Calotropis Procera Triggered Biochemical and Molecular Alterations In Breast Cancer Caused By DMBA In Female Albino Rats

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KEYWORDS

Calotropis procera , Anticancer , Plant extract, Breast cancer, Rat, Lipid profile

ABSTRACT

Objectives: To investigate the *in vivo* anticancer effect of *Calotropis procera* on breast cancer induced in female albino rats with 7, 12-dimethyl benz (a) anthracene (DMBA).

Materials and study design: Fresh leaves of *C. procera* were obtained from 20 plant specimens collected from various regions in the Dawadmi governorate, Riyadh province, KSA (latitude 24.75387 and longitude 46.773686) in August 2022. Three extracts were obtained from *C. procera* leaves (aqueous, chloroform, and ethanolic extracts) were investigated for their anticancer activity on female albino rats that were classified into five equal groups (n=30): group 1 (control), group 2 (DMBA control), group 3 (DMBA + aqueous extract), group 4 (DMBA + chloroform extract), and group 5 (DMBA + ethanolic extract).

Results: The animals in groups 3, 4, and 5 treated with *C. procera* leaf extract exhibited a significant decrease in body weight compared to the animals in group 2 who were only given DMBA, and the survival analysis demonstrated a highly significant disparity between the groups, with the DMBA group displaying a greater incidence of tumors in comparison to the groups treated with *C. procera*. Biochemical findings indicated a notable variation in the average levels of cholesterol, TG, HDL and LDL between the groups.

Conclusion The results imply that aqueous, chloroform, and ethanolic *C. procera* leaf extracts may have preventive properties against tumor initiation and promotion.

1. Introduction

Breast cancer is one of the most common malignancies in women and the second leading cause of cancer-related death worldwide [1]. The incidence of breast cancer has been steadily increasing in recent years in Arab countries, with a significant number of cases being diagnosed only in advanced stages of the disease [1]. Among Saudi Arabian women, the incidence of breast cancer has progressively increased, and the disease occurs at an earlier age compared with that in western countries [2].

Common methods for the treatment of cancer are known to have undesirable effects, and alternative treatment options are limited; hence, there is an urgent need to develop new drugs from natural products that are more effective and exert fewer harmful effects. Several studies have focused on this issue [3], [4] and [5]. Many agents derived from natural sources exhibit anticancer properties without causing considerable adverse effects and, hence, play a vital role in developing new drug [6], [7], and [8]. Plants belonging to the Asclepiadaceae family exhibit a wide range of therapeutic activities. These plants are used in traditional medicine to treat ulcers, leprosy, tumors, hemorrhoids, hematological problems, blood pressure, respiratory diseases, and liver and spleen ailments. Furthermore, these plants exert anticoagulant, purgative, anthelmintic, antipyretic, analgesic, antimicrobial, anti-inflammatory, and neuromuscular blocking effects [9]. Moreover, the stems, flowers, latex, and leaves of plants from the family Asclepiadaceae contain compounds known as cardiac glycosides, which have been investigated

for their antiproliferative effects [10]. Calotropis procera (Asclepiadaceae) is a herb that has been commonly used in folk medicine to treat various diseases for more than 1500 yeas [11].

C. procera is a wild-growing tropical plant widely distributed across Asia, Africa, and the Northeast of Brazil and is one of the versatile medicinal plants. It has often been used for treating fever, joint pain, constipation, and muscular spasms in Saudi Arabia [12]. Several active compounds are present in almost all parts of *C. procera*. However, the total amount or the relative distribution of any given plant may vary based on ecological factor [13]

According to a previous study, *C. procera* has a high latex producing capacity and is well known for its toxic effects [14]. Various chemical constituents in extracts from this plant exert inhibitory effects on tumor and cancer cell lines. Mainly, the roots and latex parts of *C. procera* have anticancer potential owing to the presence of calotropinol, proceragenin, calotoxin, hydroxyketone, procesterol, multiflorenol, cyclosadol, βsitostenone, uzarigenine, βanhydroepidigitoxigenin, pentacyclictriterpinoids, [15], [16] and cysteine proteinase [17]. Hence, in the present study, we aimed to investigate the in vivo antiproliferative effects of *C. procera* on breast cancer induced by 7,12-dimethyl benz(a)anthracene (DMBA) in animal experiment designed models.

