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Cardioprotective Effects of the Aqueous Leaf Extract of *Asystasia vogeliana* via Modulation of VCAM-1 and p53 in Anemia-Induced Rats

Basiru Olaitan Ajiboye [1], Olawale Razaq Ajuwon [2], Abdullateef Olamide Babalola [1], Damilare Emmanuel Bamigboye [1], Olumide Adebola Alonge [1], Oluwatosin Aliu [1], Raimot Olabisi Ojedokun [3], Sunday Abimbade [4], Mary Abiola Okesola [5], Precious Eseose Agboinghale [6], Ajoke Fehintola Idayat Akindele [7], Oluwafemi Adeleke Ojo [8], Babatunji Emmanuel Oyinloye [9] and Gamal Moustafa Mahmoud Abdelfattah [10,*]

[1] Phytomedicine and Molecular Toxicology Research Laboratory, Department of Biochemistry, Federal University Oye-Ekiti, Oye-Ekiti 371104, Ekiti State, Nigeria
[2] Redox Biology and Phytomedicine Research Laboratory, Department of Biochemistry, Federal University Oye-Ekiti, Oye-Ekiti 371104, Ekiti State, Nigeria
[3] Nutritional Biochemistry Unit, Department of Chemical Sciences, Fountain University Osogbo, Osogbo 230284 Osun State, Nigeria
[4] Natural Product Laboratory, Department of Chemistry, Federal University Oye-Ekiti, Oye-Ekiti 371104, Ekiti State, Nigeria
[5] Biochemistry Unit, Department of Chemical Sciences, Augustine University, 106103 Ilara-Epe, Lagos, Nigeria
[6] Department of Chemistry and Biomolecular Sciences, Faculty of Science, University of Ottawa, Ottawa, ON K1N 6N5, Canada
[7] Environmental Management and Toxicology Unit, Department of Biosciences and Biotechnology, Faculty of Sciences, University of Medical Sciences, Ondo City 351104, Ondo State, Nigeria
[8] Institute of Biomedicine, University of Turku, FI-20014 Turku, Finland
[9] Phytomedicine and Molecular Drug Discovery Laboratory (PMDDL), Department of Biochemistry, Federal University Oye-Ekiti, Oye-Ekiti 371104, Ekiti State, Nigeria
[10] Laboratory of Growth Regulators, Palacký University and Institute of Experimental Botany, The Czech Academy of Sciences, Šlechtitelů 27, 78371 Olomouc, Czech Republic
* Correspondence: G.M.Abdelfattah@ljmu.ac.uk or gamal.abdelfattah01@upol.cz
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Abstract: This study evaluates the therapeutic potential of *Asystasia vogeliana* (AV) aqueous leaf extract on the expression of vascular cell adhesion molecule (VCAM) and the tumor suppressor protein p53 in the cardiac tissue of rats rendered anemic with 2,4-dinitrophenylhydrazine (DNPH). Forty rats were randomly assigned to five groups: a normal control group, a DNPH-induced group without treatment, two DNPH-induced groups receiving AV extract at 200 and 400 mg/kg body weight, and a DNPH-induced group treated with 200 mg/kg of vitamin B_{12} as a standard reference. All treatments lasted for 29 days. At the conclusion of the study, the animals were sacrificed, and their heart tissues were harvested for biochemical and molecular assessments. Serum lipid profiles and expression levels of VCAM and p53 proteins were assessed. The results showed that AV-treated anemic rats exhibited significant improvements in lipid parameters and a marked downregulation of VCAM and p53 expression compared to untreated anemic rats ($p < 0.05$). Additionally, a reduction in pro-inflammatory cytokines associated with cardiac injury was observed. These findings suggest that *A. vogeliana* aqueous extract may have cardioprotective and anti-inflammatory effects in anemia-induced cardiac damage. Further investigations are warranted to fully elucidate its mechanisms and therapeutic potential.

Keywords: *Asystasia vogeliana*; cardiac injury; 2,4-dinitrophenylhydrazine; liver damage; gene expression

1. Introduction

Asystasia vogeliana Benth. (Figure 1), commonly referred to as Vogel's Coromandel or Asian Coromandel, is a perennial under-shrub belonging to the Acanthaceae family. This flowering plant is native to tropical regions of Africa and Asia, and it has subsequently naturalized in various parts of the world. The morphological characteristics of AV include simple, opposite, ovate leaves arranged in a decussate pattern. The plant features

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zygomorphic flowers, often accompanied by colorful bracts [1]. In Nsukka, Enugu State, Nigeria, the plant is locally known as “ogwu iba ocha nanya”, which translates to “drug for treatment”.

Traditionally, *A. vogeliana* has been used in local medicine to address various conditions, including hepatitis [2]. This highlights the significant role of AV in traditional healing practices. The therapeutic effects of AV are often amplified when used in conjunction with other plants. Notable combinations include *Cassia alata, Cymbopogon citratus*, and *Citrus aurantifolia juice*. Indigenous practices utilizing these combinations have demonstrated increased efficacy in managing health issues such as malaria, chronic fever, gonorrhea, and leprosy [1].



Figure 1. *Asystasia vogeliana* Benth. plant and flowers.

The presence of alkaloids, flavonoids, saponins, triterpenes, and steroids was confirmed through phytochemical screening. Furthermore, the *in vitro* antioxidant activities, including lipid peroxidation, ferric reducing antioxidant power (FRAP), total antioxidant capacity (TAC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, hydroxyl radical scavenging assay, as well as total flavonoid and total phenolic contents, were analyzed and reported [3,4].

Vascular Cell Adhesion Molecule-1 (VCAM-1) is an essential endothelial adhesion molecule that modulates inflammatory responses. It enables the recruitment and transmigration of immune cells, such as macrophages and T lymphocytes, into target tissues and is widely expressed within the vascular system. The expression of VCAM-1 is upregulated by various stimuli, including reactive oxygen species and pro-inflammatory cytokines like interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). Recent studies have linked VCAM-1 to various immune-related disorders, including rheumatoid arthritis, asthma, transplant rejection, and cancer [5]. Furthermore, VCAM-1 has been recognized as a potential biomarker for endothelial damage, underscoring its significance in understanding disease mechanisms and developing diagnostic and therapeutic strategies [6,7].

The p53 protein functions as a transcription factor essential for the cellular response to stress. It targets a broad range of genes and microRNAs. As a tumor suppressor, p53 primarily halts cell cycle progression or induces apoptosis in response to DNA damage. When p53 function is compromised, the accumulation of unrepaired DNA damage can lead to cancer. Researchers have dedicated significant efforts to explore specific sequences of DNA known as p53 response elements (RE), which p53 recognizes [8]. Moreover, p53 regulates its tumor-suppressing effects through three interconnected mechanisms: temporary cell cycle arrest (quiescence), permanent cell cycle arrest (senescence), and programmed cell death (apoptosis). By activating these mechanisms, p53 helps prevent neoplastic transformation and offers protection against cancer development [9].

It has been reported that 2,4-dinitrophenylhydrazine (DNPH) has been used to induce anemia in animal models [10]. When DNPH is injected into animals, it undergoes autoxidation, producing reactive oxygen species (ROS). This process causes harmful cellular reactions, resulting in anemia [10]. Therefore, the objective of this study is to evaluate the relative gene expression of VCAM and p53 in the hearts of DNPH-induced rats treated with AV aqueous leaf extract. Also, this study will evaluate serum lipid profiles, atherogenic indices, phosphatase, transaminase, lactate dehydrogenase, and ATPase activities, as well as redox status among other parameters in DNPH-induced rats administered AV aqueous leaf extract.

2. Materials and Methods

2.1. A. vogeliana Benth. Leaves Identification and Authentication

The leaves of *A. vogeliana* were procured from a local farm in Ikole-Ekiti, Ekiti State, Nigeria on 20 October 2025. The plant material was identified and authenticated by Dr. Odewo at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria, where a voucher specimen (FHI 114032) was deposited for future reference.

