Effect of *Aloe vera* on some serum bioactive parameters in male Wistar albino rats

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**Abstract**
For centuries, *Aloe vera* has been hailed as a natural remedy with a plethora of health benefits. The present study aimed to evaluate the impact of *A. vera* on their haematological, biochemical, and immunological parameters. Twenty male Wistar albino rats were taken and split into two groups. Group I (Treatment Group) received a prepared aqueous *A. vera* extract at a concentration of 50 mg/kg of body weight for a full 30 days. Group II (Control Group) was given 0.9% normal saline as a placebo. The study found that the rats that received the *A. vera* extract had significantly (*p*<0.05) higher packed cell volume (14.8±1.08) compared to the control group (11.26±0.45). However, there were no significant changes in the levels of hemoglobin (Hb), red blood cell count (RBC), mean corpuscular hemoglobin (MCH) concentration, white blood cells (WBC) count, platelets count, and mean corpuscular volume (MCV). The serum alanine transaminase (ALT) concentration (U/L) was significantly (*p*<0.05) lower in the treated group (39.54±7.74) than in the control group (77.68±6.96), while there were no significant changes in the levels of urea and creatinine compared to the control group. The plasma levels of proinflammatory markers (TNFa, IL-6, and IFNy) showed no significant differences between both groups. Overall, *A. vera* showed promise as a natural remedy for improving certain aspects of health, but more research is needed to understand its effects fully.

**Keywords:** *Aloe vera*, Bioactive factors, Haematological parameters, Immunological parameters

**INTRODUCTION**

*Aloe vera* has been used for its healing properties for thousands of years. From boosting the immune system to improving digestion and promoting healthy skin (Nimma et al., 2017; Choudhary, S, 2018). It has enabled its use for centuries to treat various ailments (Maan et al., 2018). It is not just ancient cultures that recognize the power of *A. vera*. It is still widely used in many modern societies, particularly in Asia, where countries like Africa, India and China rely on it as an essential component of their traditional medicine (Martínez-Burgos et al., 2022). *A. vera* has played such a significant role in medical practices that it was used in horde medicine as far back as 2000 years ago and is a vital ingredient in many food products. Its versatility and effectiveness make it a must-have in any natural health complementary therapy (Nirala et al., 2020).

Some of its polysaccharides have been found to have incredible therapeutic benefits. These benefits range from boosting the immune system to facilitating wound healing and even repairing damage caused by radiation exposure (Minjares-Fuentes et al., 2018). *A. vera* has also been found to have antimicrobial and antioxidant properties. In recent years, researchers have become increasingly interested in the potential benefits of *A. vera* on blood-related disorders. Studies have shown that it may positively impact haematological parameters, including blood cells, which could be promising news for those suffering from blood-related conditions (Amber et al., 2021). It also contains anthraquinones, compounds that have been found to have anti-inflammatory and antioxidant properties (Kumar et al., 2019; Nikookalam et al., 2019; Ebrahim et al., 2020; Saleem et al., 2022). This means that *A. vera* not only has the potential to heal and protect the body from disease, but it may also help reduce inflammation and promote overall health and wellness.

1. There is uncertainty regarding the impact of *A. vera* on hematological parameters and blood-related indices. For example, an earlier study conducted by Yiftikhar et al. (2015) documented that *A. vera* extract normalized the
changes in the levels of white blood cell counts, red blood cell counts, platelet counts, and blood indices induced by diclofenac in white albino rats, moreover, Im et al., (2014) has reported that A. vera gel has increased T cells and erythrocytes in mice model. While these studies suggest that A. vera may positively impact haematological parameters. Unlike these studies that have used A. vera products to protect against the toxic effects of cytotoxic drugs or ameliorate the potential erythropenia due to pathological impacts of disease status, the present study aimed to evaluate the effect of A. vera on some blood bioactive parameters in healthy Wistar albino rats without any interventions.

MATERIALS AND METHODS

Aloe vera extract preparation
Plant material A. vera (L.) specimens were obtained from local herbal markets. In this experiment, A. vera leaves were used for the extraction. In brief, the leaves were washed 2-3 times with tap water and then ground with 50 ml of distilled water using a pestle and sterilized mortar. The resulting homogenized liquid was filtered twice through cheesecloth and then centrifuged at 5,000 rpm for 10 minutes. The supernatants were collected and diluted with 50 mL of distilled water to obtain a concentration of 50 mg/kg of body weight (Guo and Mei, 2016; Ebrahim et al., 2020). The research was conducted in 2022 at the Department of Biology, College of Science, University of Tikrit, Iraq.

