Archives of Advances
in Biosciences

Original Article:

Chemotherapeutic Potential of Methanol Leaf Extract of *Telfairia occidentalis* on Oxidative Stress, Hepatic, Hematological, and Biochemical Alterations in DMBA-Induced Breast Cancer in Wistar Rats

Hassan Abdulsalam Adewuyi[1]*, Chinedu Sebastine Ugwu[1], Sakariyau Waheed Adio[2], Monsurat Ololade Nasiru[3], Adeola Victor Kolawole[4], Adeola Elizabeth Adeniji[5], Saheed Kayode Tairu[6], Toheeb Adedolapo Jumah[7], Adepeju Matilda Adekoya[8], Tosin Titus Olaniran[9], Emmanuel Oluseyi Adeniyi[10]

1. Department of Biochemistry, Federal University of Technology, PMB 65 Minna, Niger State Nigeria
2. Department of Chemistry and Biochemistry, College of Science, Old Dominion University, Norfolk Virginia USA
3. Department of Biological Sciences, Southern Illinois University Edwardsvile
4. Department of Health, Uk Health Security Agency
5. Department of Biochemistry Ladoke Akintola University of Technology Ogbomoso Nigeria
6. Department of Biological Sciences, Kwara State University Nigeria
7. Department of Human Anatomy, Ahmadu Bello University Zaria
8. Department of Cell Biology and Genetics University of Lagos Nigeria
9. Department of Pure and Applied Science Lautech Ogbomoso Nigeria
10. Department of Health Lagos State Health Service Commission, State Hospital Gbagada Lagos Nigeria

Cite this article as: Hassan Abdulsalam Adewuyi, Chinedu Sebastine Ugwu1, Sakariyau Waheed Adio, Monsurat Ololade Nasiru, Adeola Victor Kolawole, Adeola Elizabeth Adeniji, Saheed Kayode Tairu, Toheeb Adedolapo Jumah, Adepeju Matilda Adekoya, Tosin Titus Olaniran, Emmanuel Oluseyi Adeniyi (2025). Chemotherapeutic Potential of Methanol Leaf Extract of Telfairia occidentalis on Oxidative Stress, Hepatic, Hematological, and Biochemical Alterations in DMBA-Induced Breast Cancer in Wistar Rats . *Archives of Advances in Biosciences*, *16*(1), 1–8. https://doi.org/10.22037/aab.v16i1.49245

https://journals.sbmu.ac.ir/aab/article/view/49245

Article info:
Received: 25 Jul 2025
Accepted: 27 Jul Dec 2025
Published: 04 Aug 2025

*** Corresponding author:**

Hassan Abdulsalam Adewuyi, MSc.

Address: Department of Biochemistry, Federal University of Technology, PMB 65 Minna, Niger State Nigeria

E-mail:
hasselbatch @gmail.com

Abstract

Introduction: *Telfairia occidentalis* is a tropical plant widely grown in West Africa, traditionally used for treating several health issues. This study examined the effects of methanol and water extracts of *T. occidentalis* leaves on oxidative stress markers, liver function, blood parameters, and biochemical indices in Wistar rats with breast cancer induced by 7,12-dimethylbenz[a]anthracene.

Materials and Method: A randomized design was used with five groups of rats (10 per group), treated with T. occidentalis extracts at 100, 200, and 400 mg/kg doses. The methanol extract was prepared using Soxhlet extraction (1:8 plant: solvent ratio). DMBA (30 mg/kg in corn oil) was administered orally once weekly for 4 weeks. Standard techniques were applied for phytochemical screening and toxicity testing. Antioxidant enzymes, liver markers, blood cells, glucose, and cholesterol were analyzed.

Results: Phytochemical screening showed varied levels of bioactive compounds. No toxicity was noted up to 400 mg/kg. Results showed significant ($p < 0.05$) dose-dependent improvement: MDA, liver enzymes, WBC, glucose, and cholesterol levels decreased significantly, while SOD, CAT, and Hb levels increased in extract-treated rats compared to the cancer group.