2.2. Chemicals and Enzyme Kits

The following chemicals were utilized in this study: 2,4-dinitrophenylhydrazine, acetic acid, *n*-hexane, ethanol, ammonium hydroxide, dilute ammonium hydroxide, and dimethyl sulfoxide (DMSO). All reagents utilized in this study were sourced from Sigma-Aldrich (Taufkirchen, Germany) and were of analytical grade. Enzyme assay kits employed for the biochemical analyses were obtained from Randox Laboratories (Crumlin, UK).

2.3. A. vogeliana Extract Preparation

The leaves of *A. vogeliana* were air-dried for seven days and subsequently ground into a fine powder using an electric blender. A total of 500 g of the powdered material was extracted in 1.5 L of distilled water on a mechanical shaker. The mixture was allowed to macerate for 72 h with intermittent agitation to facilitate optimal extraction. Following maceration, the mixture was filtered through a fine mesh cloth, and the filtrate was concentrated using a rotary evaporator. The concentrated extract was then freeze-dried to yield 50 g of powdered aqueous extract. The extract yield was approximately 10%, and it was used for subsequent experiments.

2.4. Animals and Anemia Induction

Forty (40) male Wistar rats were procured from the Animal House of Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria. Male rats were used in this study to avoid physiological and hormonal variations associated with the estrous cycle in female rats, which may influence experimental outcomes. The animals were acclimatized for one week prior to the commencement of the experiment, during which they had unrestricted access to standard feed and water. All experimental procedures were conducted in accordance with the ethical guidelines approved by the Faculty of Science Ethics Committee, Federal University Oye-Ekiti (FUOYE), under Approval Number FUOYEFSC 201122-REC2024/008.

After acclimatization, a dosage of 40 mg/kg body weight of 2,4-dinitrophenylhydrazine (DNPH) was prepared in a solvent composed of dimethyl sulfoxide (DMSO) and water in a 1:12 ratio. This solution was administered to 32 of the rats over three consecutive days to induce anemia. Induction of anemia was confirmed by a decrease in packed cell volume (PCV), hemoglobin (Hb), red blood cell (RBC) count, and other relevant hematological parameters compared to baseline measurements [10]. Blood samples for these analyses were collected from the tail of each rat. Following blood collection, the rats were divided into five groups, with each group consisting of eight rats, as follows:

Group 1: Control.
Group 2: DNPH-induced rats without treatment (untreated).
Group 3: DNPH-induced rats administered 200 mg/kg body weight of aqueous *A. vogeliana* leaf extract.
Group 4: DNPH-induced rats administered 400 mg/kg body weight of aqueous *A. vogeliana* leaf extract.
Group 5: DNPH-induced rats administered 200 mg/kg body weight of vitamin B12 [10].

2.5. Sacrifice and Sample Collection

On the 29th day of the experiment, the animals were humanely sacrificed under anesthesia induced by halothane. Blood samples were collected via cardiac puncture into plain sample bottles for serum preparation. The collected blood was centrifuged at 1500 rpm for 5 min to obtain the serum, which was subsequently used for biochemical analyses. The hearts were excised, rinsed with a buffer solution, and subsequently homogenized. The resulting homogenate was centrifuged at 3000 rpm for 15 min to separate cellular debris.

2.6. Biochemical Parameters

The serum lipid profile and levels of creatine kinase MB, phosphatase, and lactate dehydrogenase were assessed using kits provided by the respective manufacturers. Atherogenic indices were calculated using formulas reported in the literature [11]. The concentrations of cytokines, specifically tumor necrosis factor-alpha (TNF-α) and interleukin-10 (IL-10), were determined using enzyme-linked immunosorbent assay (ELISA) kits in

accordance with the manufacturer's instructions. The evaluation of redox status was carried out according to the procedures outlined in the respective enzyme kits. The activities of ATPase were assessed using methods described in the literature [12].

2.7. Molecular Gene Expression Analysis of VCAM and p53

Total RNA was extracted from the heart tissue using the Quick-RNA Miniprep™ kit manufactured by Zymo Research Corporation, Irvine, CA, USA. The concentration and purity of the isolated RNA were determined spectrophotometrically at 260 nm. Subsequently, the RNA was reversed transcribed into complementary DNA (cDNA) using a cDNA synthesis kit. Polymerase chain reaction (PCR) amplification of the VCAM and p53 genes was performed using OneTaq®® 2X Master Mix manufactured by New England Biolabs, Inc. (NEB), Ipswich, MA, USA. and gene-specific primers obtained from Inqaba Biotec, Hatfield, South Africa, as presented in Table 1. Actin-beta was employed as the housekeeping gene for normalization. Quantification of the relative expressions of VCAM and p53 was performed using ImageJ software (Version 1.54p).

Table 1. Primer sequence.

Parameter	Forward Primer	Reverse Primer
VCAM-1	5′-ACTGTGACCTGTCAGCGAAG-3′	5′-CTCCCGTGTACAAGTGGTCC-3′
p53	5′-CCCCTGAAGACTGGATAACTGT-3′	5′-ATTAGGTGACCCTGTCGCTG-3′
Actin-Beta	5′-GCAAGGATACTGAGAGCAAGAG-3′	5′-CATCTCCCTCACAATTCCATCC-3′

2.8. Data Analysis

Data were analyzed using GraphPad Prism version 6.0 software. Differences among experimental groups were assessed using one-way analysis of variance (ANOVA). Results are expressed as mean ± standard deviation (SD), and differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Effect of Aqueous Extract from A. vogeliana on Serum Lipid Profiles in 2,4-Dinitrophenylhydrazine-Induced Rats

The impact of the aqueous extract from *A. vogeliana* leaves on serum lipid profiles in 2,4-dinitrophenylhydrazine (DNPH)-induced rats is depicted in Figure 2. Figure 2A presents the serum total cholesterol concentrations in DNPH-induced rats. No significant difference was observed in total cholesterol levels between the groups treated with 200 mg/kg (200AV) and 400 mg/kg (400AV) of *A. vogeliana* aqueous extract when compared with the normal control (NC) and the standard control (induced + Vitamin B_{12}) groups. However, a significant decrease ($p < 0.05$) in total cholesterol concentration was observed in the treated groups (200AV and 400AV) as well as in the standard control group when compared with the untreated induced group.

Figure 2B presents triglyceride (TG) concentrations. Similar to total cholesterol, TG levels showed no significant differences ($p < 0.05$) between the induced rats receiving 200AV and 400AV of *A. vogeliana* and those in the NC and standard control groups. However, a significant decrease ($p < 0.05$) in TG concentrations was noted in the treated groups compared to untreated rats.

Figure 2C shows the concentrations of very low-density lipoprotein cholesterol (VLDL-C). The VLDL-C levels did not significantly differ among the groups treated with 200AV and 400AV of *A. vogeliana* compared to NC and standard control. Nevertheless, significant decreases ($p < 0.05$) in VLDL-C concentration were recorded in the treatment groups compared to untreated induced rats.

Figure 2D shows the serum high-density lipoprotein cholesterol (HDL-C) concentrations. A significant increase ($p < 0.05$) in HDL-C levels was observed in the standard control group and in DNPH-induced rats treated with 200 mg/kg (200AV) and 400 mg/kg (400AV) of *A. vogeliana* aqueous extract compared to the untreated induced group. Although a slight increase ($p > 0.05$) was noted, it was not statistically significant in some treatment comparisons.

Figure 2E presents the serum low-density lipoprotein cholesterol (LDL-C) concentrations. There were no significant differences in LDL-C levels between rats treated with 200AV and 400AV of *A. vogeliana* aqueous extract and those in the normal control (NC) and standard control groups. However, a significant reduction ($p < 0.05$) in LDL-C concentrations was observed in the treatment groups compared to the untreated induced rats.

