Advancement in Medicinal Plant Research
Vol. 13(1), pp. 9-16, January 2025
DOI: 10.30918/AMPR.131.24.032
ISSN: 2354-2152
Full Length Research Paper

Effects of *Artemisia vulgaris* L. and *Cannabis sativa* L. on body weight and haematological parameters of DMBA-induced female wistar rats

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Accepted 31 December, 2024

ABSTRACT

The use of medicinal plants in managing toxicological and pathological conditions has garnered significant attention due to their bioactive components and therapeutic potential. *Artemisia vulgaris* L. (*A. vulgaris*) and *Cannabis sativa* L. (*C. sativa*) are notable for their diverse pharmacological properties, including antioxidant, anti-inflammatory, and restorative effects. This study investigates their impact on body weight and hematological parameters in female wistar rats exposed to 7,12-dimethylbenz[a]anthracene (DMBA), a carcinogenic compound known to induce systemic toxicity and hematological disruptions. Understanding the protective and restorative effects of these plants could provide insights into alternative therapeutic approaches for mitigating DMBA-induced toxicity and its associated complications. The study aimed to evaluate the effects of leaf extracts of *A. vulgaris* L. and *C. sativa* L. on cancer using animal models. Sixty-three female albino rats were used and divided into nine groups of seven animals each. Group I (control) was fed a normal diet. Groups II to IX were orally administered 20 mg/kg of 7,12-dimethylbenz[a]anthracene (DMBA) to induce tumors. Group II received no treatment, while Group III was treated with tamoxifen (6.6 mg/kg). Groups IV, V, and VI were treated with 100 mg/kg, 200 mg/kg, and 400 mg/kg of *A. vulgaris* extract, respectively. Similarly, Groups VII, VIII, and IX were treated with 100 mg/kg, 200 mg/kg, and 400 mg/kg of *C. sativa* extract, respectively. A significant reduction in body weight was observed in DMBA-induced animals. However, treatment with *A. vulgaris* and *C. sativa* extracts resulted in a gradual and significant ($p \leq 0.05$) increase in body weight across all treated groups. Additionally, treatment with the plant extracts demonstrated a significant ($p \leq 0.05$) increase in hemoglobin and packed cell volume levels compared to the untreated DMBA group. A decrease in neutrophil and platelet levels was observed in the treated groups compared to the DMBA group, although this decrease was not statistically significant ($p > 0.05$). The findings of this study suggest that *A. vulgaris* and *C. sativa* extracts may mitigate anemia and other hematological disruptions associated with breast cancer, highlighting their potential as complementary therapeutic agents.

Keywords: Breast cancer, *Artemisia vulgaris*, *Cannabis sativa,* body weight, hematological parameters, DMBA.

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INTRODUCTION

Therapeutic plants are widely used in herbal treatments due to their curative properties (Hassan and Abdul, 2012). Certain plants containing bioactive components have been confirmed to exhibit anticancer activities (Wang et al., 2012). *Artemisia vulgaris*, a member of the Asteraceae family, is known to contain pharmacological compounds. The diverse applications of this plant species are attributed to its rich chemical composition, which includes essential oils, flavonoids, sesquiterpene lactones, phenolic acids, coumarins, and other metabolites. The essential oil content in *A. vulgaris* contributes significantly to its use as a culinary spice in

various regions worldwide (Obistioiu et al., 2014). Additionally, the leaves of *A. vulgaris* have a high essential oil (EO) content, comprising cineole, α-pinene, camphene, camphor, and ketones (Radulović et al., 2013). Halina et al. (2020) reported that the most frequently identified volatile compounds in *A. vulgaris* extracts include caryophyllene oxide, 1,8-cineole, sabinene, camphor, camphene, α-thujone, and β-thujone.