Study animals
The animals, 20 male albino rats (Wistar strain) weighing 180–200 g, were obtained from the Animal House/University of Mosul for the study. The animals were housed in wire mesh cages with a controlled light cycle (12 h of light and 12 h of darkness), fed commercial rat chow ad libitum, and given plenty of water (Abdulkader et al., 2022b; Abdulkader et al., 2022a). The study rats were divided into two groups, each including ten rats. One group (Group I) was assigned as a treatment group and received the prepared extract orally at a concentration of 50 mg/kg of body weight for 30 days, and another group, the control group (Group II), received 0.9% normal saline. The study was approved ethically and registered in the College of Pharmacy [UoM 03 on 16.11.2022].

Laboratory assessment
To assess hematological parameters, 5 mL of blood was collected from each animal via the retro-orbital venous plexus and placed in tubes containing lithium heparin. Approximately 2.5 mL of the blood was centrifuged for 10 minutes at 4,000 rpm to obtain plasma for immunological and biochemical tests.

Haematological tests
Various haematological parameters were analyzed to gain insight into the subject’s health status. The packed cell volume (PCV %) was determined using the microhematocrit method, which is a widely used technique to gauge the proportion of red blood cells to plasma in a blood sample. The red blood cell (RBC, cells/ml) and white blood cell (WBC, cells/ml) counts were estimated using the hemocytometer, a device that accurately counts blood cells in a specific volume. These parameters were crucial in determining the overall health of the subject. The haemoglobin (Hb, g/dl) concentration was also measured using the cyanmethemoglobin technique, a precise and reliable method to measure the amount of haemoglobin in the blood. The haemoglobin is an essential protein that binds to oxygen and helps transport it throughout the body. Hence, measuring its concentration provides crucial information about the subject's blood oxygen-carrying capacity. Furthermore, the mean corpuscular volume (MCV, femtoliters), mean corpuscular haemoglobin (MCH, picograms) and mean corpuscular haemoglobin concentration (MCHC, g/dl) were subsequently calculated. These parameters were calculated using mathematical formulae considering the RBC count and the haemoglobin concentration (Khabbazi et al., 2014; Ofem et al., 2015). These parameters provide essential information about the red blood cells' size, shape, and colour and are used to diagnose various blood disorders and anaemia (Iftikhar et al., 2015; Shediwah et al., 2019).

Biochemical tests
This study utilized a variety of biochemical parameters to assess the health and wellness of the subjects involved. One such parameter was total protein (TP), which was measured using a reagent that binds to the peptide bonds within the protein molecules, resulting in a colour change that can be measured spectrophotometrically. Another parameter measured was albumin concentration utilizing bromocresol green (BCG) to bind to albumin, forming a complex that can also be measured spectrophotometrically at 596 nm. Serum alanine transaminase (ALT, pg/ml) activity is an enzyme that helps metabolize amino acids in the liver. Aspartate aminotransferase (AST, pg/ml) activity, this enzyme is also involved in amino acid metabolism but is found in both the liver and other organs, including the heart and muscles. Finally, alkaline phosphatase (ALP, pg/ml) activity was evaluated (Abdullah et al., 2022; Al-Shakarchi et al., 2023b; Al-Shakarchi et al., 2023a). This enzyme is involved in various processes within the body, including bone formation and liver function (Abdullah et al., 2022; Al-Shakarchi et al., 2023b; Al-Shakarchi et al., 2023a). Finally, a biochemical autoanalyzer (Model Name: Mispa Viva, Swiss) was used to evaluate plasma levels (mg/100ml) of urea and creati-
nine according to the manufacturer's instructions.

**Immunological parameters**

Elisa kits (Sunlong -China) were used to evaluate the plasma levels (pg/ml) of TNF, IL6, and IFNγ according to the instruction of the manufacturer (Alsadoon and Abdullah, 2023; Faisal et al., 2019).

**Statistical analysis**

A t-test was used to compare parameters’ means of treatment and control groups using GraphPad Prism version 4.00 for Windows. All data were presented as means with standard error deviation. The statistical significance was considered when the value of $P$ was ≤ 0.05.

**RESULTS AND DISCUSSION**

The effects of *A. vera* on haematological parameters after one month of administration to Wistar albino rats are summarized in Table 1. The Hb, PCV, RBC, MCHC, and MCH levels were increased after oral administration of the extract; however, only the rise in PCV (%) was statistically significant ($P ≤ 0.05$).

The treatment group (Group 1) showed a significant decrease ($P = 0.001$) in their plasma levels of ALT compared to the control group. The treatment group also showed non-significant ($P > 0.05$) differences in AST and ALP plasma levels compared to the control group. Moreover, there were no significant changes ($P > 0.05$) in the levels of urea and creatinine between the two parameters compared to the control group (Table 1). Compared with the control group, plasma levels of TNF, IL6, and IFN- in rats that were given 50 mg/kg of the *A. vera* extract showed no significant differences ($P > 0.05$) (Table 3).