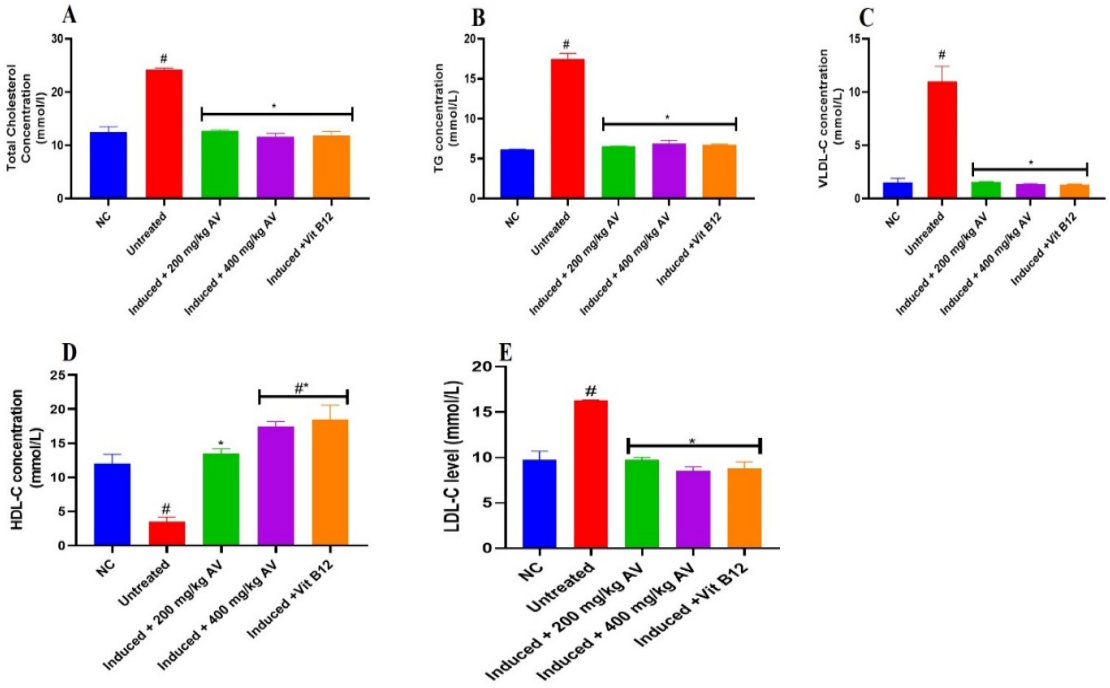


Figure 2. Effect of *A. vogeliana* aqueous leaf extract on serum lipid profiles in 2,4-dinitrophenylhydrazine-induced rats. (**A**) Total cholesterol concentrations, (**B**) triglyceride (TG) concentrations, (**C**) very low-density lipoprotein cholesterol (VLDL-C) concentrations, (**D**) high-density lipoprotein cholesterol (HDL-C) concentrations, (**E**) low-density lipoprotein cholesterol (LDL-C) concentrations. Each value is a mean of eight determinations ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. untreated. ***Legend***: NC: Normal Control, AV: *A. vogeliana*, Vit: Vitamin.

3.2. Effect of Aqueous Extract from A. vogeliana on Lipids Atherogenic in 2,4-Dinitrophenylhydrazine-Induced Rats

The effect of the aqueous leaf extract of *A. vogeliana* on lipid atherogenic indices in DNPH-induced rats is presented in Figure 3. Figure 3A illustrates the atherogenic index of plasma (AIP). No significant differences in AIP were observed between DNPH-induced rats treated with 200 mg/kg (200AV) and 400 mg/kg (400AV) of *A. vogeliana* compared to the normal control (NC) and standard control groups. However, a significant reduction ($p < 0.05$) in AIP was observed in the treated groups relative to the untreated induced rats.

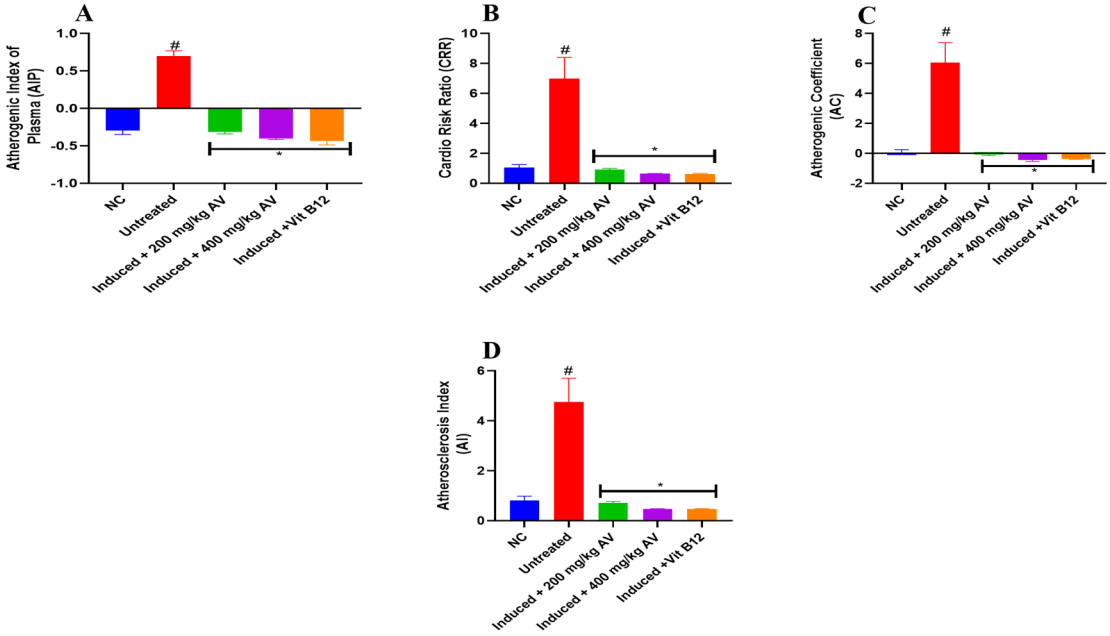


Figure 3. Effect of *A. vogeliana* leaf aqueous extract on lipid atherogenic indices in 2,4-dinitrophenylhydrazine-induced rats. (**A**) Atherogenic index of plasma (AIP), (**B**) Cardio risk ratio (CRR), (**C**) Atherogenic coefficient (AC), (**D**) Atherosclerosis index (AI). Each value is a mean of eight determinations ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. untreated. ***Legend***: NC: Normal Control, AV: *A. vogeliana*, Vit: Vitamin.

Figure 3B displays the cardio risk ratio (CRR). Similar to AIP, no significant differences in CRR were evident for induced rats treated with 200AV and 400AV of *A. vogeliana* compared to NC and standard control. Nevertheless, a significant decrease ($p < 0.05$) in CRR was observed in the treated groups when compared to untreated rats.

Figure 3C presents the findings for the atherogenic coefficient (AC). There were no significant differences in AC among induced rats treated with 200AV and 400AV of *A. vogeliana* compared to NC and standard control. Nevertheless, a significant decrease ($p < 0.05$) in AC was recorded in the treated groups relative to the untreated rats.

Figure 3D illustrates the atherosclerosis index (AI). No significant differences in AI were found among induced rats receiving 200AV and 400AV of *A. vogeliana* when compared to NC and standard control. However, a significant decrease ($p < 0.05$) in AI was observed in the treatment groups compared to untreated induced rats.

3.3. Effect of Aqueous Extract from A. vogeliana on Heart Phosphatase Activities in 2,4-Dinitrophenylhydrazine-Induced Rats

The results of heart phosphatase activities in DNPH-induced rats treated with *A. vogeliana* aqueous extract are shown in Figure 4. Figure 4A presents the specific activity of acid phosphatase. No significant differences in acid phosphatase activity were observed among induced rats receiving 200AV and 400AV compared to NC and standard control groups. However, a significant increase ($p < 0.05$) in acid phosphatase specific activity was noted in the treated groups compared to the untreated induced rats.