Cannabis sativa L., a dioecious plant from the Cannabaceae family, is distributed globally and holds significant therapeutic value (Hartsel et al., 2016). *C. sativa* is used as a palliative therapy or as a co-administration with primary treatments for various diseases (Hartsel et al., 2016). The plant contains a diverse range of bioactive compounds, including cannabinoids, nitrogenous compounds, sugars, terpenoids, fatty acids, and flavonoids (McPartland and Russo, 2001). Mammary cancer, the most prevalent cancer among women worldwide (Mohamed et al., 2015), develops when normal cellular mechanisms regulating survival, proliferation, and differentiation are disrupted. Despite advancements in treatment strategies such as surgery, chemotherapy, and radiation, the management of metastatic breast cancer continues to present significant clinical challenges (Mantas et al., 2016).

Hematological parameters are valuable indicators of the adverse effects of foreign compounds on an animal's blood constituents (Arika et al., 2016). Anemia is a common condition among cancer patients, including those with cervical, ovarian, and endometrial cancers. Anemic patients often experience symptoms such as shortness of breath, fatigue, and reduced energy levels. Hemoglobin (Hb) and packed cell volume (PCV) levels are indirectly associated with an increased risk of cardiac failure in cancer patients (Rana et al., 2015). Furthermore, systemic inflammatory responses have been identified as critical factors affecting survival in several malignancies, with white blood cells serving as key mediators. Angiogenesis, a crucial step in tumor growth, progression, and metastasis, also involves platelets (Mantas et al., 2016). This study aims to investigate the effects of selected plants on body weight and hematological indices in wistar rats with DMBA-induced breast cancer.

MATERIALS AND METHODS

Collection of plant materials

The plants used in this study included:

- *Artemisia vulgaris* L. leaves, which were harvested from the A-Z Biotechnology Limited Medicinal Plants Plantation in Rayfield, Jos, Plateau State, Nigeria. The plant was authenticated by the Herbarium Unit of the Department of Plant Science, University of Jos, Nigeria, with the voucher number JUHN21000361.
- *Cannabis sativa* L. leaves, which were collected from the National Drug Law Enforcement Agency (NDLEA) Command Headquarters, Jos.

Ethics Approval

The study protocol, with the reference number F17-00379, was approved by the University of Jos Ethical Committee for Laboratory Animal Care and Experimentation.

Preparation of aqueous extracts of the plants

The plants were pulverized, and the aqueous extract of each plant sample was prepared following the method described by Gupta et al. (2013). A quantity of 200 g of the fine powder was macerated in 400 mL of distilled water for 24 hours. The macerate was then filtered using muslin cloth followed by filter paper (No. 3). The filtrate was concentrated using a water bath at 60°C, yielding a dark green residue weighing 23.3 g. The extract was stored in a freezer until further use.

Experimental animals

A total of 63 apparently healthy female Wistar rats were purchased from the Animal House of the Faculty of Pharmaceutical Sciences, University of Jos. The rats were 120 days old and weighed between 150 g and 250 g. They were housed under laboratory conditions, fed a standard food ration (MLMX), and provided with potable water ad libitum. The MLMX feed consisted of millet (60%), groundnut (16%), soybeans (16%), crayfish (5%), and palm oil (3%).

Induction of breast cancer in experimental female wistar rats

The method used for the induction of breast cancer in the experimental rats was based on Akuru et al. (2019) with slight modifications. Mammary gland tumors were induced in female wistar rats via a single intraperitoneal injection of 20 mg of 7,12-dimethylbenz(a)anthracene (DMBA) dissolved in 1 mL of sesame oil. Weekly physical examinations were conducted to monitor the development of tumors, which were expected to appear

as swellings. The rats were also subjected to weekly weight measurements to determine if tumor development was associated with weight loss. Swellings in each rat's mammary glands were examined by palpation.

By the eighth week following DMBA administration, the rats were re-examined to confirm tumor development. A biopsy was conducted on the swellings to confirm malignancy, following the procedure described by John-Kennedy et al. (2013). Mammary tumors were aseptically excised, fixed in 10% formaldehyde, and embedded in paraffin. Tissue sections (5 µm thick) were stained with hematoxylin-eosin and examined under a research microscope by a histopathologist, as described by Barros et al. (2004) and John-Kennedy et al. (2013).