2. Methodology

Plant collection and extraction methods

Fresh leaves of *C. procera* were obtained from 20 plant specimens collected from various regions in the Dawadmi governorate, Riyadh province, KSA (latitude 24.75387 and longitude 46.773686) during August 2022. The leaves were identified and authenticated by the Department of Botany, College of Science, Shaqra University, Dawadmi governorate, Riyadh province, KSA.

The aqueous, chloroform, and ethanolic extracts of *C. procera* leaves were prepared according to previously described protocols[19] and stored dry at 4°C until use. Briefly, 200 g of the leaves were dried in the shade, ground, and soaked in 500 mL of water, chloroform, and ethanol for 72 h at room temperature. The supernatant was filtered through a Whatman No. 4 filter paper, centrifuged (at 600 × g for 10 min), and dried in lyophilizer (Christ Beta 1-8 K, Bio block Scientific, Germany) for 72 h. A final mass of 30 g was obtained from the raw powdered extract.

Animals, materials, and groups

The study done in accordance with the guidelines of the National Council for the Control of Animal Experimentation and all trials were carried out with approval from the Animal Experimental Ethics Committee of Shaqra University, KSA, following internationally accepted procedures (License no. ERC_SU_20230014). A total of 150 Wistar breed female (40-day-old) rats were divided equally into five groups, as follows:

Group 1 (n = 30) control group:

The animals were fed with a standard diet. Water was provided *ad libitum* throughout the experimental period.

Group 2 (n = 30), DMBA control group:

The animals were orally administered 80 mg/kg DMBA in sunflower oil via gavage to induce a tumor and were fed with a standard diet. Water was provided *ad libitum* throughout the experimental period as described by Kaur *et al.* [13].

Group 3 (n = 30), DMBA + aqueous extract of C. procera leaves:

The animals were orally administered 80 mg/kg DMBA in sunflower oil via gavage to induce a tumor and were fed with a standard diet. Water was provided *ad libitum* throughout the experimental period, and aqueous extract of *C. Procera* leaves dissolved in 1% carboxymethylcellulose (CMC) was orally administered at 5 mg/kg/day for 4 weeks.

Group 4 (n = 30), DMBA + chloroform extract of C. procera leaves:

The animals were orally administered 80 mg/kg DMBA in sunflower oil via gavage to induce a tumor and were fed with a standard diet. Water was given *ad libitum* throughout the experimental period, and the chloroform extract of *C. procera* leaves dissolved in 1% CMC was orally administered at 5 mg/kg/day for 4 weeks.

Group 5 (n = 30), DMBA + ethanolic extract of C. procera leaves:

The animals were orally administered 80 mg/kg DMBA in sunflower oil via gavage to induce a tumor and were fed with a standard diet. Water was given *ad libitum* throughout the experimental period, and the ethanolic extract of *C. procera* leaves dissolved in 1% CMC was orally administered at 5 mg/kg/day for 4 weeks. All groups were kept in an environment with standard temperature and light (21 ± 1°C and 12-h dark/light cycle).

Leaf extract administration

The leaf extract was prepared and dissolved in 1 mL of distilled water and injected intraperitoneally with 0.5 mL of saline solution (5 mg/kg of body weight/day). The treatment was performed between 10.00 a.m. and 11.00 a.m. for 4 weeks according to Ahmad, 2020 [18].

Histopathological evaluation

The rats were palpated on a weekly basis to determine the presence of mammary tumors. Tumor development was pathologically seen in the 6^{th} week after DMBA administration. Tissue samples were sent to the pathology laboratory in 10% formalin for histopathological examination. Furthermore, 4-µm thick sections from paraffin-embedded tissues were stained with hematoxylin–eosin (H&E) and examined under a light microscope (Olympus BX50). The presence of the tumor was pathologically detected, and based on microscopic examination of tumor size, the tumor grade was reported. The tumor volume was estimated according to the method of Saddiq et al., 2022 [20]. Briefly, the solid tumor was considered a prelate ellipsoid with one long axis and two short axes. The two short axes were measured with vernier calipers. The tumor volume was calculated using the following formula:

Tumor volume = 0.5 × length (cm) × width^2 (cm)

Biochemical studies

Blood was collected with and without anticoagulant and the serum was centrifuged at 5000 rpm for 15 min to obtain a clear supernatant and stored at −80°C until its use for further biochemical analysis. Breast tissue was homogenized in ice-cold lysis buffer. The homogenates were centrifuged for 15 min at 16,000 rpm at 4 °C. Supernatants were collected and used in biochemical and molecular studies.

