients suffering from acute inflammation which reacted with the “c” polysaccharide antibody of the pneumococcus capsule (8). It is made as a homopentameric protein called native CRP (nCRP), which is capable of permanently dissociating into five distinct monomers called monomeric CRP (mCRP) at sites of infection and inflammation (9). CRP is synthesized primarily in liver hepatocytes but also by smooth muscle cells, macrophages, endothelial cells, lymphocytes, and adipocytes (10). There is evidence indicating that oestrogen, given as hormone substitution therapy, affects CRP concentrations among elderly persons (9). CRP has traditionally been used as a marker of cardiac events and infection; however, there is currently increasing evidence that it also plays essential functions in inflammatory mechanisms and host response to disease, such as the complement cascade, cellular death, nitric oxide release, phagocytosis, and cytokine production, especially tumor necrosis factor- α and interleukin-6 (9). Similar to many mediators of inflammatory processes, CRP has pleiotropic effects (11). Both “pro-inflammatory” and “anti-inflammatory” activities have been described (12). CRP binds to carbohydrates on microorganisms, such as phosphocholine, and activates the classical complement pathway of innate immunity by activation of C1q in the presence of calcium (13). CRP has been reported to play a protective role in a variety of inflammatory disorders, including protecting mice from a deadly challenge with bacterial lipopolysaccharide and other inflammatory mediators (11). Furthermore, CRP has been shown to prevent the onset and progression of experimental allergic encephalomyelitis, which is an animal model for multiple sclerosis (14). It has also been shown to reduce neutrophil and protein infiltration into lung tissues in a mouse model of chemotactic factor-induced alveolitis (15).

White blood cells defend the body against transmissible diseases and perform an important function in the body's immunity and inflammatory disorders (16). The classification of white blood cell (WBC) is vital since it can help hematologists diagnose some immunological disorders, as well as numerous blood conditions, such as certain types of cancer and leukemias (17). The analysis procedure can be done by automatic and manual approaches to count and classify WBC (16).

There is a need for effective anti-inflammatory treatments for several diseases. Inflammatory diseases are among the major global health challenges, affecting millions of people worldwide. Conventional anti-

inflammatory drugs are available; however, they often have side effects that limit their long-term use. Plant-derived compounds, on the other hand, have been employed for the treatment of numerous disorders for millennia and have fewer adverse effects. *Aloe vera* is a plant that has been traditionally used to treat inflammation, but there is a need for scientific validation of its anti-inflammatory effect in an animal model. This study aimed to investigate the potential of *Aloe vera* methanolic extract to reduce formalin-induced inflammation by measuring its effects on total white blood cell (TWBC) count and CRP levels in rats. The study seeks to determine the optimal dose and duration of treatment with *Aloe vera* extract to effectively reduce inflammation while avoiding potential side effects.

Materials and Methods

Study Area

The study was carried out at Kwara State University, Malete, Nigeria.

Collection, Identification, and Preparation of Plant Sample

Fresh leaves of *Aloe vera* were collected from a garden in Ilorin, Kwara State. The *Aloe vera* plant was identified and authenticated at the Department of Plant Biology, University of Ilorin, Ilorin, Kwara State, with a reference number: UILH/001/1370/2022. The leaves were rinsed with tap water to remove dirt and air-dried at ambient temperature (25 ± 2 °C) for 2 weeks. Then, 200 g of the dried leaves was milled to a fine powder using an electric grinder.

Preparation of Methanolic Extract

First, 100 g of the powder prepared by each drying method was soaked in 500 mL of methanol for 48 hours. Subsequently, the crude extracts were filtered with filter paper (Whatman No.1) to separate the filtrate from the residue. The solid-to-solvent ratio was 1:5. The methanolic extract was concentrated in a rotary evaporator. The resulting extract (residue) was weighed and kept at 4 °C until analysis.