Conclusion: The findings suggest that *T. occidentalis* leaves are rich in antioxidants and have liver-protective potential, making them a promising option for managing oxidative stress. The extract's safety also supports its possible use in future clinical trials.

Keywords: *Telfairia occidentalis*, phytochemicals, oxidative stress, toxicity, liver enzymes

1. Introduction

Breast cancer is a major public health issue and a leading cause of cancer mortality worldwide [1]. Its development involves both genetic and environmental factors. Oxidative stress plays a key role in this process, pointing to the importance of antioxidants and preventive agents [2]. Breast cancer is a multifactorial disease, and its prevention and treatment require a comprehensive approach. As breast cancer is complex, finding new and effective natural therapies is essential [3]. Several studies have investigated the potential of natural products in breast cancer prevention and treatment. These studies have shown promising results, highlighting the importance of continued research in this area.

Telfairia occidentalis, also called fluted pumpkin, is a tropical vegetable used traditionally for managing illnesses, including cancer [4]. Research has shown its antioxidant, anti-inflammatory, and DNA-protective effects [5, 6].

Although *T. occidentalis* is known for its health benefits, its role in preventing breast cancer is not well studied. This work aims to assess how its ethanol and aqueous leaf extracts affect oxidative stress, liver function, blood, and biochemical markers in rats with DMBA-induced breast cancer.

Discovering new plant-based antioxidants is key to cancer prevention strategies. Due to its rich antioxidant content, *T. occidentalis* could be useful in reducing oxidative stress and possibly preventing cancer. More studies are needed to confirm these benefits and understand how it works.

Natural products have long been part of traditional healing. Their value in treating breast cancer is gaining interest. *T. occidentalis*, with its antioxidant and anti-mutagenic properties, may help prevent or manage breast cancer. This study adds to the understanding of how this plant might be used in future cancer therapy.

2. Materials and Methods

Plants collection

Fresh *T. occidentalis* leaves were harvested from the Biological Garden at the Federal University of Technology, Minna, Niger State in April 2023. Plant identification was confirmed by Mr. Dangana M.C. from the Department of Biological Sciences, FUT Minna. All reagents used were obtained from Sigma Chemical Co., USA, while the equipment used was provided by the Department of Biochemistry, FUT Minna.

Experimental animals

Sixty rats weighing between 125 and 160 grams were sourced from the animal breeding unit of the National Veterinary Research Institute, Vom, Nigeria. The animals were housed in plastic cages at 22 ± 3 °C, with 50 ± 5% relative humidity, and a 12-hour light/dark cycle. Rats had free access to clean water and standard pellet feed.

Plant Material and Extraction

The extraction process followed the method described by a previous study [7]. Leaves were rinsed, air-dried, and ground to a fine powder using an electric blender. The powder was extracted with methanol in a 1:8 ratio, heated for two hours in a distillation flask. The mixture was filtered and concentrated to yield the crude extract, and percentage yield was calculated using:

Percentage yield = (Weight of extract / Weight of powdered sample) × 100

Quantitative Phytochemical Analysis

The content of key phytochemicals in the extracts was determined using standard methods [8]. Alkaloids were measured using the method in [9], total flavonoids by [10], tannins by [11], phenolic acids by [12], and saponins by [13].

Acute Toxicity Study

The study was conducted using rats divided into five groups of ten. Group 1 served as the control and received 1 mL of distilled water, while groups 2 through 5 received 100, 200, 400, and 800 mg/kg of the extract. The animals were monitored for toxicity signs and mortality over 24 hours.