Tissue Homogenates were weighed and rinsed in 5-10 mL PBS* to remove excess blood. The cells were lysed by ultrasonication followed by centrifugation at 5000×g for 5 minutes.

Lipid profile

Estimation of lipid profile: Plasma levels of cholesterol, triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and total cholesterol (TC) were measured by standardized enzymatic procedures, using kits supplied by Roche Diagnostics (Mannheim, Germany) on the Olympus AU 400 automated clinical chemistry analyzer. Low density lipoprotein cholesterol (LDL-C) was calculated according to formula of Friedwald et al. [21].

Statistical analysis

The data were statistically analyzed using SPSS 21.0 computer software, and the arithmetic means and standard deviations of all parameters were calculated. Kolmogorov–Smirnov test was applied to determine the homogeneity of the data, and the data were found to display a normal distribution. One-way analysis of variance was employed to identify the differences among groups, and the origin of the

differences was detected using Duncan's multiple range test. Differences with $P < 0.05$ were accepted as significant.

Result and Discussion

Changes in Body Weight

Table (1) and Figure (1a) depict the alterations in body mass within various cohorts as compared to the control group exhibiting normalcy. The rats belonging to the control group (group 1) displayed a typical growth pattern and achieved a standard weight gain, reaching a value of 327.6 ± 10.5 g over a span of 6 months. Within all groups exposed to the carcinogen or *C. procera* (groups 2, 3, 4, and 5), the subjects exhibited a regular augmentation in body mass during the initial 3 weeks, only to subsequently experience a noticeable decline in comparison to the control group, particularly in group 2. The mean value ± standard deviation of body weight (g) in group 2 was 215.6 ± 12.6 and exhibited a significantly ($P < 0.05$) lower mass compared to the other groups. Additionally, the animals subjected to DMBA and *C. procera's* aqueous, chloroform, and ethanolic extracts (3, 4, and 5) also displayed significant changes in body weight. Group 1, treated with different extracts of *C. procera* leaves, experienced less weight loss compared to group 2 treated with DMBA only, indicating that these extracts have a beneficial effect on preventing breast cancer progression.

Table 1: The mean values ± DEV of the body weight (gm) in different groups during the period of the experiment.

Groups / Months	G 1	G 2	G 3	G 4	G 5
1	99.9±2.68	100.0±1.66	100.4±1.34	100.4±1.44	100.6±1.51
2	158.3±8.21	130.2±7.68	130.8±5.44	131.7±4.73	135.7±5.35
3	233.5±4.39	159.4±4.14	180.5±4.60	198.0±10.24	206.8±8.53
4	270.3±4.25	184.8±5.78	205.9±6.72	215.0±4.26	218.2±4.12
5	302.5±3.38	191.5±8.13	222.7±5.08	228.6±6.47	233.1±6.27
6	333.1±7.56	219.1±6.75	269.4±13.11	276.5±14.51	279.3±6.53

Data are expressed as mean ± SEM. **P*<0.001 compared with the control Group 2. ***P*<0.001 compared with normal control Group 1.
G1: Control untreated group
G2: DMBA treated group
G3: (DMBA + aqueous leave extract) treated group;
G4: (DMBA + Chloroform leave extract) treated group
G5: (DMBA + Ethanol leave extract) treated group

Survival Rate

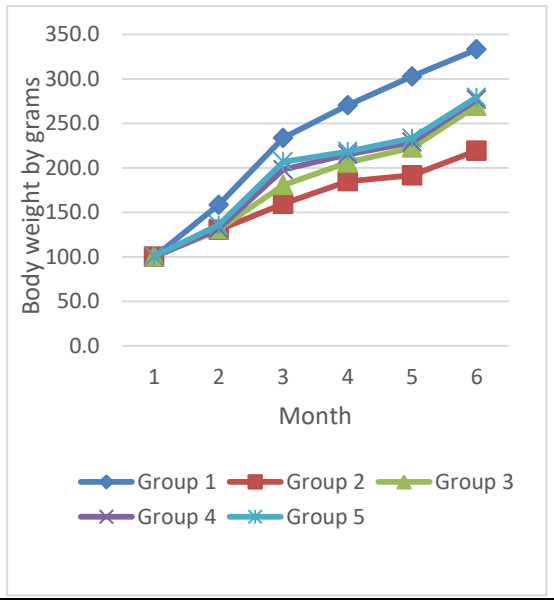
Table (2) and Figure (1b) illustrate the survival rates of experimental animals during the duration of treatment. The application of survival analysis revealed a highly significant disparity between the group that solely received the carcinogen and the groups that were administered *C. procera* extract alongsidethe carcinogen ($P < 0.001$) over the course of treatment.