Experimental design

The experiment was conducted over 14 weeks, following a modified protocol described by Akuru et al. (2019). The experimental animals were divided into the following groups:

- **Group I:** Normal rats, not induced with cancer, fed with MLMX and water.
- **Group II:** Carcinogenic control rats, induced with 20 mg/kg of DMBA, untreated.
- **Group III:** Carcinogenic control rats, induced with 20 mg/kg of DMBA, treated with a standard drug (tamoxifen, 20 mg/kg).
- **Groups IV, V, and VI:** Cancerous rats treated with aqueous leaf extract of *A. vulgaris* at doses of 100, 200, and 400 mg/kg body weight, respectively.
- **Groups VII, VIII, and IX:** Cancerous rats treated with aqueous leaf extract of *C. sativa* at doses of 100, 200, and 400 mg/kg body weight, respectively.

Body weight measurements

The body weights of the experimental animals were recorded regularly throughout the experiment. Initial body weights were measured before treatments, and final weights were recorded to assess the effects of the treatments. Changes in body weight were expressed as average percentage variations.

Hematological analysis

Blood samples were collected from each rat into EDTA tubes for hematological analysis. Parameters such as red blood cell (RBC) count, hemoglobin concentration, packed cell volume (PCV), platelet count, white blood cell (WBC) count, and differential white cell counts were analyzed using an automated hematology analyzer (Sysmex KX-3).

Statistical analysis

Data were analyzed using GraphPad Prism software (version 8.0). Significant differences between groups were determined using one-way analysis of variance (ANOVA) in a completely randomized design. Results were expressed as means ± standard deviations, with a significance level of 95% confidence ($p \leq 0.05$). All experiments were conducted in triplicate, and values were averaged.

RESULTS

Upon induction of cancer, there was a significant decrease in the body weight of the rats. The lowest weight was recorded in the group treated with 100 mg/kg of the aqueous leaf extract of *A. vulgaris* before the carcinogen was administered. Significant differences ($P \leq 0.05$) in body weight were observed among the experimental groups treated with different extracts of *A. vulgaris*. A general increase in the rats' body weights was noted after six weeks of treatment, as shown in Figure 1. Figure 2 illustrates the weights of DMBA-treated Wistar rats administered an aqueous concentrate of the leaf extract of *C. sativa*.

The results of the hematological analysis are presented in Table 1. The PCV levels and hemoglobin concentrations in all treated groups were significantly higher ($P \leq 0.05$) than in the control group. When compared to the DMBA-untreated group, these levels were higher but not significantly different ($P \leq 0.05$). The red blood cell indices of DMBA-treated Wistar rats given aqueous concentrates of different plants showed no significant differences ($P \leq 0.05$) among all groups.

Table 2 presents the blood platelet concentrations of DMBA-treated Wistar rats administered aqueous concentrates of *A. vulgaris* and *C. sativa*. The DMBA group showed a significant increase ($P \leq 0.05$) in platelet concentration ($753.00 \pm 0.00 \times 10^9$/L) compared to the control group ($585.00 \pm 0.00 \times 10^9$/L). The other groups showed no significant differences ($P \leq 0.05$) but had higher values than the DMBA group.

The results for white blood cell (WBC) concentrations of DMBA-treated Wistar rats given aqueous concentrates of different plants are shown in Table 3. The WBC concentrations in the DMBA A.v100, DMBA C.v400, and DMBA C.v200 groups were significantly higher ($P \leq 0.05$) compared to the control and DMBA-untreated groups. Other groups showed no significant differences ($P \leq 0.05$) when compared to the control and DMBA groups.