Figure 4B illustrates the specific activity of alkaline phosphatase. The alkaline phosphatase activity in rats receiving 400AV did not differ significantly from the standard control group but was significantly higher ($p < 0.05$) than that in the 200AV group. Additionally, a significant increase ($p < 0.05$) in alkaline phosphatase specific activity was found in treated rats receiving both 200AV and 400AV compared to untreated induced rats.

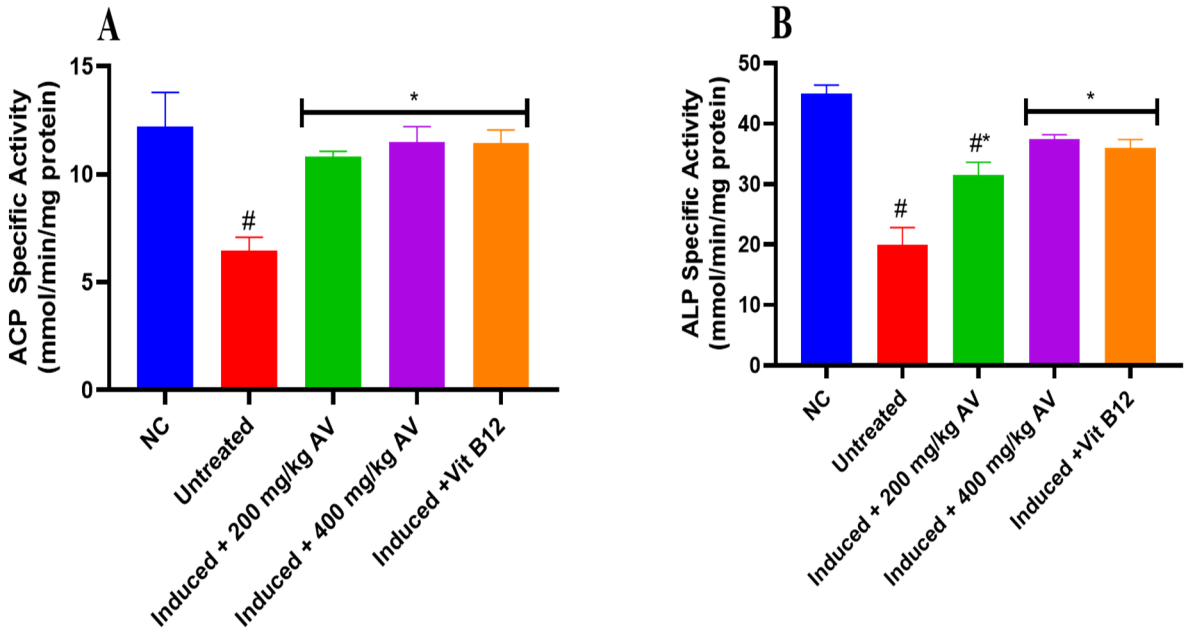


Figure 4. Effect of *A. vogeliana* leaf aqueous extract on heart phosphatase activities in 2,4- dinitrophenylhydrazine-induced rats. (**A**) Specific activity of acid phosphatase (ACP), (**B**) Specific activity of alkaline phosphatase (ALP). Each value is a mean of eight determinations ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. untreated. ***Legend***: NC: Normal Control, AV: *A. vogeliana*, Vit: Vitamin, ALP: Alkaline phosphatase, ACP: Acid phosphatase

3.4. Effect of Aqueous Extract from A. vogeliana on Heart Transaminase Activities in 2,4-Dinitrophenylhydrazine-Induced Rats

The influence of *A. vogeliana* aqueous extract on heart transaminase activities in DNPH-induced rats is illustrated in Figure 5. Figure 5A displays the specific activities of alanine transaminase (ALT). No significant differences were found in ALT specific activities among the induced rats receiving 200AV and 400AV of *A. vogeliana* compared to NC and the standard control. However, a significant increase ($p < 0.05$) in ALT specific activities was observed in the treated groups compared to untreated induced rats, indicating a positive response to the extract.

Figure 5B presents the specific activities of aspartate transaminase (AST). The AST specific activity in the 200AV group did not differ significantly from that of the standard control group but was significantly lower ($p < 0.05$) than AST activity in the 400AV group. A notable increase ($p < 0.05$) in AST specific activities was detected in induced rats receiving both doses of *A. vogeliana,* along with normal control and standard control groups, compared to untreated induced rats.

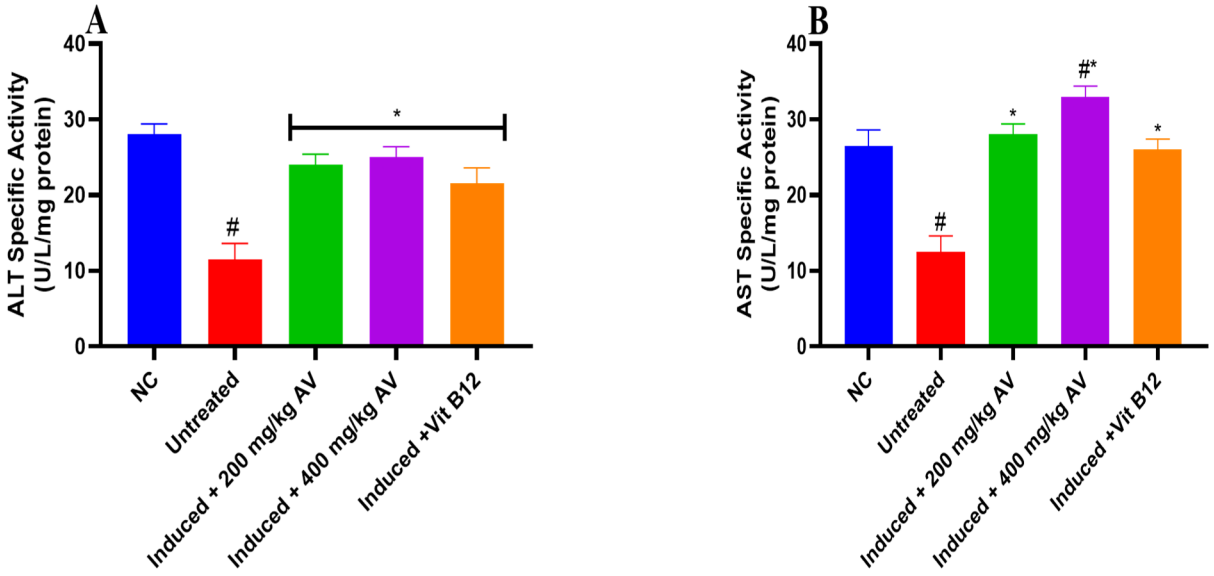


Figure 5. Effect of *A. vogeliana* leaf aqueous extract on heart transaminase activities in 2,4-dinitrophenylhydrazine-induced rats. (**A**) Specific activity of alanine aminotransferase (ALT), (**B**) Specific activity of aspartate aminotransferase (AST). Each value is a mean of eight determinations ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. untreated. ***Legend***: NC: Normal Control, AV: *A. vogeliana*, Vit: Vitamin, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase

3.5. Effect of Aqueous Extract from A. vogeliana on Heart Lactate Dehydrogenase (LDH) and Creatine Kinase MB (CKMB) Activities in 2,4-Dinitrophenylhydrazine-Induced Rats

The impact of *A. vogeliana* aqueous extracts on heart LDH activity in DNPH-induced rats is presented in Figure 6. Figure 6A illustrates the LDH activity levels. Induced rats receiving 400AV displayed LDH activity that was not significantly different from the standard control group but was significantly elevated ($p < 0.05$) compared to those receiving 200AV. A marked increase ($p < 0.05$) in LDH activity was also evident in induced rats receiving both 200AV and 400AV, as well as in NC and standard control groups compared to untreated induced rats.