At the six-month mark of the experiment, it was observed that the group of animals treated solely with the carcinogen (group 2) exhibited the lowest survival rate among all groups (26.7%) and succumbed earlier than the control group (100%) and the other treated groups. These other groups displayed survival rates of 66.7% for the DMBA + Aqueous leaf extract-treated group, 70% for the DMBA + chloroform leaf extract-treated group, and 63.3% forthe DMBA + ethanol leaf extract-treated group.

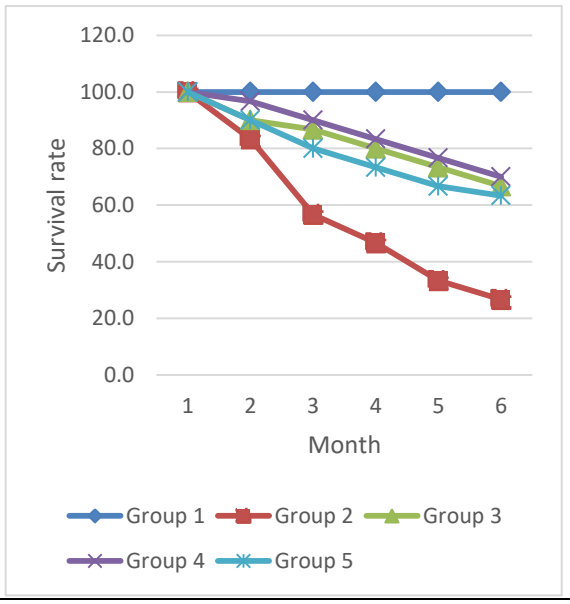
Table 2: The survival rate in different groups during the period of the experiment.

Groups / Months	G 1	G 2	G 3	G 4	G 5
1	100.0	100.0	100.0	100.0	100.0
2	100.0	83.3	90.0	96.7	90.0
3	100.0	56.7	86.7	90.0	80.0
4	100.0	46.7	80.0	83.3	73.3
5	100.0	33.3	73.3	76.7	66.7
6	100.0	26.7	66.7	70.0	63.3

G1: Control untreated group; G2: DMBA treated group; G3: (DMBA + aqueous leave extract) treated group; G4: (DMBA + Chloroform leave extract) treated group; G5: (DMBA + Ethanol leave extract) treated group



(1a)



(1b)

Figure 1: Body weights and survival rate changes in different groups.

(1a): The mean values of body weights in the control and other treated groups during the experiment period (6 Months)
(1b): The Survival rate in different groups during the experiment period (6 Months)
G1: Control untreated group; G2: DMBA treated group; G3: (DMBA + aqueous leave extract) treated group; G4: (DMBA + Chloroform leave extract) treated group; G5: (DMBA + Ethanol leave extract) treated group.

Histopathology

Histopathological studies of mammary tissues revealed tumor incidence of 100% (30/30) in rats treated with DMBA alone (group 2), which decreased to 66.6% (18/30), 50% (15/30), and 43.3% (13/30) in groups 3, 4, and 5, respectively. The difference between the tumor incidence rates was statistically significant ($P < 0.05$). The final tumor incidence rate of the DMBA-only treated group was 1.5, 2.0, and 2.3 folds higher than that of groups 3, 4, and 5, respectively (Table 3 and Fig. 2a).

The number of tumors, the mean values of the tumor diameter, and the volume of breast tumors at the end of the intervention are listed in Table 3 and depicted in Fig. 2b, Fig. 2c, and Fig. 2d. The first tumors were reported 110 days after receiving DMBA in all groups, and the incidence increased during the period. Examination of the mammary gland tissue in group 1 (control) revealed the normal histological architecture of the mammary glands, which was composed of branching ducts surrounded by connective tissue, adipose tissue, and a few acini (Fig. 3a).

Examination of the mammary gland sections from DMBA rats (group 2) revealed destruction of the mammary gland architecture, and the normal surrounding adipose tissue was poorly detected and occupied by intense inflammatory reactions with marked fibrosis. In addition, the glandular tissue was completely replaced by dense populations of malignant tumor cells (Fig. 3b and Fig. 3c).

Table 3: Tumor evolution in treatment groups.