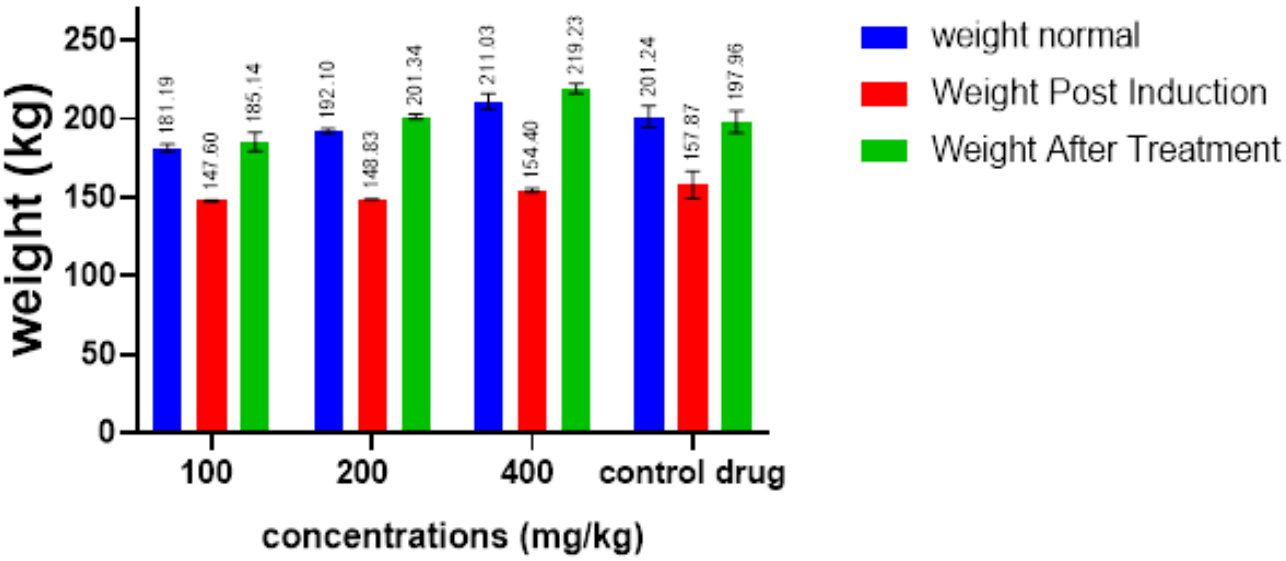

Figure 1. Effects of aqueous leaf extract of *Artemisia vulgaris* on body weights in DMBA-induced cancer wistar rats.

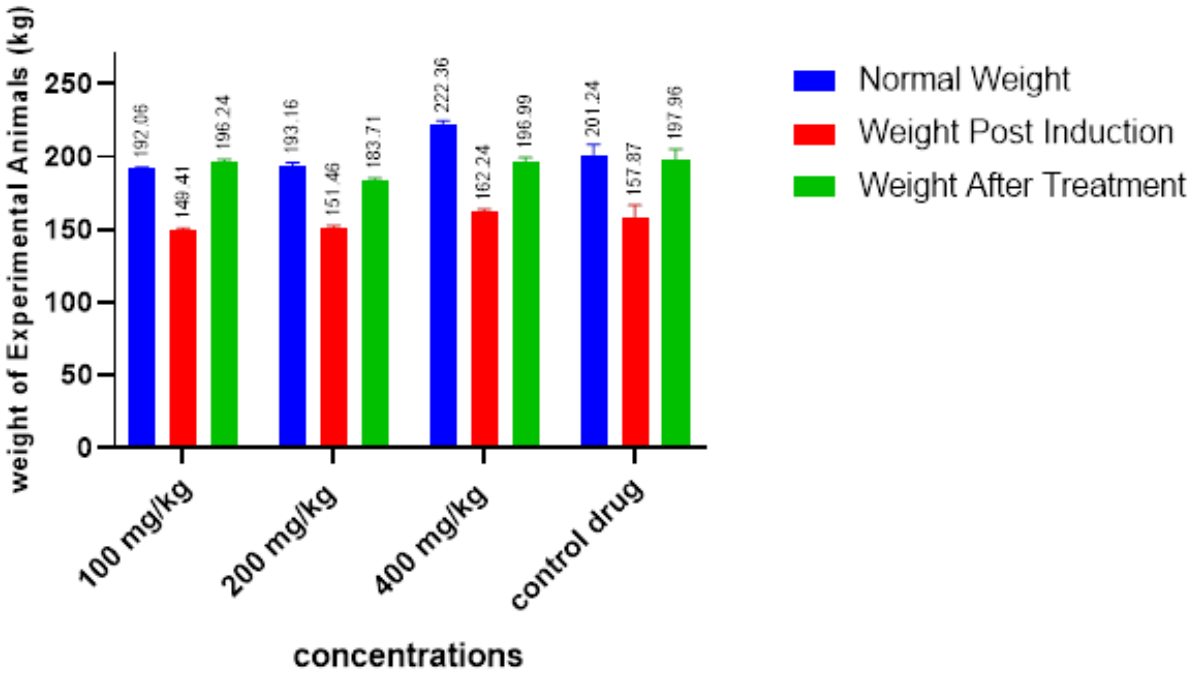

Figure 2. Effects of aqueous leaf extract of *Cannabis sativa* on body weights in DMBA-induced cancer wistar rats.