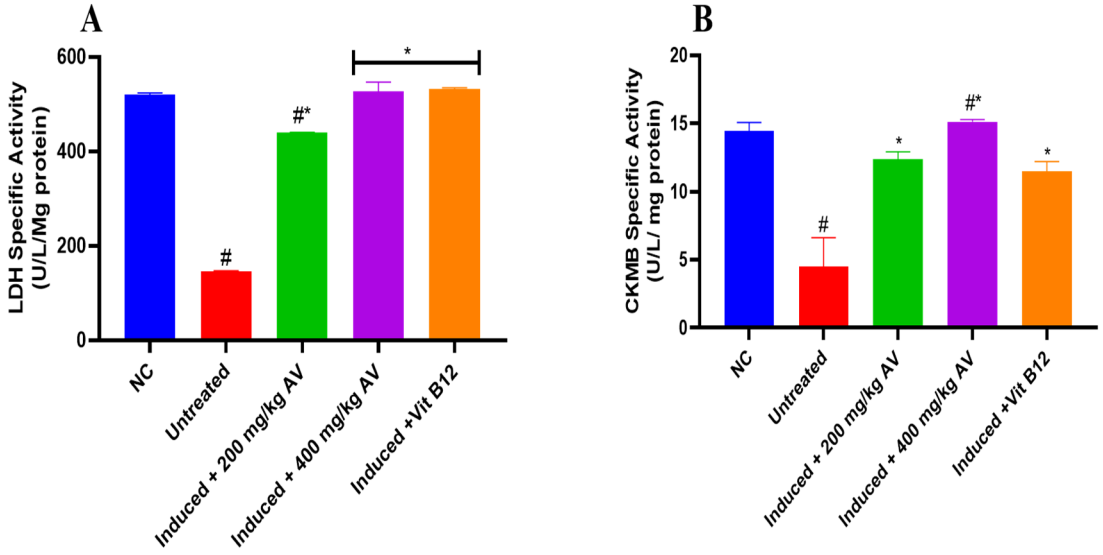


Figure 6. Effect of *A. vogeliana* leaf aqueous extract on heart lactate dehydrogenase (LDH) and creatine kinase MB (CKMB) activities in 2,4-dinitrophenylhydrazine-induced rats. (**A**) Specific activity of lactate dehydrogenase

(LDH), (**B**) Specific activity of creatine kinase-MB (CKMB). Each value is a mean of eight determinations ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. untreated. ***Legend***: NC: Normal Control, AV: *A. vogeliana*, Vit: Vitamin, LDH: Lactate dehydrogenase, CKMB: Creatine kinase-MB

Figure 6B shows the results for CKMB activity. The CKMB specific activity in induced rats treated with 200AV did not significantly differ from the standard control group; however, it was significantly lower ($p < 0.05$) than the CKMB activity in rats treated with 400AV.

3.6. Effect of Aqueous Extract from A. vogeliana on Heart ATPase Activities in 2,4-Dinitrophenylhydrazine-Induced Rats

The impact of *A. vogeliana* aqueous extracts on heart ATPase activities in DNPH-induced rats is depicted in Figure 7. Figure 7A illustrates the specific activity of Na^+/K^+-ATPase. Induced rats receiving 400AV exhibited no significant difference in Na^+/K^+-ATPase activity compared to the standard control group. However, this activity was significantly higher ($p < 0.05$) than that in the group receiving 200AV. A significant increase ($p < 0.05$) in Na^+/K^+-ATPase specific activity was noted in both treatment groups as well as in NC and standard control groups compared to untreated induced rats.

Figure 7B illustrates the specific activity of Ca^{2+}/Mg^{2+}-ATPase. A significant decrease ($p < 0.05$) in Ca^{2+}/Mg^{2+}-ATPase activity was observed in rats treated with 200 mg/kg (200AV) and 400 mg/kg (400AV) of *A. vogeliana* aqueous extract when compared with the standard control group. Conversely, a significant increase ($p < 0.05$) in Ca^{2+}/Mg^{2+}-ATPase activity was recorded in both treatment groups relative to the untreated induced rats.

Figure 7C presents the specific activity of Mg^{2+}-ATPase. No significant differences were observed in Mg^{2+}-ATPase activity among DNPH-induced rats treated with 200AV and 400AV compared to the normal control (NC) and standard control groups. However, a significant increase ($p < 0.05$) in Mg^{2+}-ATPase specific activity was observed in the treated groups and the standard control relative to the untreated induced rats.

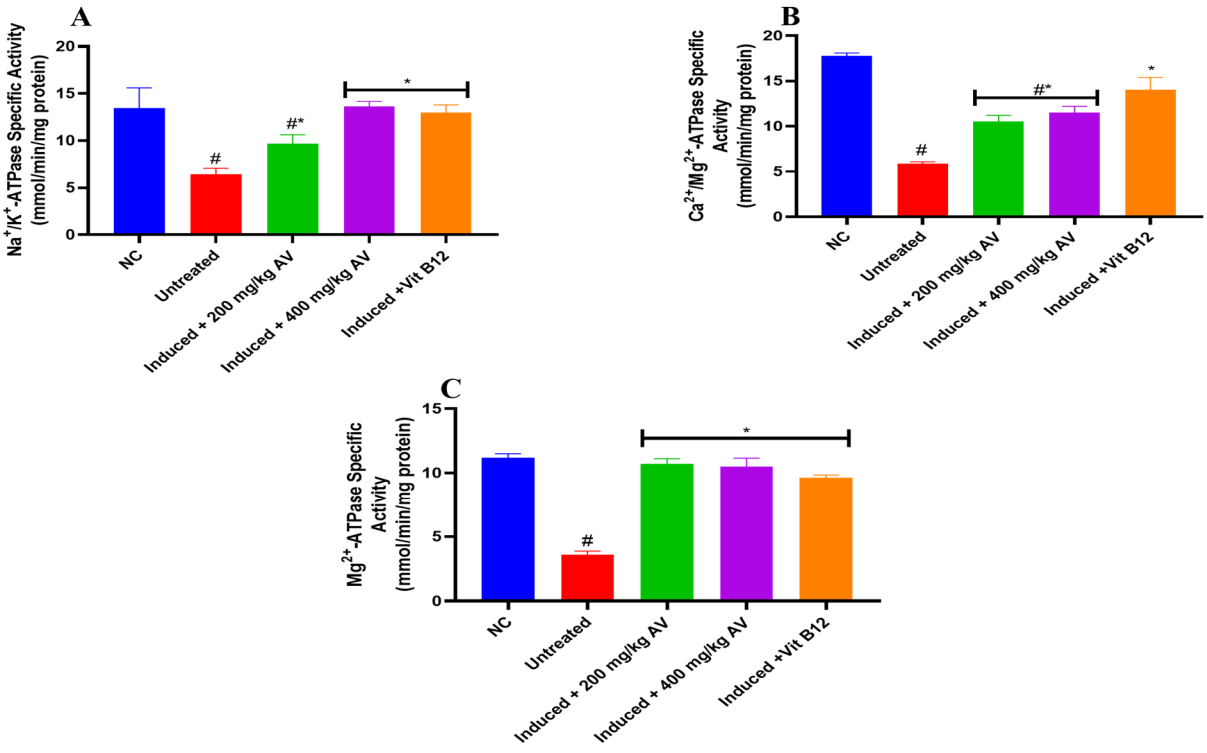


Figure 7. Effect of *A. vogeliana* leaf aqueous extract on heart ATPase activities in 2,4-dinitrophenylhydrazine-induced rats. (**A**) Specific activity of Na^+/K^+-ATPase, (**B**) Specific activity of Ca^{2+}/Mg^{2+}-ATPase, (**C**) Specific activity of Mg^{2+}-ATPase. Each value is a mean of eight determinations ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. untreated. ***Legend***: NC: Normal Control, AV: *A. vogeliana*, Vit: Vitamin.