Phytochemical Analysis

The *Aloe vera* extract was subjected to standard phytochemical analyses to determine different constituents, including alkaloids, tannins, flavonoids, and phenolics (18,19).

Experimental Animals

Twenty-five Wistar rats, weighing 120–200 g, used for this study were purchased from an animal farm in Ilorin, Nigeria. The rats were kept in a spacious and well-ventilated cage at ambient temperature (25 ± 2 °C) and under natural dark/light cycles. They were allowed to acclimatize for 5 days and were given feed and water *ad libitum*.

Induction of Inflammation in Experimental Animals

For induction of inflammation in rats, formalin 5% was

diluted with distilled water and injected intraperitoneally at a dose of 2 mg/kg for 2 days. Inflammation was considered to be induced following an increase in the CRP level of the rats.

Experimental Design

This was an experimental study on the assessment of CRP and TWBC in Wistar rats with inflammation, following the administration of methanolic extract of *Aloe vera*. Adult Wistar rats weighing 120–200 g were used in the study. The rats were selected at random and divided into 5 groups (groups A, B, C, D, and E). Each group had 5 animals. All the rats were numbered group-wise and individually.

- Group A was labelled as “normal control” and given only rat feed and water.
- Group B was labelled as “positive control” and given rat feed, water, and 0.2 mL of formalin.
- Group C received feed, water, 0.2 mL of formalin, and 200 mg of methanolic extract of *Aloe vera* for 2 weeks.
- Group D received feed, water, 0.2 mL of formalin, and 400 mg of methanolic extract of *Aloe vera* for 2 weeks.
- Group E received feed, water, 0.2 mL of formalin, and 600 mg of methanolic extract of *Aloe vera* for 2 weeks. This was done using an oral gavage once daily (9–10 AM).

Animal Sacrifice

The animals were sacrificed 12 hours after the last treatment. Whole blood was collected from the heart via cardiac puncture into tubes containing ethylene di-amine tetra acetate (EDTA) using a sterile syringe and needle and dispensed into respective dry specimen bottles that were labelled accordingly. The haematological analyses were carried out as the blood sample was collected.

Laboratory Analysis

C-reactive Protein Assay

The plasma CRP level was determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit from Arbor Assays according to the method used in a previous study (20).

White Blood Cell Count

TWBC count was determined using a Sysmex haematology autoanalyzer manufactured in Japan (21).

Results

Phytochemical Concentration of Different Constituents in the *Aloe vera* Plant

Table 1 shows the concentration of the different phytochemical constituents in the *Aloe vera* extract. This result revealed the presence of active constituents in the *Aloe vera* extract, including flavonoids, phenolics, alkaloids, and tannins.

Effects of *Aloe vera* on the Total White Blood Cell Count

Table 2 shows the mean TWBC count of groups A, B, C, D, and E. This study found the mean value of TWBC to vary among groups. The lowest mean value was found in group C (rats received feed, water, 0.2 mL of formalin, and 200 mg of methanolic extract of *Aloe vera*) ($8.60 \pm 0.74 \times 10^9$ cells/L), while the highest mean value was found in group B (positive control) ($16.12 \pm 3.02 \times 10^9$ cells/L). Relative to groups A, C, and D, a significant increase in the TWBC ($13.92 \pm 4.76 \times 10^9$ cells/L) was observed in group E (rats received feed, water, 0.2 mL of formalin, and 600 mg of methanolic extract of *Aloe vera*).

Effects of *Aloe vera* on the C-Reactive Protein

Table 3 shows the mean CRP level of Groups A, B, C, D, and E. The mean value of CRP varied among groups. The lowest mean value was found in group C (rats received feed, water, 0.2 mL of formalin, and 200 mg of methanolic extract of *Aloe vera*) (0.056 ± 0.003 mg/dL), while the highest mean value was found in group A (negative control) (0.058 ± 0.002 mg/dL). The mean CRP level of group C was significantly lower compared to other groups.