Experimental Design and Procedures

Fifty rats were assigned into five groups: a control group, a DMBA-induced cancer group, and three groups treated with *T. occidentalis* extracts at 100, 200, and 400 mg/kg. All groups, except the healthy control, were induced with 7,12-dimethylbenz[a]anthracene (DMBA) at a dose of 30 mg/kg body weight, administered orally once per week for four consecutive weeks. Following DMBA induction, rats in the treatment groups were administered methanol extract of T. occidentalis at 100, 200, and 400 mg/kg body weight for 28 days to evaluate its chemotherapeutic potential. Blood was collected on day 29 and analyzed for hematological

and biochemical parameters using the method in [14].

Although histopathological or imaging techniques were not employed to confirm tumor development, the success of DMBA induction was inferred from biochemical alterations, including elevated malondialdehyde (MDA), liver enzyme levels, and decreased antioxidant activity. These changes align with widely reported biomarkers indicative of DMBA-induced carcinogenesis in animal models.

Biochemical parameters

Thiobarbituric acid reactive substances (TBARS) were analyzed using the procedure by [15]; superoxide dismutase (SOD) activity by [16]; catalase (CAT) activity by [17]. Liver enzymes alanine transaminase (ALT) and aspartate transaminase (AST) were measured with the Reitman-Frankel method [18]. Alkaline phosphatase (ALP) was assessed using the method in [19]. Hemoglobin (Hb) and white blood cell (WBC) counts were measured using the Neubauer hemocytometer [20]. Glucose and cholesterol were determined using the glucose oxidase [21] and cholesterol oxidase methods [22], respectively.

Statistical Analysis

All data were analyzed with SPSS version 20.0 (IBM, USA). One-way ANOVA was used to evaluate differences between groups, and the least significant difference (LSD) test was employed for post hoc comparisons.

3. Results

Quantitative Phytochemical Analysis

T. occidentalis leaves contained moderate levels of alkaloids (12.56 ± 1.23 mg/g). Flavonoids were present in the highest concentration (20.45 ± 2.12 mg/g), followed by phenolic acids (15.67 ± 1.56 mg/g) and tannins (10.23 ± 1.12 mg/g). Saponins were found in the lowest quantity (8.34 ± 0.98 mg/g).

Table 1. Quantitative phytochemical Composition of *T. occidentalis* leaf extract

Phytochemical	T. occidentalis Leaf Extract (mg/g)
Alkaloids	12.56 ± 1.23^{c}
Flavonoids	20.45 ± 2.12^{a}
Phenolic acids	15.67 ± 1.56^{b}
Saponins	8.34 ± 0.98^{e}
Tannins	10.23 ± 1.12^{d}

Values are presented in Mean ± SEM of three replicate determinations. Values with different superscript alphabets down the colunm are significantly different ($p < 0.05$).

Acute toxicity of Methanol extract of T. occidentalis on Wistar rats

No deaths occurred at 100, 200, or 400 mg/kg doses, indicating good tolerance. At 800 mg/kg, two out of ten rats died, showing possible toxicity at higher levels. Observed symptoms included sluggishness and lack of appetite. The LD50 lies between 400 mg/kg and 800 mg/kg.

Table 2. Acute toxicity of Methanol extract of *T. occidentalis* on Wistar rats

Dose (mg/kg)	Mortality	Signs of Toxicity
100	0/10	None
200	0/10	None
400	0/10	None
800	2/10	Lethargy, loss of appetite, death

LD_{50} is greater than 400mg/kg body weight and less than 800 mg/kg bodyweight

Effect of Methanol extract of T. occidentalis of Oxidative Stress Marker of Wistar rats

Significant ($p < 0.05$) differences in oxidative stress parameters (MDA, SOD, CAT) were noted across all groups. The breast cancer group had elevated MDA and reduced SOD and CAT levels compared to the treated groups. Rats treated with T. occidentalis (100–400 mg/kg) showed marked improvements, with enhanced antioxidant enzyme activity and reduced lipid peroxidation.