3.7. Effect of Aqueous Extract from A. vogeliana on Heart Redox Status in 2,4-Dinitrophenylhydrazine-Induced Rats

The effects of *A. vogeliana* aqueous extracts on heart redox status in DNPH-induced rats are shown in Figure 8. Figure 8A illustrates the specific activity of catalase. A significant decrease ($p < 0.05$) in catalase activity was

detected in the treatment groups receiving 200AV and 400AV compared to the standard control. In contrast, a significant increase ($p < 0.05$) in catalase activity was observed in treated groups compared with untreated rats, yielding results comparable to the normal control group.

Figure 8B shows the specific activity of superoxide dismutase (SOD). No significant differences in SOD specific activity were observed among induced rats receiving 200AV and 400AV compared to the standard control group. However, a significant increase ($p < 0.05$) in SOD specific activity was noted in treated rats compared to untreated groups, reflecting results from the normal control.

The GST activity in rats received 200AV and 400AV did not differ significantly from that in standard control. However, a significant increase ($p < 0.05$) in GST activity was documented in the treated groups relative to the untreated rats, demonstrating values aligned with the normal control (Figure 8C).

Figure 8D details the specific activity of glutathione peroxidase (GPx). There were no significant differences among induced rats receiving 200AV compared to the standard control; however, its activity was significantly lower ($p < 0.05$) than that in the group receiving 400AV. Notably, a significant increase ($p < 0.05$) in GPx activity was observed in both treated groups and the normal control relative to untreated rats.

Figure 8E reveals the glutathione (GSH) concentrations. No significant differences were noted among rats receiving 200AV and 400AV compared to the standard control. Yet, a significant increase ($p < 0.05$) in GSH concentrations was recorded in treated groups compared to untreated rats, reflecting results akin to the normal control.

Figure 8F presents the concentrations of malondialdehyde (MDA). MDA concentrations in induced rats receiving 400AV exhibited no significant difference relative to the standard control but were lower ($p < 0.05$) than those in rats receiving 200AV. In both treated groups, significant decreases ($p < 0.05$) in MDA concentrations were observed compared to untreated induced rats, yielding results similar to those of the normal control.

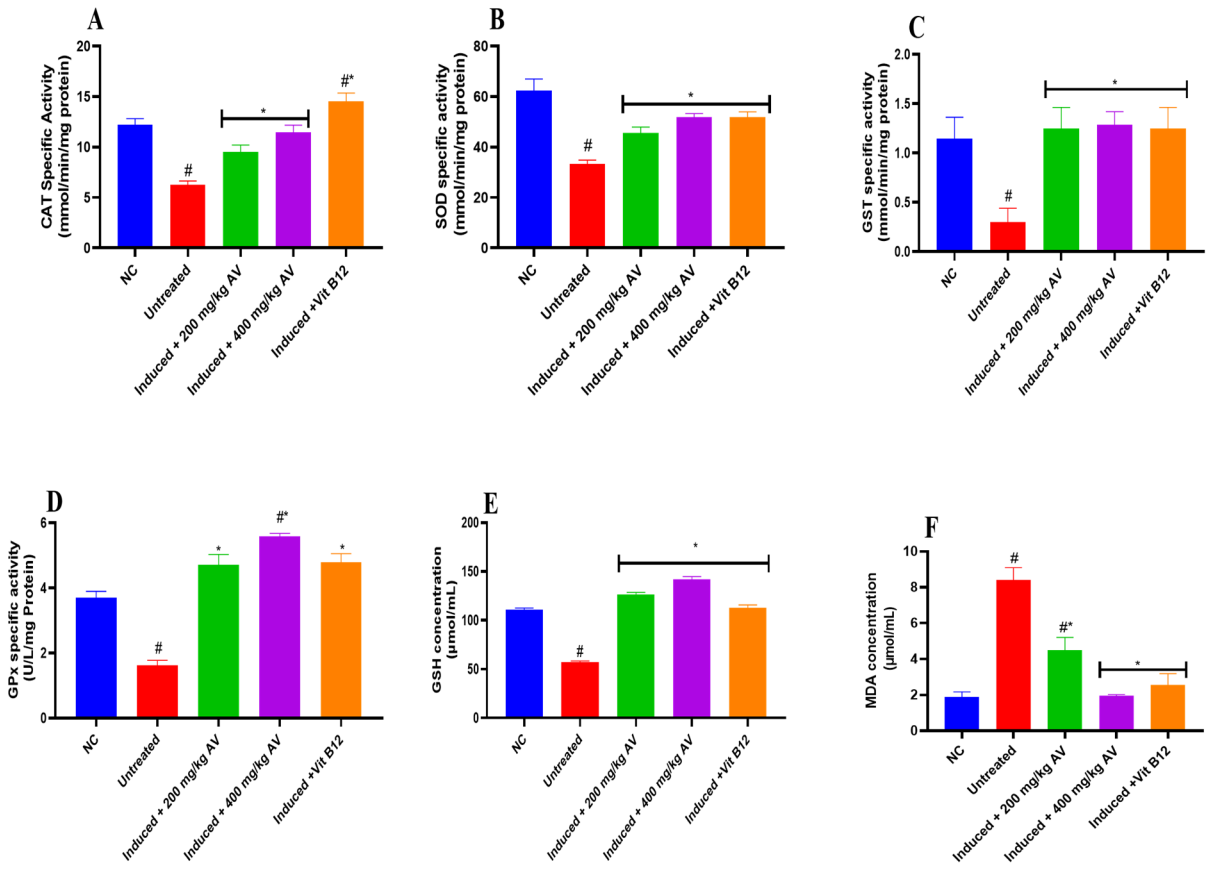


Figure 8. Effect of *A. vogeliana* leaf aqueous extract on heart redox status in 2,4-dinitrophenylhydrazine-induced rats. (**A**) Specific activity of catalase (CAT), (**B**) Specific activity of superoxide dismutase (SOD), (**C**) Specific activity of glutathione-S-transferase (GST), (**D**) Specific activity of glutathione peroxidase (GPx), (**E**) Reduced glutathione (GSH) concentration, (**F**) Malondialdehyde (MDA) concentration. Each value is a mean of eight determinations ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. untreated. ***Legend***: NC: Normal Control, AV: *A. vogeliana*, Vit: Vitamin, CAT: Catalase, SOD: Superoxide dismutase, GST: Glutathione-S-transferase, GPX: Glutathione peroxidase, GSH: Reduced glutathione

3.8. Effect of Aqueous Extract from A. vogeliana on Heart Cytokine Levels in 2,4-Dinitrophenylhydrazine-Induced Rats

The impact of *A. vogeliana* aqueous extracts on heart cytokine levels in DNPH-induced rats is illustrated in Figure 9. Figure 9A depicts the concentrations of interleukin-10 (IL-10). Significant differences in IL-10 concentrations were identified among rats treated with 200AV and 400AV of *A. vogeliana* in comparison to the standard control group. Additionally, a notable decrease ($p < 0.05$) in IL-10 concentrations was noticed in both treated groups compared to the untreated group. The results in the treated groups were comparable to those of the normal control group.

Figure 9B presents the concentrations of tumor necrosis factor-alpha (TNF-α). There was a significant difference in the level of TNF-α in the induced rats treated with 200AV and 400AV of *A. vogeliana* compared to the standard control. Further, there was a significant reduction ($p < 0.05$) in TNF-α levels in the treated groups compared to untreated rats, with outcomes mirroring those of the normal control group.