The research conducted by Ofem et al. (2015) investigated the effects of *A. vera* gel on the blood health of Albino rats. Their findings revealed that when given orally, *A. vera* extract has no significant effects on the levels of various haematological parameters such as haemoglobin (Hb), packed cell volume (PCV), and red blood cell (RBC) count. In comparison, Ofem et al. (2015) reported a significant increase in WBCs and platelet counts. However, only the increase in Hb level was statistically significant in the present study. The observed increase in Hb levels may be attributed to various bioactive compounds in *A. vera*, such as polysaccharides and anthraquinones (Lin et al., 2017). Polysaccharides have been found to have immunomodulatory properties (Ipshita et al., 2018) and can stimulate the production of several blood elements, including haemoglobin and RBCs (Kim et al., 2018; Ayesha Noor et al., 2017). In addition, anthraquinones possess anti-inflammatory and antioxidant properties that can help to protect the blood cells from oxidative stress and inflammation (Maan et al., 2018). This study’s findings on *A. vera*’s effects on haematological parameters are incongruent with the findings of other research investigating this plant’s potential benefits. For instance, in a study conducted by Tafi et al. (2019) on rainbow trout found that the trout’s red blood cell count, haemoglobin levels, and packed cell volume significantly increased. Furthermore, Rajasekaran et al. (2010), and his team showed that *A. vera* gel extract had the power to safeguard blood cells in rats with diabetes, not only improving their blood cell counts and preventing further damage. This research highlights the potential of *A. vera* in treating diabetes and its complications. These studies highlight the potential of *A. vera* as a natural remedy for improving haematological parameters in individuals with diabetes. While more research is needed to fully understand the mechanisms underlying these effects, these findings offer promising insights into the potential health benefits of *A. vera* and its potential role in managing blood-related conditions.

It was found that oral administration of *A. vera* extract significantly ($p=0.001$) reduced alanine aminotransf erase (ALT) plasma in albino rats. ALT is a liver enzyme released into the bloodstream when the liver is damaged or inflamed. The observed reduction in ALT levels suggests that *A. vera* may have a protective effect on liver function. Although the changes were not statistically significant, it is worth noting that the plasma levels of aspartate aminotransferase (AST) and alkaline phos-

<table>
<thead>
<tr>
<th>Parameters (mean±SE)</th>
<th>Treatment group (n=10)</th>
<th>Control (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>44.2±3.79</td>
<td>42.4±3.78</td>
</tr>
<tr>
<td>PCV(%)</td>
<td>14.8±1.08*</td>
<td>11.26±0.45</td>
</tr>
<tr>
<td>RBC (x10⁶/µl)</td>
<td>7.18±0.39</td>
<td>6.79±0.42</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>21.02±0.46</td>
<td>19.69±0.35</td>
</tr>
<tr>
<td>MCHC(g/dl)</td>
<td>34.90±0.87</td>
<td>32.93±1.10</td>
</tr>
<tr>
<td>WBCs(x10⁶/µl)</td>
<td>7.74±2.09</td>
<td>8.26±0.77</td>
</tr>
<tr>
<td>Platelets(x10⁶/µl)</td>
<td>113.6±13.89</td>
<td>117.4±11.57</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>59.43±1.16</td>
<td>57.7±2.42</td>
</tr>
</tbody>
</table>

*Significant difference at $P ≤ 0.05$.

<table>
<thead>
<tr>
<th>Parameters (mean±SE)</th>
<th>Treated group (n=10)</th>
<th>Control (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/l)(10)</td>
<td>39.54±7.74**</td>
<td>77.68±6.96</td>
</tr>
<tr>
<td>AST(U/l)(10)</td>
<td>105.24±8.42</td>
<td>99.74±5.08</td>
</tr>
<tr>
<td>ALP(U/l)(10)</td>
<td>110.31±6.88</td>
<td>118.32±9.16</td>
</tr>
<tr>
<td>Albumin (g/dL) (10)</td>
<td>2.12±0.17</td>
<td>2.10±0.11</td>
</tr>
<tr>
<td>Urea (mg/100ml)</td>
<td>12.73±0.65</td>
<td>12.77±0.48</td>
</tr>
<tr>
<td>Creatinine (mg/100ml)</td>
<td>0.28±0.05</td>
<td>0.27±0.04</td>
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</table>
Table 3. Immunological parameters of rats received Aloe vera extract versus control group

<table>
<thead>
<tr>
<th>Parameters (mean±SE)</th>
<th>Treated group (n=10)</th>
<th>Control (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα (pg/ml)</td>
<td>21.98±0.89</td>
<td>23.54±1.21</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>7.14±1.04</td>
<td>6.74±0.41</td>
</tr>
<tr>
<td>IFNγ (pg/ml)</td>
<td>19.41±1.24</td>
<td>20.82±0.84</td>
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No significant difference reported.