Parameter	G1	G2	G3	G4	G5
% Tumor incidence	0.00% (0/30)	100.00 % (30/30)	66.6 % (18/30)	50 % (15/30)	43.3 % (13/30)
No. of tumors (per tumor bearing rat)	0.00	3.23± 0.201	1.93± 0.219	1.87± 0.243	1.53± 0.124
Tumor diameter (mm)	0.00	43.88±5.86	29.98±5.06	27.38±4.47	26.72±4.21
Tumor volume (cm^3) rat-1	0.00	28.24±1.334	19.24±0.781	17.56±0.698	15.72±0.690

G1: Control untreated group; G2: DMBA treated group; G3: (DMBA + aqueous leave extract) treated group; G4: (DMBA + Chloroform leave extract) treated group; G5: (DMBA + Ethanol leave extract) treated group

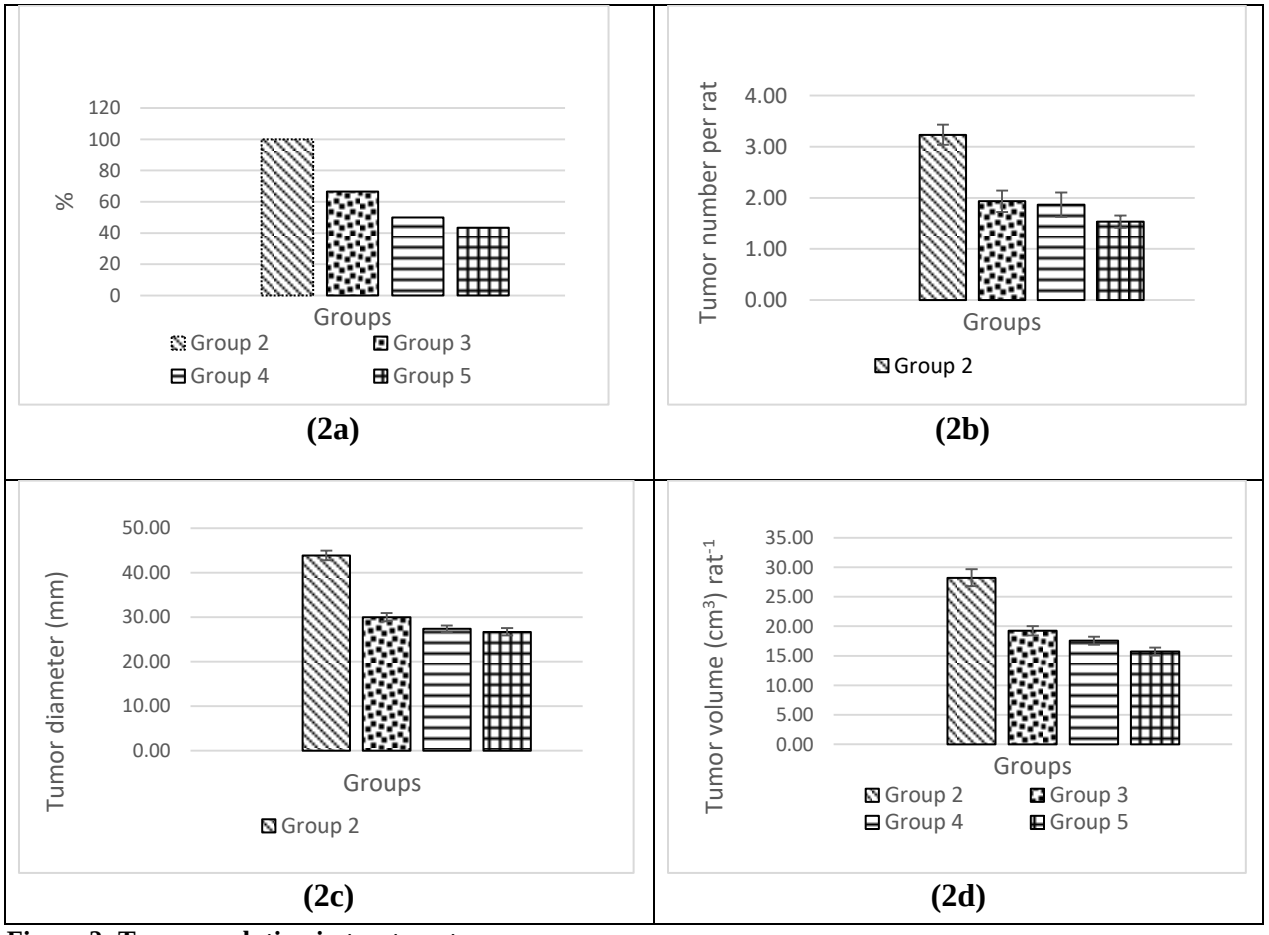


Figure 2: Tumor evolution in treatment groups.
(2a): Tumor incidence in different treatment groups
(2b): Tumor number (per tumor-bearing rat) in different treatment groups
(2c): Tumor diameter (mm) in different treatment groups
(2d): Tumor volume (cm^3) in different treatment groups
G1: Control untreated group; G2: DMBA treated group; G3: (DMBA + aqueous leave extract) treated group; G4: (DMBA + Chloroform leave extract) treated group; G5: (DMBA + Ethanol leave extract) treated group.