Table 1. Concentration of Phytochemicals in the *Aloe vera* Extract

Phytochemicals	Concentration
Total flavonoids	131.18 QE mg/g
Total phenolics	1130.5 GAE/g
Alkaloids	163 mg/100 g
Tannins	3.56 mg/100 g

QE: quercetin equivalent; GAE: gallic acid equivalent

Table 2. Effects of Aloe Vera Methanolic Extract on Total White Blood Cell Count ($\times 10^9$ cells/L)

Groups	Mean \pm SD	P Value
Group A	8.92 \pm 2.48	0.500
Group B	16.12 \pm 3.02	0.003*
Group C	8.6 \pm 0.74	0.19
Group D	9.8 \pm 1.18	0.09
Group E	13.92 \pm 4.76	0.03*

* Statistically significant at P value < 0.05

Note. Group A: Normal control (water+feed); Group B: Positive control (water+feed+formalin); Group C: Feed+water+200 mg of methanolic extract of *Aloe vera*; Group D: Feed+water+400 mg of methanolic extract of *Aloe vera*; Group E: Feed+water+600 mg methanolic extract of *Aloe vera*. Reference range: Total white blood cell count = $4.5\text{--}13.0 \times 10^9$ cells/L.

Table 3. Effects of Aloe Vera on C-reactive Protein level (mg/dL)

Groups	Mean \pm SD	P Value
Group A	0.058 \pm 0.002	0.42
Group B	0.061 \pm 0.006	0.14
Group C	0.056 \pm 0.003	0.04*
Group D	0.061 \pm 0.005	0.08
Group E	0.064 \pm 0.012	0.16

* Statistically significant at P value < 0.05 .

Note. Group A: Normal control (water+feed); Group B: Positive control (water+feed+formalin); Group C: Feed+water+200 mg of methanolic extract of *Aloe vera*; Group D: Feed+water+400 mg of methanolic extract of *Aloe vera*; Group E: Feed+water+600 mg methanolic extract of *Aloe vera*. Reference range: The normal C-reactive protein level is less than 0.9 mg/dL.

Discussion

Aloe vera, a medicinal plant belonging to the Asphodelaceae family, has been demonstrated to be capable of combating oxidative stress (22). The natural products obtained from it are divided into two groups: gel and latex. The gel is a clear mucilage produced by the pulp of the leaves, and the latex (juice) is a yellowish bitter substance derived from the outermost layer of the leaves (23). *Aloe vera* has demonstrated several therapeutic effects. It has been shown to prevent tumor growth in mice, alleviate respiratory tract disorders, and cardiovascular system disorders (24). It is also efficient in the treatment of radiation-induced dermatitis and has anti-atherogenic, anti-ulcer, and immune-stimulatory effects (25). *Aloe vera* has been shown to protect cells from damage caused by various toxic substances (26). White blood cells are important components of the immune system because they protect the body from transmissible infections and foreign bodies. Leucocytes or WBC are immune system cells that help the body fight infections, illness, and foreign elements (27).

The flavonoid concentration in the study was determined to be 131.18 QE mg/g. Flavonoids are phenolic compounds that are abundant in fruits, vegetables, and plants. They have antioxidant, and anti-inflammatory qualities, according to a previous study (28). In the same vein, the phenolic content was determined to be 1130.5 GAE/g. Phenolic compounds are a broad family of plant chemicals that have been demonstrated to have antioxidant and anti-inflammatory activities (29). They are thought to be responsible for the health advantages of eating fruits, vegetables, and other plant-based foods. The alkaloid content was determined to be 163 mg/100 g. Alkaloids have been proven to possess anti-inflammatory activities (30). Finally, the concentration of tannins was determined to be 3.5635 mg/100 g. Tannins are phenolic compounds found in plants that have antioxidant and anti-inflammatory activities (31).