For neutrophil and eosinophil levels, no significant differences (P ≤ 0.05) were observed among all groups. Regarding lymphocyte levels, the DMBASTD group (73.48 ± 0.60%), DMBA A.v400 group (76.20 ± 0.00%), and DMBA C.s100 group (64.40 ± 0.00%) had significantly higher values (P ≤ 0.05) compared to the DMBA-untreated groups, while other groups showed no significant differences (P ≤ 0.05).

In terms of monocyte count, the DMBA A.v100 group had a significantly lower value (P ≤ 0.05) compared to the

Table 1. Red cell indices of DMBA-treated wistar rats given aqueous extract of the various plants.

Groups	RBC ($X10^{12}$/L)	HB (g/L)	PCV (%)
Control (MLMX)	6.26 ± 0.49^{a}	6.40 ± 0.00^{c}	24.50 ± 0.00^{b}
DMBA	6.73 ± 0.98^{a}	9.18 ± 0.00^{b}	52.30 ± 0.00^{a}
DMBA+STD	8.84 ± 1.14^{b}	16.12 ± 0.76^{a}	$56.32\pm.123^{b}$
DMBA+A.v100	8.89 ± 0.91^{b}	13.57 ± 0.80^{a}	55.21 ± 0.72^{a}
DMBA+A.v200	7.56 ± 1.07^{b}	16.70 ± 0.00^{a}	54.30 ± 0.00^{a}
DMBA+A.v400	7.86 ± 0.00^{b}	14.90 ± 0.00^{a}	64.80 ± 0.00^{a}
DMBA+C.s100	8.54 ± 0.00^{b}	15.80 ± 0.00^{a}	66.90 ± 0.00^{a}
DMBA+C.s200	8.27 ± 0.00^{b}	16.10 ± 0.00^{a}	59.30 ± 0.00^{a}
DMBA+C.s400	7.82 ± 0.00^{b}	15.00 ± 0.00^{a}	45.60 ± 0.00^{a}
L.S.D.	0.74		
S.F.	****		

Values are stated as Mean ± SEM. Values in a column with a similar alphabetical superscript do not contrast significantly p≤0.05. L.S.D = Least Significant Different, S.F. = Significant Figure, RBC = Red Blood Cell, HB = Hemoglobin, PCV = Packed Cell Volume.

Table 2. Blood platelet concentration of DMBA-treated wistar rats, given-aqueous extracts of the various plants.

Groups	PL ($X10^{9}$/L)
Control	585.00 ± 0.00^{c}
DMBA	753.00 ± 0.00^{a}
DMBA+STD	845 ± 49.77^{b}
DMBA+A.v100	486.29 ± 1.25^{c}
DMBA+A.v200	325.00 ± 0.00^{a}
DMBA+A.v400	474.00 ± 0.00^{c}
DMBA+C.s100	543.00 ± 0.00^{c}
DMBA+C.s200	504.00 ± 0.00^{c}
DMBA+C.s400	474.00 ± 0.00^{c}
L.S.D.	0.74
S.F.	****

Values are stated as Mean ± SEM values in a column with a similar alphabetical superscript do not contrast significantly p≤0.05. L.S.D. = Least Significant Different, S.F. = Significant Figure, PL= Platelet.

Table 3. Erythropoietic indices of DMBA treated albino rats given aqueous extracts of the various plants.