The mammary gland carcinoma detected in the DMBA group was graded as moderately differentiated (grade II) carcinoma characterized by poor tubular formation, increased mitotic figures per microscopic field, and marked nuclear and cellular pleomorphism. On the contrary, DMBA groups treated with *C. procera* leaf extract exhibited significant regression of the tumor grade, which was scored as well-differentiated (grade I) carcinoma (Fig. 3d, Fig. 3e, and Fig. 3f).

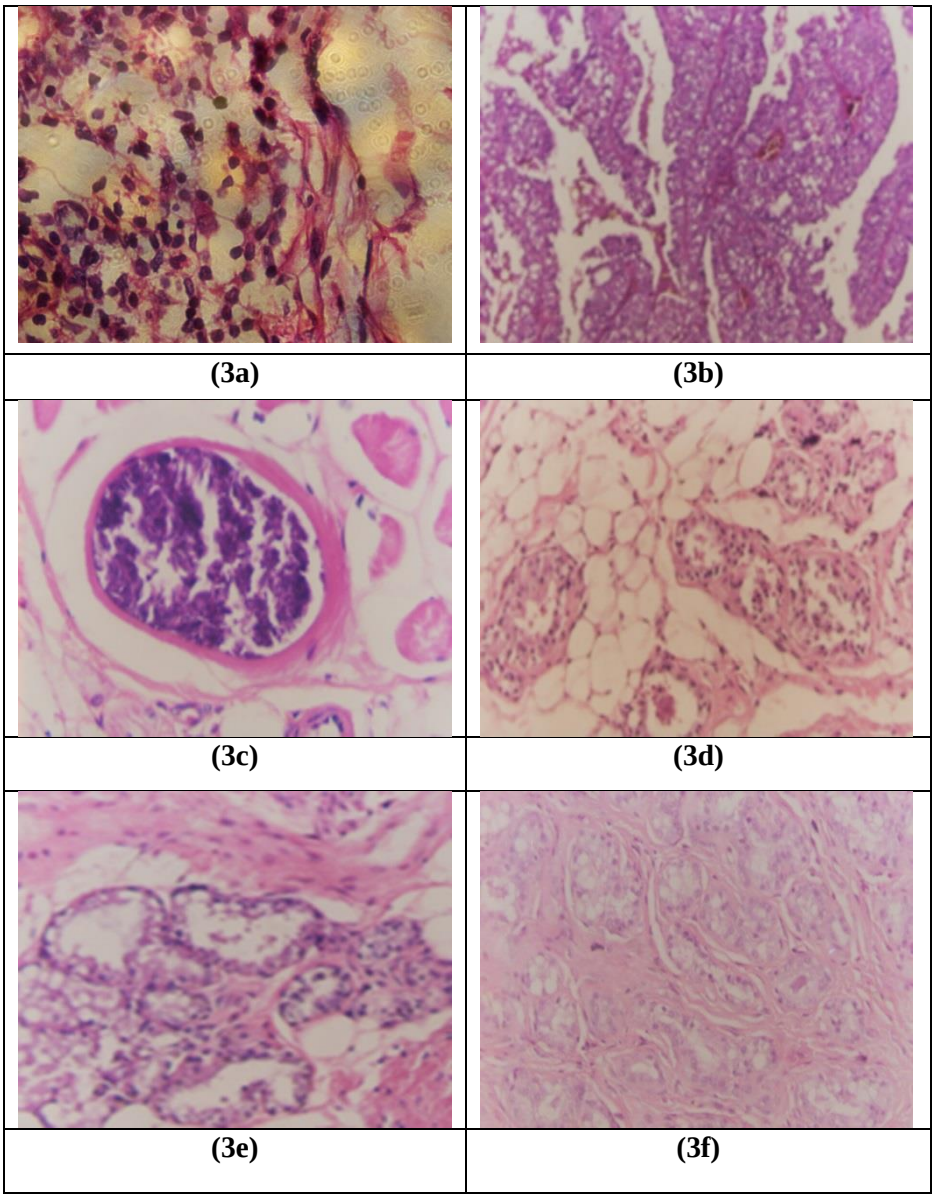


Figure 3: Histological section of the mammary gland in experimental groups.
(3a): Histological section of normal mammary gland of femal rat fed on standarad diet, showing normal duct and normal acini.
(3b): Histological section of mammary gland shows papillary adenocarcinoma. This type of cancer is characterized by tumor cell nests containing glandular spaces. Low power view. (H & E 125). The microphotograp from DMBA treated group.
(3c): Histological section of mammary gland shows hyperplastic ductal cells progecting in the lumen forming a papillary configuration (benign intraductal papilloma). Low power view. (H & E 125). The microphotograp from DMBA treated group.
(3d): Histological section of mammary gland showing ductal hyperplasia.The microphotograp from DMBA+ aqueous leave extract group.
(3e): Histological section of mammary gland showing mild hyperplasia characterized by an increase in ductal epithelial cells. The epithelial cells do not cross the duct lumen. The microphotograp from DMBA+ Chloroform leave extract group.
