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Studies on the Toxicity and Anticancer Properties of Stem-Bark Extract of *Prosopis africana* Plant

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This study investigated the anticancer and hepatoprotective potential of *Prosopis africana* stem bark fractions in 7,12-dimethylbenz[α]anthracene (DMBA)-induced mammary cancer in female albino Wistar rats. The stem bark was extracted using ethanol, and fractions (F1, F2, F3) were obtained via column chromatography. Acute toxicity testing revealed dose-dependent mortality at an LD_{50} of 5000 mg/kg, indicating low toxicity. DMBA induction significantly elevated cancer antigen 125 (CA125) levels (48.52 IU/L vs. normal control: 14.48 IU/L), confirming tumor development. Treatment with *Prosopis africana* fractions, particularly F1 at 500 mg/kg, markedly reduced CA125 levels (32.49 IU/L, 33% decrease), demonstrating potent anticancer activity. Additionally, DMBA-induced liver damage, evidenced by elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, was significantly mitigated by F1 (ALT: 30.66 IU/L; AST: 38.32 IU/L), suggesting hepatoprotective effects. Fraction F1 exhibited superior efficacy compared to F2 and F3. These findings highlight *Prosopis africana*'s therapeutic potential as a natural anticancer and hepatoprotective agent, supporting its use in traditional medicine.

Keywords: Anticancer, Breast, Mortality, *Prosopis africana*, Toxicity

Introduction

Medicinal plants and human beings have a unique relationship since time immemorial. Humanity's vital interest in plants, primarily as a source of food, shelter, and clothing, dates back to the very origin of human civilization. Tribal people have traditionally used medicinal plants as treatments for various illnesses, such as rheumatism, diabetes, inflammation, stomach problems, and even insect poisoning. Because of their distinct chemical characteristics, various bioactive substances, including phenolic compounds, flavonoids, terpenoids, saponins, and essential oils, have been extracted from these plants and have shown protective effects.

Prosopis africana (Guill. & Perr.) Taub (Mimosaceae) is a plant commonly found in sub-Saharan Africa, and it has been used in traditional medicine for centuries due to its various therapeutic properties. The stem bark of this plant has been reported to contain several phytochemicals, such as alkaloids, flavonoids, tannins, and saponins, among others, which have been linked to its anti-inflammatory, analgesic, antidiabetic, and antimicrobial activities. Despite its widespread use in traditional medicine, however, there is a lack of adequate scientific data on the toxicity profile of *Prosopis africana*.

Cancer is a complex and multifaceted disease characterized by the uncontrolled growth and spread of abnormal cells. It arises due to genetic mutations that disrupt normal cellular functions, leading to uncontrolled proliferation, evasion of cell death, and metastasis. Cancer is a leading cause of death worldwide, with an estimated 10 million deaths in 2020 alone. The disease manifests in various forms, including carcinomas, sarcomas, leukemias, lymphomas, and central nervous system cancers, each with distinct biological behaviors and treatment challenges.

Medically speaking, breast cancer is considered the most frequent malignant tumor that begins in the breast cells and spreads to other parts of the body. Breast cancer is a global disease of significant burden, and its incidence continues to rise globally, accounting for a high mortality rate in women. The number of new cases and deaths as a result of breast cancer in the United States is estimated to be 268,600 and 41,760, respectively, according to the American Cancer Society. The incidence of breast cancer in Black women is lower than that of White women, but the death rate in Black women is higher, possibly due to differences in diet and lifestyle. The prevalence of breast cancer in Nigeria has surpassed that of cervical cancer and may be attributed to factors such as delayed first childbirth, shorter duration of breastfeeding, family history of breast cancer, and late menopause, as reported by previous literature. The 7,12-dimethylbenz[α]anthracene (DMBA) is a procarcinogen specifically used to induce breast cancer in experimental rats, undergoing metabolic activation to the carcinogenic dihydrodiol epoxide. The carcinogenic and mutagenic activity of DMBA requires metabolic activation by mixed-function oxidases located

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in rat liver microsomes. The dihydrodiol epoxide binds with adenine residues of deoxyribonucleic acid, resulting in mutagenesis and carcinogenesis.

The aim of this work is to investigate medicinal plant extracts (*Prosopis africana* stem bark) and study some of the therapeutic properties that might be responsible for its potential as an anticancer agent.