phatase (ALP) in rats who were administered A. vera extract did show some variation. This could suggest that A. vera extract may have some impact on liver function in the experimental animal model used, although further research is needed to confirm this hypothesis (Amber et al., 2021). The study conducted by Tariq et al. (2014) investigated the impact of 0.5% A. vera leaf powder on ALT and AST levels, as well as antioxidant status and liver damage in Japanese quail at 35 days of age. The results revealed no significant difference between the groups treated with A. vera and the control group, suggesting that A. vera supplementation did not significantly affect these parameters.

On the other hand, Fallah (2015) reported that the serum activity of alkaline phosphatase, AST, and ALT were reduced in broiler chickens at 42 days of age when treated with gel (1.5 or 3.0%) in drinking water compared to the control group. This suggests that A. vera supplementation may have a negative impact on liver function in broiler chickens. Sinha et al. (2017), also investigated the effects of A. vera leaves alcoholic extract supplementation on Jabalpur colour birds (32 weeks of age), reporting that supplementation with 2, 5 or 7 g/kg diet decreased lipid peroxidation, increased antioxidant status, and provided protection to both liver and kidney tissues. This suggests that A. vera leaves alcoholic extract may have a positive impact on liver health in birds when supplemented at appropriate levels. The liver and kidneys are vital organs that play important roles in the body’s metabolic processes. They filter toxins and waste products from the blood and maintain the body’s fluid and electrolyte balance. Therefore, any damage or dysfunction to these organs can have serious consequences on overall health. A. vera has been shown to potentially protect liver and kidney function in animal studies (Rajasekaran et al., 2010). The discrepancies in the results of these aforementioned studies may be due to differences in the dose, duration, and mode of administration of A. vera, as well as differences in the study population and the underlying disease or condition being studied. The present study has revealed no impact of A. vera on renal function tests. In contrast, a study conducted by dos Santos et al. (2021) discovered that this plant has a protective effect on the kidneys of diabetic rats since researchers found that A. vera significantly decreased urea and creatinine levels significantly. Another study by Bahmani et al. (2016) found that A. vera has a protective effect on the kidneys of rats with induced kidney damage. Iftikhar et al. (2015) reported that A. vera provided kidney functional protection in Albino rabbits. These studies mentioned above have been conducted on a rat model when there is induced damage, while in the present study, the rats were normally healthy and exposed to A. vera extract. The effect of A. vera contents has been studied for many years and there have been a variety of findings regarding its effects on inflammation and immune function. In a study by Wahedi et al. (2017), using Aloesin present in A. vera has shown to induce increased cytokine levels and growth factors (Wahedi et al., 2017). Specifically, they looked at the levels of IL-β, IL-6, TGF-β, and TNF-α, which are all involved in the immune response to infection and inflammation. Nevertheless, this study was done on supernatant collected from scratch cell line model in vitro, in which case there is no interference of other surrounding milieu factors. An earlier study by Naini et al. (2021) showed that A. vera gel reduced TNF or IL levels in rat ulcerative colitis models. The present study concluded that A. vera extract has shown no changes in measured proinflammatory markers (TNFα, IL-6, and INF). In contrast, Nimma et al. (2017) have shown that A. vera can potentially reduce inflammation in the oral mucosa after dental extraction. However, this evaluation was based on healing scores and reduced pain/swelling without measuring cytokine levels (Nimma et al., 2017). Similarly, a study by Kim et al. (2010) investigated the anti-inflammatory effects of A. vera gel in ulcerative colitis model rats. The model used in the present study was normal healthy rats without any disease induction, whereas most of these models used A. vera in a disease model in which inflammation is induced. This point might explain the discrepancy between current study results compared to published ones. However, this study provides new insight into already available information because most studies mentioned above focus on the protective effects of A. vera against pathological disease abnormalities on blood indices or against chemical intervention-induced blood abnormalities; thereby, this study gives a clear idea about the adverse effects of A. vera faraway from the interaction with induced pathological conditions.

Conclusion

The present study concluded that A. vera did not affect measured hematological parameters (Hb, RBCs, MCHC, WBCs, platelets, and MCV) with elevated PCV. The study also showed reduced ALT liver enzyme without impacting other measured liver parameters (AST, ALP, albumin, urea, and creatinine). There was no impact of A. vera on the proinflammatory markers (TNFα,
IL-6, and IFNγ). The present study recommends using A. vera even in normal individuals without any remarkable side effects.

Conflict of interest
The authors declare that they have no conflict of interest.

REFERENCES