(3f): Histological section of the mammary gland shows fibroadenoma, in which overgrowth occurs in both epithelium and connective tissues. On section, they are paler and somewhat less vascular than carcinomas.The microphotograp from DMBA+ Ethanol leave extract group.

Examination of mammary gland sections from female rats that received aqueous, chloroform, or ethanolic extracts of *C. procera* leaves in addition to DMBA (groups 3, 4, and 5) showed significant differences between the animals.

Animals treated with aqueous extract of *C. procera* leaves (group 3) demonstrated ductal hyperplasia (Fig. 3d). Furthermore, those treated with the chloroform extract (group 4) showed signs of malignancy, including mild hyperplasia characterized by an increase in ductal epithelial cells. The epithelial cells did not cross the duct lumen (Fig. 3e). Additionally, animals treated with the ethanolic extract (group 5) showed fibroadenoma in which overgrowth occurred in both epithelial and connective tissues, which were paler and somewhat less vascular than the carcinomas (Fig. 3f).

Biochemical studies

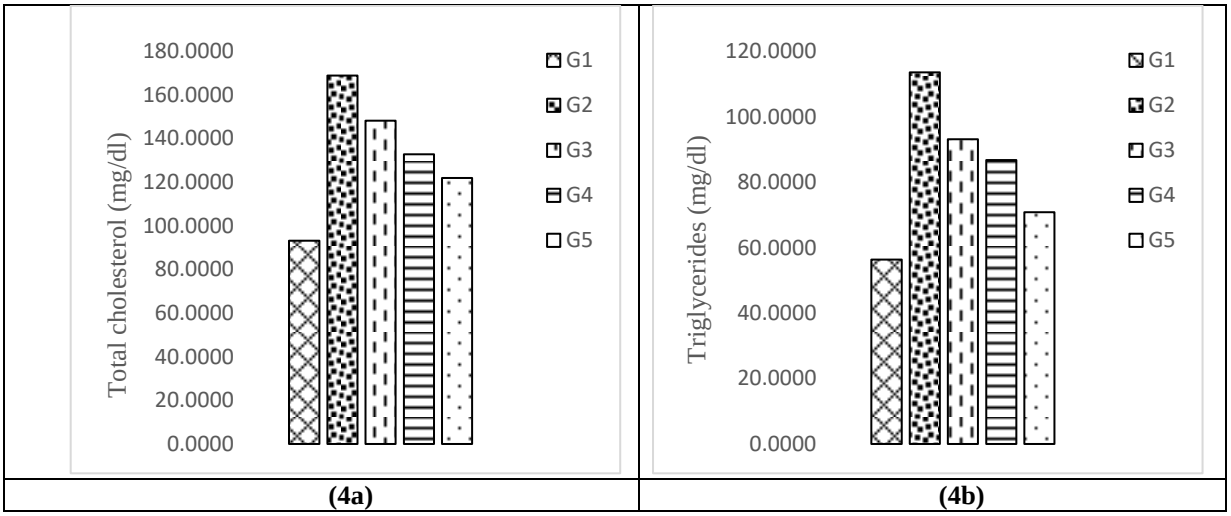
The levels of Total Cholesterol and Triglycerides or lipoproteins (LDL and HDL) are significantly elevated in DMBA treated animals (Group 2) when compared to control rats (Group 1). Experimental animals showed alteration in the serum levels of total Cholesterol and Triglycerides in addition to lipoproteins (LDL and HDL), significant (P >0.05) change after-treatment of the experimental animals in groups 3,4, and 5 with leaf extract when compared with the DMBA group or control group (Table 4 and Figures 4a, 4b, 4c and 4d).