Materials and Methods

Sample Collection, Preparation, and Extraction

The stem bark of *Prosopis africana* was collected from Girei Local Government Area of Adamawa State, Nigeria, and dried under shade at room temperature in the Chemistry Department, Modibbo Adama University, Yola, Adamawa State. The sample was ground to powder using a mortar and pestle. Soxhlet extraction method was used to extract the crude extract of *Prosopis africana* stem bark using ethanol as the solvent.

Chromatographic Analysis of Prosopis africana Ethanolic Extract

Column chromatography and thin-layer chromatography were employed to separate and identify compounds in the *Prosopis africana* ethanolic extract. The chromatographic techniques facilitated the isolation of various fractions, which were analyzed for their constituent compounds. Column chromatography was carried out using ethyl acetate and n-hexane as the mobile phase. After preparation of the column, the stationary phase was packed into the column. The sample was loaded, followed by elution with the mobile phase, which separated the compounds based on polarity, and the fractions were collected as the mobile phase eluted from the column.

Preparation of 7,12-Dimethylbenz[α]anthracene (DMBA) (Carcinogen Substance)

Exactly 20 g of DMBA was dissolved in 100 mL of water.

Animal Collection

Female albino rats weighing between 120 g ± 20 g were obtained from the National Veterinary Research Institute (NVRI), Plateau State, Nigeria. The rats were housed in propylene cages and provided with a standard grower diet (Vital Feeds) and water. The rats were maintained under laboratory conditions of temperature (28°C) and a 12-hour light and dark cycle for seven days to allow acclimatization before the commencement of the experiment.

Sub-acute Toxicity Test

The acute toxicity test was carried out according to the method described by Lorke (1983) and adopted by Karber (2007) with slight modifications. The principle employed was to administer the test limit according to body weight. Twenty albino rats of 120 g ± 20 g were grouped into five groups of four rats each. The animals in each group received a specific dose, starting from group one, which received the lowest dose of 1000 mg/kg, to the highest dose of 5000 mg/kg. The animals were observed for 24 hours for changes in behavior as well as mortality. The LD_{50} was calculated using the arithmetical method of Karber, as follows:

$$LD_{50} = LD_{100} - \sum \left[\frac{a \times b}{n} \right]$$

where, LD_{50} = Median lethal dose, LD_{100} = Least dose required to kill 100%, a = Dose difference, b = Mean mortality, n = Group population.

Experimental Design of the Cancer Test

Forty rats were randomly divided into eight equal groups of five rats each. Group 1 served as the normal control (no DMBA inducement). Breast carcinogen (DMBA) was induced at a dose of 15 mg/kg body weight in groups 2, 3, 4, 5, 6, 7, and 8. Group 2 served as the experimental control. After 4 weeks of inducing cancer, the rats were treated with *Prosopis africana* fractions (F1, F2, and F3) at 250 mg/kg and 500 mg/kg body weight for 4 weeks consecutively.

Histological Investigation

Mammary gland biopsies were excised from the experimental animals of each group after collecting blood samples. The biopsies were fixed in a 10% neutral formalin solution. The sections were processed in an alcohol-xylene series, embedded in paraffin, and sections of 0.45 microns were cut using microtome techniques. After microtomy, they were stained with hematoxylin and eosin. The different sections were examined microscopically for the evaluation of histopathological changes.

Statistical Analysis

The results were presented as mean value ± standard error of mean (SEM). One-way ANOVA was used for multiple comparisons of groups.

Results and Discussion

Acute Toxicity Test of Prosopis africana Using Albino Wistar Rats

The acute toxicity test of *Prosopis africana* stem bark extract is shown in Table 1. No deaths were observed in groups 1–3, which received doses of 1000–3000 mg/kg. One death was observed in group 4, which received a dose of 4000 mg/kg. Two deaths were observed in group 5, which received a dose of 5000 mg/kg. All animals survived phase one of the acute toxicity test. Only the animals administered with the highest doses of 4000 and 5000 mg/kg of the ethanolic extract died; therefore, the LD_{50} of the extract was calculated as 4812.5 mg/kg.

Table 1 Acute toxicity test of *Prosopis africana* stem bark extract

Group	Dose (mg/kg)	Dose Diff. (a)	Dead	Mean Dead (b) Product (a × b)
1	1000	1000	0	0
0				
2	2000	1000	0	0
0				
3	3000	1000	0	0
0				
4	4000	1000	1	0.25
250				
5	5000	1000	2	0.5
500				

Column Chromatographic Analysis of Prosopis africana Stem Bark Crude Extract

The results of the column chromatographic analysis are presented in Table 2. The largest fraction (Fraction 1) is dark brown in color and represents about 32.4% of the total mass. Fractions 2 and 3 are brown and yellow in color, respectively, and represent 20.8% and 12.4% of the total mass, respectively. These results can be useful for identifying the presence of specific compounds, optimizing chromatography conditions, and scaling up purification processes.