Groups	WBC ($X10^{9}$/L)	N (%)	L (%)	M (%)
Control	6.50 ± 0.00^{c}	11.88 ± 1.53^{c}	69.30 ± 0.00^{a}	14.60 ± 2.71^{a}
DMBA	14.80 ± 0.00^{a}	16.20 ± 0.00^{a}	61.98 ± 0.79^{b}	10.20 ± 2.71^{ab}
DMBA+STD	16.12 ± 6.96^{b}	20.42 ± 0.00^{a}	73.48 ± 0.60^{a}	6.10 ± 0.42^{b}
DMBA+A.v100	16.40 ± 0.00^{b}	100.00 ± 0.00^{a}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
DMBA+A.v200	9.50 ± 0.00^{b}	16.70 ± 0.00^{a}	68.90 ± 0.00^{b}	14.40 ± 0.00^{c}
DMBA+A.v400	15.10 ± 0.00^{b}	18.60 ± 0.00^{a}	76.20 ± 0.00^{a}	5.20 ± 0.00^{c}
DMBA+C.s100	15.10 ± 0.00^{a}	25.80 ± 0.00^{b}	64.40 ± 0.00^{a}	9.80 ± 0.00^{b}
DMBA+C.s200	18.30 ± 0.00^{a}	31.60 ± 0.00^{b}	59.30 ± 0.00^{b}	9.10 ± 0.00^{b}
DMBA+C.s400	26.40 ± 0.00^{a}	30.00 ± 0.00^{a}	60.00 ± 0.00^{c}	10.00 ± 0.00^{b}
L.S.D.	0.28			
S.F.	****			

Values are stated as Mean ± SEM. Values in a column with alike alphabetical superscript do not contrast significantly p≤0.05. WBC = White Cell count, N= neutrophil, L= Leukocytes, M= Monocyte count.

control and DMBA-untreated groups.

DISCUSSION

Effects of *A. vulgaris* and *C. sativa* extracts on body weight in female breast cancer rats

Body weight is a crucial parameter for studying the toxic effects of chemicals or substances. In the present study, the body weight gain of the DMBA-treated group declined compared to the control group. The decrease in body weight in DMBA-treated rats indicates the negative effects of the carcinogen. This finding aligns with Rajendran et al. (2019), who reported that DMBA reduced rats' body weight due to altered energy metabolism associated with tumor development. Additionally, the metabolism of DMBA produces reactive oxygen species (ROS), which disrupt normal biochemical processes, leading to weight loss (Rajendran et al., 2019).

Remarkable improvement in the body weights of rats was observed in the DMBA group treated with leaf extracts of *A. vulgaris* and *C. sativa*. Weekly monitoring showed a gradual increase in weight among extract-treated groups, though the differences were not statistically significant. These results suggest that treatment with *A. vulgaris* and *C. sativa* leaf extracts improved the body weight of DMBA-treated rats. This finding is consistent with Ebtihal et al. (2022), who reported a 9.47% increase in body weight over four weeks in DMBA-induced rats treated with aqueous leaf extract of *A. annua* (400 mg/kg).

Complete blood count and erythropoietic indices

A complete blood count reflects the cellular immune response in cancer patients (Rana et al., 2015). The erythropoietic indices of DMBA-administered albino rats treated with aqueous extracts of the plants at various concentrations are presented in Table 1. Significant increases ($p \leq 0.05$) in packed cell volume (PCV) and hemoglobin concentration were observed in all treated groups compared to the control and DMBA groups. Although the red blood cell (RBC) count increased in all treated groups, the difference was not statistically significant ($p \leq 0.05$).

PCV, hemoglobin, and RBC are markers of anemia, which increases the risk of death in cancer patients due to heart failure (Arika et al., 2016; Akinbami et al., 2013). Anemia in cancer patients often results from bleeding, nutritional deficiencies, bone marrow damage, tumor infiltration, or malignancy (Ali, 2014). The anti-anemic properties of the plants could be attributed to their high iron content, reducing the risk of heart failure in cancer patients. These findings are consistent with those of Akuru, Amadi, and Abbey (2019) and Zingue et al. (2018), who reported increased PCV, MCHC, MCV, RBC, and hemoglobin levels following treatment with *Sorghum vulgare*, *Eremomastax polysperma*, *Brillantaisia owariensis*, and *Acacia seyal* in DMBA-induced mammary tumor models.