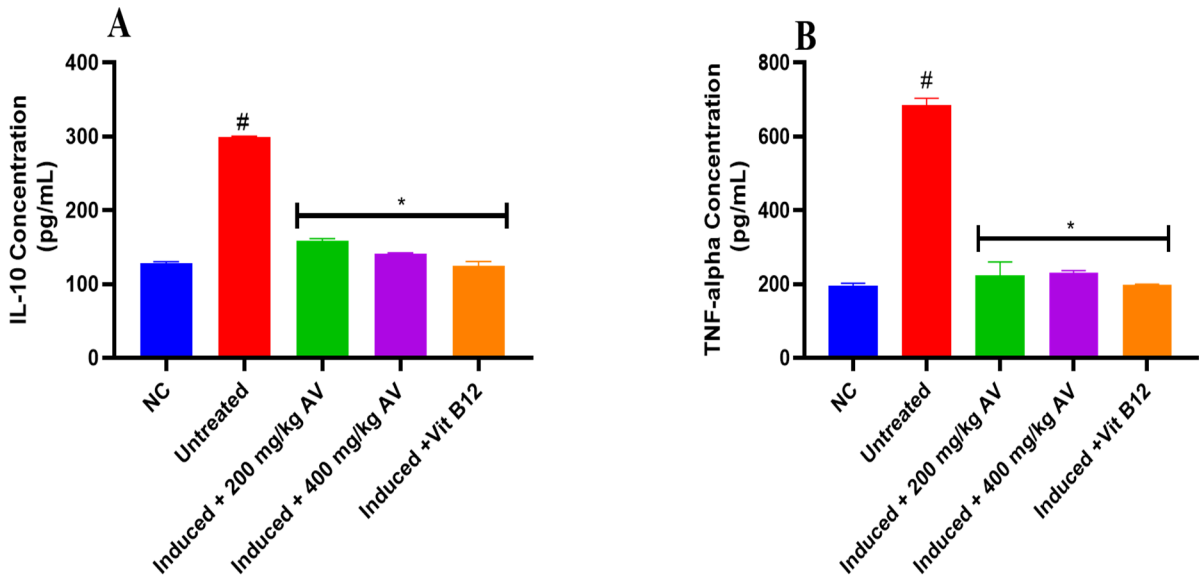


Figure 9. Effect of *A. vogeliana* leaf aqueous extract on heart cytokine levels in 2,4-dinitrophenylhydrazine-induced rats. (**A**) Interleukin-10 (IL-10) concentration, (**B**) Tumor necrosis factor-alpha (TNF-α) concentration. Each value is a mean of eight determinations ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. untreated. ***Legend***: NC: Normal Control, AV: *A. vogeliana*, Vit: Vitamin, TNF-alpha: Tumor necrosis factor, IL-10: Interleukin 10.

3.9. Effect of Aqueous Extract from A. vogeliana on Relative Gene Expressions of VCAM and P53 in 2,4-Dinitrophenylhydrazine-Induced Rats

The relative gene expressions of vascular cell adhesion molecule (VCAM) and tumor suppressor protein p53 following treatment with aqueous extracts from *A. vogeliana* leaves in DNPH-induced rats are illustrated in Figure 10. Figure 10A displays the relative expressions of VCAM. There was no significant difference in VCAM expression among induced rats receiving 400AV of *A. vogeliana* compared to the standard control group. However, VCAM expression was significantly lower ($p < 0.05$) in these rats compared to those receiving 200AV. Furthermore, significant decreases ($p < 0.05$) in VCAM expressions were noted in induced rats treated with both 200AV and 400AV as well as in the standard control group compared to untreated induced rats. The treated groups exhibited results comparable to the normal control group.

Figure 10B presents the relative expressions of p53. No significant difference in p53 expression was observed in DNPH-induced rats treated with 400 mg/kg (400AV) of *A. vogeliana* compared to the standard control group. However, p53 expression was significantly lower ($p < 0.05$) in these rats than in those receiving 200AV of *A. vogeliana*. A significant decrease ($p < 0.05$) in p53 expression was observed in groups treated with both 200AV and 400AV, as well as in the standard control group, compared to untreated induced rats. Notably, the treated groups displayed lower p53 expression levels than those in the normal control group.

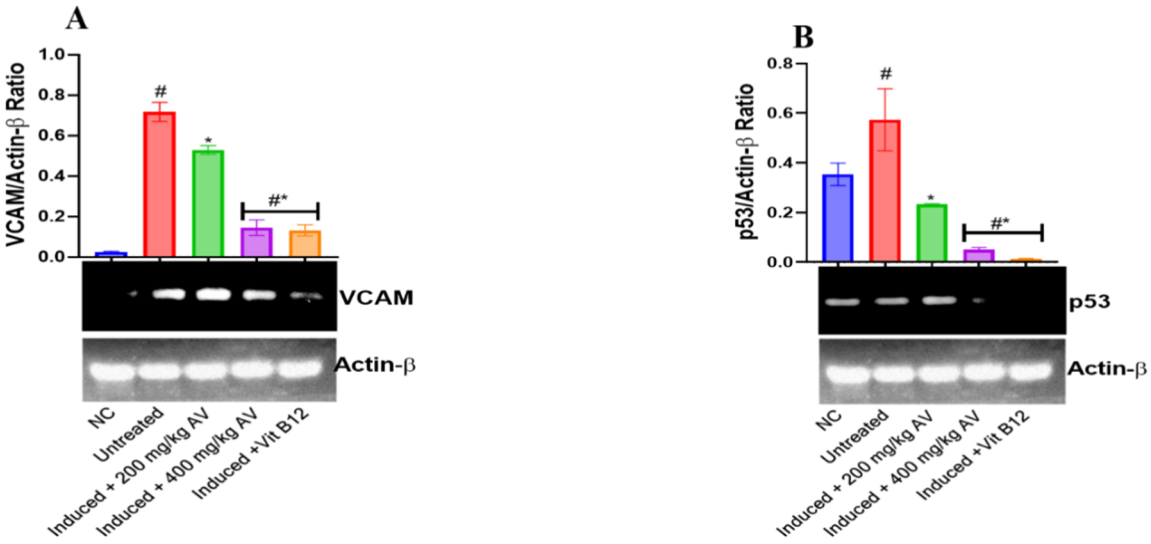


Figure 10. Effect of *A. vogeliana* leaf aqueous extract on relative gene expressions of VCAM and p53 in 2,4-dinitrophenylhydrazine-induced rats. (**A**) Relative gene expression of VCAM, (**B**) Relative gene expression of p53. Each value is a mean of eight determinations ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. untreated. ***Legend***: NC: Normal Control, AV: *A. vogeliana*, Vit: Vitamin.

4. Discussion

The phenylhydrazine-induced anemia model in Wistar rats is a widely recognized and reliable method for evaluating antianemic agents due to its rapid onset and reproducibility. Phenylhydrazine induces hemolytic anemia by generating oxidative stress, which damages mature red blood cells, leading to reduced hemoglobin levels, hematocrit, and erythrocyte count [13]. This model is particularly suitable for assessing the therapeutic potential of *A. vogeliana* aqueous extract, as it allows for the evaluation of its effects on lipid profiles, enzyme activities, and the expression of key markers such as vascular cell adhesion molecule (VCAM) and tumor suppressor protein p53 in the hearts of anemic rats.

Antioxidants play a crucial role in safeguarding biological systems against oxidative damage induced by reactive oxygen species (ROS), which can adversely affect lipids, proteins, and DNA. These antioxidants include radical scavengers such as vitamins and enzymes like superoxide dismutase (SOD), as well as phenolic compounds like flavonoids, anthocyanins, and polyphenols, which are known for their stable redox characteristics and ability to neutralize reactive radicals [14]. Increasing health challenges have stimulated interest in natural remedies, particularly plant-based foods rich in nutrients and bioactive phytochemicals [15,16]. Green leafy vegetables, including *A. vogeliana*, possess valuable nutritional properties that may contribute to health promotion and disease prevention [17].

Phytochemical analyses of *A. vogeliana* have revealed flavonoids, tannins, and phenolic compounds with antioxidant properties that may contribute to its cardioprotective effects [3,4,18]. Similarly, *Ocimum gratissimum* extracts have shown antioxidant and anti-inflammatory effects in diabetic cardiomyopathy models by modulating genes such as p53 and VCAM [19]. These findings suggest that the potential cardioprotective and anti-inflammatory activities of *A. vogeliana* may be linked to its flavonoid and polyphenolic constituents.