Table 2 Column Chromatographic Analysis of *Prosopis africana* Stem Bark Crude Extract

Fractions	Mass (g)	Percentage Yield	Color
1	8.1	32.4	Dark brown
2	5.2	20.8	Brown
3	3.1	12.4	Yellow

Anticancer Investigation of the Fractions of Prosopis africana Stem Bark

Table 3 shows the results of investigating the anticancer activity of fractions of *Prosopis africana* stem bark on cancer antigen 125 (CA125) and liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST). CA125 is a tumor marker for ovarian cancer. The normal control group had a mean CA125 level of 14.480 ± 0.679 IU/L, which is within the normal range. The negative control group had a significantly higher mean CA125 level of 48.523 ± 1.418 IU/L, indicating the presence of cancer. The treatment groups (F1, F2, and F3) showed a dose-dependent decrease in CA125 levels, with the highest dose (500 mg/kg) showing the most significant reduction. This suggests that the fractions of *Prosopis africana* stem bark may have anticancer activity.

ALT and AST are liver enzymes that are often elevated in response to liver damage or disease. The normal control group had mean ALT and AST levels of 21.183 ± 0.580 IU/L and 27.646 ± 1.191 IU/L, respectively, which are within the normal range. The negative control group had significantly higher mean ALT and AST levels, indicating liver damage or disease. The treatment groups showed a dose-dependent decrease in ALT and AST levels, with the highest dose (500 mg/kg) showing the most significant reduction. This suggests that the fractions of *Prosopis africana* stem bark may have hepatoprotective activity.

Overall, the results suggest that the fractions of *Prosopis africana* stem bark may have anticancer and hepatoprotective activities, with the highest dose (500 mg/kg) being the most effective. However, further studies are needed to confirm these findings and to determine the mechanisms of action.

Table 3 Anticancer Investigation of *Prosopis africana* Fractions on CA125, ALT, and AST

Group	CA125 (IU/L)	ALT (IU/L)	AST (IU/L)
Normal control	14.480 ± 0.679	21.183 ± 0.580	27.646 ± 1.191
Negative control	48.523 ± 1.418	43.453 ± 2.283	50.026 ± 0.549
F1 250	39.286 ± 0.448	34.910 ± 0.998	42.183 ± 0.455
F1 500	32.487 ± 0.857	30.660 ± 0.308	38.320 ± 1.211
F2 250	40.863 ± 0.759	39.453 ± 0.355	42.010 ± 0.929
F2 500	39.416 ± 0.591	35.310 ± 0.927	40.150 ± 0.364
F3 250	40.830 ± 0.601	39.816 ± 0.268	40.776 ± 1.345
F3 500	40.190 ± 0.383	37.796 ± 0.606	37.883 ± 0.627

Conclusion

The study investigated the therapeutic potential of *Prosopis africana* stem bark fractions (F1, F2, F3) in DMBA-induced mammary cancer in rats. Acute toxicity tests showed no mortality at doses ≤ 3000 mg/kg, with an LD_{50} exceeding 5000 mg/kg, indicating low toxicity. Anticancer activity assessment revealed that DMBA induction elevated CA125 levels (48.52 IU/L vs. normal 14.48 IU/L). Fraction F1 (500 mg/kg) showed the strongest reduction in CA125 (32.49 IU/L, 33% decrease). Fractions F2 and F3 had moderate effects (15–18% reduction). Hepatoprotection studies indicated that DMBA increased ALT (43.45 IU/L) and AST (50.03 IU/L), suggesting liver damage, but F1 (500 mg/kg) restored ALT to 30.66 IU/L and AST to 38.32 IU/L, close to normal levels.

In conclusion, *Prosopis africana* extract is safe at moderate doses ($LD_{50} > 5000$ mg/kg). Fraction F1 (500 mg/kg) exhibited

the strongest reduction in CA125, indicating potent antitumor activity. Additionally, F1 significantly mitigated DMBA-induced hepatotoxicity, outperforming F2 and F3. These results suggest that *Prosopis africana* fractions, especially F1, possess significant anticancer and hepatoprotective properties, supporting its traditional medicinal use.

Conflict of Interest

The authors declare no competing interest.

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