Platelet count

Table 2 presents the platelet concentrations of DMBA-treated albino rats given aqueous plant extracts. A significant increase ($p \leq 0.05$) in platelet concentration was observed in the DMBA-untreated group compared to the control group. However, treated groups showed lower platelet counts than the DMBA-untreated group, though not statistically significant ($p \leq 0.05$).

A high platelet count is associated with the prognosis of gynecological cancers (Rochet et al., 2014) due to the secretion of growth factors and cytokines that promote angiogenesis, a critical step in breast cancer metastasis (Etim et al., 2018). The reduced platelet counts observed in this study indicate the potential of these extracts in reducing angiogenesis. These findings align with Zingue et al. (2018) and Akuru et al. (2019), who reported decreased platelet concentrations following treatment with plant extracts in DMBA-induced mammary tumor models.

White blood cell count

Table 3 shows the white blood cell (WBC) levels in DMBA-administered albino rats treated with varying concentrations of plant extracts. Significant increases ($p \leq 0.05$) in WBC levels were observed in rats treated with 100 mg/kg of *A. vulgaris* and 200 mg/kg and 400 mg/kg of *C. sativa* compared to the control and DMBA-untreated groups. However, the DMBA group treated with 200 mg/kg of *A. vulgaris* exhibited the lowest WBC level, though not statistically significant ($p \leq 0.05$).

A low WBC count increases the risk of infection (James, 2013), while a higher count raises the risk of invasive breast cancer (Rochet et al., 2012). Neutrophil levels were significantly higher ($p \leq 0.05$) in rats treated with 200 mg/kg and 400 mg/kg of *A. vulgaris* compared to the DMBA and DMBA-STD groups. These findings contradict Akuru et al. (2019), who reported lower neutrophil levels in similar treatment groups, suggesting the functionality of these extracts in cancer models.

For lymphocyte count, significantly higher values ($p \leq 0.05$) were observed in DMBA-STD and DMBA-400 mg/kg *A. vulgaris*-treated groups compared to the DMBA-

untreated group. Lymphocytes play a critical role in fighting cancer, as low lymphocyte levels are associated with relapse and decreased survival rates, while higher counts improve overall survival (Akinbami et al., 2013). These findings agree with Chen et al. (2017), who reported reduced WBC, neutrophil, and lymphocyte levels in DMBA-induced mammary cancer following treatment with *Puerariae radix*.

CONCLUSION

In conclusion, this study demonstrates the potential anticancer effects of aqueous leaf extracts of *A. vulgaris* and *C. sativa* in vivo. Both plants significantly improved red blood cell indices and stabilized white blood cells, reducing the anemia and inflammation associated with cancer. These findings suggest that *A. vulgaris* and *C. sativa* are promising candidates for developing traditional medicines to manage cancer.

CONTRIBUTION TO KNOWLEDGE

This study makes the following contributions to understanding the therapeutic potential of *Artemisia vulgaris* and *Cannabis sativa* in managing body weight and hematological parameters in DMBA-induced mammary cancer models. It provides empirical evidence on the combined effects of these two plants, which have not been previously studied in the context of chemically induced cancer. The findings offer new insights into how these plants influence body weight regulation and hematological health, specifically by assessing red blood cell (RBC) count, packed cell volume (PCV), hemoglobin (Hb) levels, and other hematological indices in the Wistar rat model.

Additionally, the study explores the mechanisms through which these plants may mitigate cancer-induced anemia and weight loss, opening new avenues for therapeutic interventions. Finally, this research contributes to the broader knowledge of plant-based treatments in cancer care and lays the groundwork for future studies on the efficacy and safety of *A. vulgaris* and *C. sativa* in managing the side effects of cancer and its treatments.

Competing interests

The authors declare that they have no competing interests.

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Citation: Ejembi PE, Odeh SO, Itelima JU, Wuyep PA, **2025**. Effects of *Artemisia vulgaris* L. and *Cannabis sativa* L. on body weight and haematological parameters of DMBA-induced female wistar rats. Adv Med Plant Res, 13(1): 9-16.