Results from this study also showed that TWBC count decreased in rats, with the lowest value ($8.600 \pm 0.742 \times 10^9$ cells/L) found in the group given 200 mg of the extract, followed by 400 mg ($9.800 \pm 1.179 \times 10^9$ cells/L) and 600 mg ($13.920 \pm 4.757 \times 10^9$ cells/L). The TWBC of these three groups increased significantly ($P=0.03$), relative to the negative control ($8.920 \pm 2.476 \times 10^9$ cells/L) and positive control ($16.120 \pm 3.023 \times 10^9$ cells/L) groups. This finding agrees with the findings of a previous study (32) in which a significant increase in TWBC count was observed following treatment with *Aloe vera* gel and salt loading in rats. The increase in TWBC count in this study indicates the immune response mechanism of the immune system to inflammation. However, *Aloe vera* gel demonstrated a protective effect on the defense mechanism, as the TWBC count was significantly increased at 600 mg dose ($P=0.03$) group compared with the negative control group. The increased TWBC count is indicative of leukocytosis. This finding is consistent with a previous study (33) in which an increase was observed in the TWBC count of Wistar

rats fed *Aloe vera* compared to the salt-fed group. The leukocytosis could probably be a result of the ongoing inflammation in the treated rats.

The results also showed a significant decrease in the CRP level (0.056 ± 0.0027 mg/dL) ($P=0.04$) in the group received 200 mg of *Aloe vera* extract. This finding agrees with the results of other studies (34-37). This result suggests that oral supplementation of *Aloe vera* significantly reduced the serum CRP level in rats received 200 mg of the extract. This finding agrees with the findings of a previous study (36) which also found that oral supplementation of *Aloe vera* could significantly reduce the serum level of CRP. The exact underlying mechanisms for the *Aloe*-induced changes in the CRP level are not known. However, the anti-inflammatory effects of *Aloe vera* have been previously studied (20,38). Findings from this study (as reported above) have shown *Aloe vera* to be rich in flavonoids, phenolics, alkaloids, and tannins, all of which have anti-inflammatory activity. This might be the reason for the observed reduced CRP levels (as reported above) which are the responses of the subjects to the anti-inflammatory activities of these compounds. *Aloe vera* also contains 75 active components: amino acids, salicylic acids, lignin, sugars, minerals, enzymes, and vitamins (39), some of which have anti-inflammatory effects. For example, polysaccharides found in the gel of *Aloe vera*, such as C-glucosyl chromone and Mannose-6-phosphate, have been proven to possess antioxidant and anti-inflammatory properties (24). Furthermore, Bradykinase contributes to the reduction of prolonged inflammation (40). As a result, it is possible that *Aloe vera* decreases CRP levels via its anti-inflammatory components.

Conclusion

The results from this study showed that *Aloe vera* gel had an immune-protective effect, as evidenced by an increase in TWBC count and a drop in CRP levels. These effects may be attributed to the anti-inflammatory characteristics of *Aloe vera*, particularly due to its polysaccharides and enzymes. Overall, *Aloe vera* has the potential to be utilized as a herbal remedy to improve the immune system and reduce inflammation in the body.

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Competing Interests

The authors declare that they have no conflict of interests.

Ethical Approval

The ethical implications of this research were carefully considered throughout the study. All animal procedures were approved by the Kwara State University Animal Care and Use Committee. Our findings have important implications for public health, and we recognize the need to ensure that this research is used in an ethical and responsible manner. Ethical approval was obtained from the Centre for Research and Development, Kwara State University, Malete, Nigeria, with reference No. KWASU/CR&D/REA/2022/0010. Ethical conditions and all NIH (National Institute of Health) Guidelines for the care and use of Laboratory Animals, governing the conduct of experiments with life animals, were strictly observed as stipulated. The Institutional Animal Care and Use Committee (IACUC) at Kwara State University, Malete, approved all animal procedures and protocols used in this study.

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