Table 3. Effect of Methanol extract of *T. occidentalis* of Oxidative Stress Marker of Wistar rats

Group	MDA (nmol/mL)	SOD (U/mL)	CAT (U/mL)
Control	2.43 ± 0.35^{a}	12.56 ± 1.23^{a}	10.23 ± 0.98^{a}
DMBA 30 mg/kg	4.56 ± 0.56^{b}	6.34 ± 0.87^{b}	5.67 ± 0.76^{b}
DMBA + *T. occidentalis* (100 mg/kg)	3.45 ± 0.42^{c}	9.23 ± 1.02^{c}	7.89 ± 0.83^{c}
DMBA + *T. occidentalis* (200 mg/kg)	2.98 ± 0.36^{d}	10.56 ± 1.15^{d}	8.98 ± 0.93^{d}
DMBA + *T. occidentalis* (400 mg/kg)	2.67 ± 0.33^{e}	11.45 ± 1.23^{e}	9.67 ± 1.02^{e}

Values are presented in Mean ± SEM of three replicate determinations. Values with different superscript alphabets down the colunm are significantly different ($p < 0.05$).

Effects of Methanol extract of T. occidentalis of Serum Hepatic Indices of Rats

Serum levels of ALT, AST, and ALP were significantly ($p < 0.05$) higher in the cancer group compared to the treatment and control groups. T. occidentalis extract at 400 mg/kg caused the greatest reduction, bringing these enzyme levels closer to normal, indicating a dose-dependent hepatoprotective effect.

Table 4. Effects of Methanol extract of T. occidentalis of Serum Hepatic Indices of Rats

Group	ALT (U/L)	AST (U/L)	ALP (U/L)
Control	35.67 ± 4.23^{a}	40.56 ± 5.12^{a}	120.45 ± 10.56^{a}
DMBA 30 mg/kg	62.34 ± 6.78^{b}	65.67 ± 7.34^{b}	180.23 ± 15.67^{b}
DMBA + *T. occidentalis* (100 mg/kg)	49.56 ± 5.67^{c}	53.45 ± 6.23^{c}	150.56 ± 12.34^{c}
DMBA + *T. occidentalis* (200 mg/kg)	43.45 ± 5.12^{d}	47.67 ± 5.67^{d}	140.45 ± 11.23^{d}
DMBA + *T. occidentalis* (400 mg/kg)	39.56 ± 4.56^{e}	42.34 ± 5.12^{e}	130.56 ± 10.56^{e}

Values are presented in Mean ± SEM of three replicate determinations. Values with different superscript alphabets down the colunm are significantly different ($p < 0.05$).

Effect of Methanol extract of T. occidentalis of hematological parameters of Wistar rats

The breast cancer group showed reduced hemoglobin and elevated WBC counts compared to other groups. Treatment with extract significantly ($p < 0.05$) increased Hb levels and reduced WBC counts in a dose-dependent manner, with the most improvement seen at 400 mg/kg.

Table 5. Effect of Methanol extract of T. occidentalis of hematological parameters of Wista rats

Group	Hb (g/dL)	WBC (×10^9/L)
Control	14.56 ± 1.23a	6.78 ± 0.67a
DMBA 30 mg/kg	10.45 ± 1.12b	9.56 ± 0.98b
DMBA + *T. occidentalis* (100 mg/kg)	12.34 ± 1.15c	7.89 ± 0.78c
DMBA + *T. occidentalis* (200 mg/kg)	13.23 ± 1.23d	6.98 ± 0.67d
DMBA + *T. occidentalis* (400 mg/kg)	13.98 ± 1.23e	6.56 ± 0.56e

Values are presented in Mean ± SEM of three replicate determinations. Values with different superscript alphabets down the colunm are significantly different ($p < 0.05$).

Effect of Methanol extract of T. occidentalis of Biochemical Parameters of Wistar rats

Glucose and cholesterol levels were significantly ($p < 0.05$) elevated in the breast cancer group. Treatment with *T. occidentalis* extract caused a significant decrease in both parameters, particularly at 400 mg/kg, indicating potential anti-diabetic and lipid-lowering effects.