The evaluation of serum lipid profiles revealed significant insights. In the untreated group, total cholesterol concentrations were markedly elevated, indicating hypercholesterolemia induced by DNPH. Therefore, treatment with 200 mg/kg and 400 mg/kg of *A. vogeliana* extract significantly reduced total cholesterol levels, suggesting antihyperlipidemic effects. This aligns with findings that extracts from *Azadirachta indica* effectively lower serum cholesterol levels [20].

Similarly, triglyceride (TG) concentrations were significantly elevated in the untreated group, indicating hypertriglyceridemia, but treatment with *A. vogeliana* extract led to significant reductions in TG levels. Elevated TG levels are associated with increased risks of cardiovascular disease, stroke, and pancreatitis, highlighting the importance of *A. vogeliana* in lipid management [21]. Furthermore, very low-density lipoprotein cholesterol (VLDL-C) levels were significantly reduced in treated groups, suggesting protective benefits against peripheral artery disease and cardiovascular complications [20].

High-density lipoprotein cholesterol (HDL-C) concentrations were markedly lower in the untreated group compared to the normal control group. Low HDL-C levels are linked to an increased risk of heart disease and

inflammation [22]. In contrast, the treated groups exhibited substantial increases in HDL-C levels, indicating that AV may mitigate cardiovascular disease risk and reduce inflammation, mirroring conclusions described in the literature [23]. In addition, low-density lipoprotein cholesterol (LDL-C) levels rose significantly in the untreated group, increasing susceptibility to various cardiovascular diseases [24]. The administration of *A. vogeliana* extract significantly lowered LDL-C concentrations, further supporting its role in managing cardiovascular health.

The atherogenic index of plasma (AIP) and cardio risk ratio (CRR) also demonstrated significant increases in the untreated group due to DNPH induction. The elevated AIP levels correlate with heightened cardiovascular risk [25]. Notably, treatment with *A. vogeliana* extract effectively attenuated these increases, indicating its potential in improving cardiovascular health. The assessment of heart enzyme activities revealed further important findings. Acid phosphatase (ACP) levels decreased significantly in the untreated group, indicating potential health implications. However, administration of 200 mg/kg and 400 mg/kg of AV extract significantly elevated ACP levels, which suggests health-promoting properties consistent with findings illustrated in the literature [26].

Alkaline phosphatase (ALP) levels in the heart tissue showed significant reductions in the untreated group compared to the normal control. A low ALP activity is associated with an increased risk of conditions such as cardiac calcification and impaired endothelial function [27]. The treatment with AV extract normalized ALP levels, indicating protective effects on cardiac health. Additionally, specific activities of alanine transaminase (ALT) and aspartate transaminase (AST) showed significant reductions in the untreated group, indicating potential liver dysfunction [28]. The administration of AV extract restored ALT and AST activities to levels comparable to those in the standard control group, suggesting protective effects against hepatic injury.

Lactate dehydrogenase (LDH) and creatine kinase MB (CKMB) activities significantly decreased in the untreated group, highlighting the risk of cardiac injury due to oxidative stress. Treatment with AV extract improved LDH and CKMB levels, indicating protective effects against ischemic damage [29]. Moreover, the activities of ATPases, including Na^+/K^+-ATPase and Ca^{2+}/Mg^{2+}-ATPase, declined in the untreated group. Impairment of these enzyme activities contributes to cardiac dysfunction and increases the risk of arrhythmias [28,29]. The treatment with AV extract significantly restored these enzyme activities, suggesting enhanced cardiac contractility and improved ion homeostasis.

Notable reductions in catalase and superoxide dismutase (SOD) activities were observed in the untreated group, highlighting oxidative damage induced by 2,4-dinitrophenylhydrazine. In contrast, treatment groups exhibited increased levels of catalase and SOD, suggesting that AV enhances antioxidant defense mechanisms. This effect is comparable to the benefits seen with vitamin B_{12} treatment [30]. The untreated group also experienced significant decreases in glutathione S-transferase (GST) activity, reflecting compromised detoxification capacity due to oxidative stress. However, treatment with *A. vogeliana* extract resulted in improved GST activity, reinforcing the extract's therapeutic potential in bolstering antioxidant defenses.

In addition, glutathione peroxidase (GPx) activity was significantly diminished in the untreated group, indicating a state of oxidative stress. The treated groups, on the other hand, showed increases in GPx activity, suggesting that the extract may help mitigate oxidative damage and support overall antioxidant status [31,32]. Glutathione (GSH) concentrations were markedly lower in the untreated group, emphasizing the oxidative damage and the depletion of this vital antioxidant. Non-significant increases in GSH levels in the treated groups imply that AV may help restore GSH concentrations. Malondialdehyde (MDA) levels significantly increased in untreated groups, indicating lipid peroxidation and ongoing oxidative stress. The substantial reductions in MDA levels noted in the treated groups (200 mg/kg and 400 mg/kg) compared to the untreated group further demonstrate the efficacy of *A. vogeliana* in combating lipid peroxidation. These findings, which resemble those observed in the Vitamin B_{12} group, suggest that AV effectively protects against oxidative damage [33,34].

Biological systems respond physiologically to stress stimuli to support survival and defense. According to the published data [35], cytokines have a variety of effects on biological processes and are implicated in the etiology of numerous illnesses, particularly inflammatory ones. In line with the literature [36], the findings regarding interleukin-10 (IL-10) levels show that their concentrations were elevated in the untreated group, indicating heightened inflammatory status. The significant decrease in IL-10 levels in the treated groups suggests that AV extract has the potential to mitigate inflammation. Furthermore, elevated levels of tumor necrosis factor-alpha (TNF-α) in the untreated group reflect the inflammatory state induced by DNPH. The marked reduction in TNF-α levels observed in the treatment groups further supports the anti-inflammatory properties of AV extract.

The relative gene expression analysis indicated that vascular cell adhesion molecule (VCAM) expression significantly increased in the untreated group, suggesting enhanced atherosclerosis progression and inflammation [37]. The observed decrease in VCAM expression within the treated groups implies that AV extract may alleviate these conditions, reinforcing its potential for reducing cardiovascular disease risk. Similarly, elevated p53 expression in the untreated group signifies increased cellular stress and a higher potential for apoptosis. The significant reduction

in p53 expression among the treated groups indicates that AV extract may help alleviate cellular stress and protect against oxidative damage [38]. These findings highlight the extract's potential role in promoting cellular health and function, especially in the context of oxidative stress.

5. Conclusions

The aqueous leaf extract of *A. vogeliana* exhibited significant potential in mitigating atherosclerosis by restoring a healthy serum lipid profile. The extract markedly reduced atherogenic indices and enhanced the activities of key enzymes, including phosphatase, lactate dehydrogenase, transaminases, Na^+/K^+-ATPase, Ca^{2+}/Mg^{2+}-ATPase, and Mg^{2+}-ATPase. These results underscore the capacity of *A. vogeliana* to promote cardiovascular health through the regulation of lipid metabolism and enzymatic functions.

In addition, the extract favorably modulated biomarkers of redox status by reducing the levels of tumor necrosis factor-alpha (TNF-α) and interleukin-10 (IL-10), suggesting notable anti-inflammatory properties. This anti-inflammatory effect may contribute to the attenuation of chronic inflammation and oxidative stress commonly associated with cardiovascular disorders. Furthermore, *A. vogeliana* extract effectively regulated the gene expression of vascular cell adhesion molecule (VCAM) and tumor suppressor protein p53. The downregulation of VCAM implies a possible reduction in atherosclerotic progression, while modulation of p53 expression indicates protective effects against cellular stress and apoptosis.

Overall, these findings suggest that *A. vogeliana* extract may serve as a promising natural therapeutic agent for supporting cardiovascular health and managing conditions linked to atherosclerosis and oxidative stress. Further investigations are warranted to isolate and characterize the bioactive constituents responsible for these beneficial effects and to elucidate their underlying mechanisms of action.

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