Table 6. Effect of Methanol extract of T. occidentlis of Biochemical Parameters of Wistar rats

Group	Glucose (mmol/L)	Cholesterol (mmol/L)
Control	5.67 ± 0.56^{a}	2.34 ± 0.23^{a}
DMBA 30 mg/kg	8.45 ± 0.87^{b}	3.56 ± 0.34^{b}
DMBA + *T. occidentalis* (100 mg/kg)	6.78 ± 0.67^{c}	2.67 ± 0.26^{c}
DMBA + *T. occidentalis* (200 mg/kg)	| 6.23 ± 0.62^{d}	2.45 ± 0.24^{d}
DMBA + *T. occidentalis* (400 mg/kg)	5.98 ± 0.59^{e}	2.34 ± 0.23^{e}

Values are presented in Mean ± SEM of three replicate determinations. Values with different superscript alphabets down the colunm are significantly different ($p < 0.05$).

4. Discussion

Phytochemical screening confirmed the presence of key compounds like flavonoids, alkaloids, and phenolic acids, which are known for their antioxidant, anti-inflammatory, and antimicrobial actions [23, 24]. The abundance of flavonoids might explain the extract's protective effects against oxidative stress and inflammation, aligning with earlier findings [25].

The acute toxicity evaluation indicated that the extract is relatively safe at doses up to 400 mg/kg. The absence of toxicity signs at lower doses and the mild effects at 800 mg/kg suggest a favorable safety profile [26]. This supports its potential use in further studies and possible therapeutic applications.

The antioxidant study showed that the extract significantly ($p < 0.05$) lowered MDA levels while restoring SOD and CAT activity. These effects were dose-dependent and point to the extract's ability to combat oxidative stress, a critical factor in cancer development. The results align with previous studies supporting the antioxidant strength of *T. occidentalis* [27].

Hepatoprotective effects were also evident. The extract helped normalize ALT, AST, and ALP levels, which were elevated in the breast cancer group. The most notable improvements were observed at 400 mg/kg. This implies the extract's capacity to protect liver cells from DMBA-induced damage [28].

The hematological improvements, such as increased

Hb and reduced WBC counts, suggest that the extract has blood-protective and anti-inflammatory properties [19]. These effects may help reverse damage caused by cancer or oxidative stress.

Metabolic parameters like glucose and cholesterol were also regulated by the extract. These findings hint at its potential anti-diabetic and cholesterol-lowering effects, particularly at higher doses [21]. This supports the idea that *T. occidentalis* could help manage both cancer and related metabolic issues.

The observed chemotherapeutic effects of T. occidentalis may be attributed to its rich content of flavonoids and phenolic acids. These bioactive compounds are known to activate antioxidant defense systems through modulation of the Nrf2 signaling pathway and suppression of lipid peroxidation. Additionally, phenolic acids exert direct free radical scavenging activity, which may account for the observed improvements in oxidative stress markers, liver function, and hematological indices in treated rats.

5. Conclusion

This study confirms that *T. occidentalis* leaf extract contains strong antioxidant properties and offers protection to the liver and blood. Its ability to reduce oxidative stress and regulate key biochemical markers suggests its usefulness in managing breast cancer and related disorders. Further research is needed to validate its safety and effectiveness in human studies.

Ethical Considerations

Compliance with ethical guidelines

All experimental procedures involving animals were approved by the Animal Use and Research Committee of the Federal University of Technology, Minna, Nigeria, under protocol number BCHFUTMINNA-012023.

Funding

This study was self-funded by the authors.

Author's contributions

All authors contributed equally to the development of this article.

Conflict of interest

The authors declare no conflict of interest existed while conducting this study

Acknowledgments

The authors appreciate Mallam Shuaib Ma'aji and Mr. Prince Chukwudi Ossai from the Department of Biochemistry, FUT Minna, for their technical assistance during lab work.

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