Table 4: The mean levels of cholesterol, Triglycerides, HDL, and LDL in control, DMBA, and CP treated groups.

	G1 mean±SD	G2 mean±SD	G3 mean±SD	G4 mean±SD	G5 mean±SD	F	Sig.
Total cholesterol (TC)((mg/dL)	**(93.11±2.92)^{a}**	**(168.83±6.59) ^{d}**	**(148.06±1.81)^{c}**	**(132.69±4.09) ^{b}**	**(121.89±3.31) ^{b}**	**48.78**	**< 0.001**
Triglycerides (TG) (mg/dL)	**(56.37±1.65)^{a}**	**(113.48±1.85) ^{e}**	**(93.05±0.85) ^{d}**	**(86.74±1.96) ^{c}**	**(70.84±1.18) ^{b}**	**194.3**	**< 0.001**
HDL (mg/dL)	**(25.95±0.69)^{a}**	**(28.14±1.17)a**	**(31.52±0.92)b**	**(32.40±1.11)^{c}**	**(27.83±0.81)^{a}**	**7.8**	**< 0.001**
LDL (mg/dL)	**(55.85±3.17)^{a}**	**(117.9±6.2) ^{d}**	**(97.93±2.18)^{c}**	**(82.94±3.42) ^{b}**	**(79.89±3.19) ^{b}**	**33.5**	**< 0.001**

a, b, and c letter in the same raw differ significantly at $p < 0.05$.

G1: Control untreated group; G2: DMBA treated group; G3: (DMBA + aqueous leave extract) treated group; G4: (DMBA + Chloroform leave extract) treated group; G5: (DMBA + Ethanol leave extract) treated group.



(4a) **(4b)**

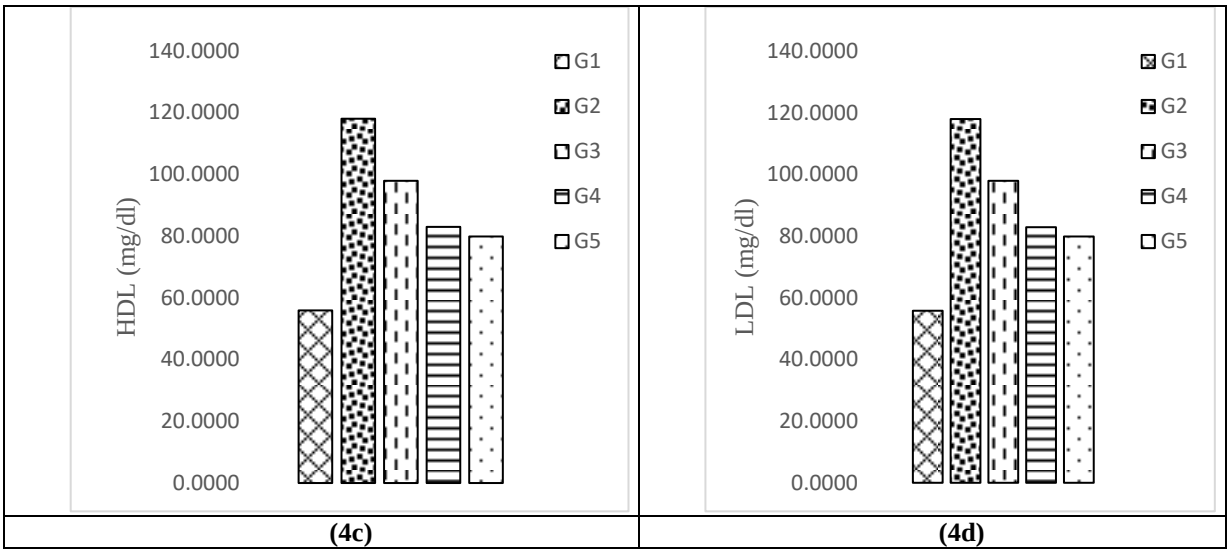


Figure 4: The mean levels of cholesterol, Triglycerides, HDL, and LDL in control, DMBA, and CP treated groups.
(4a): The mean levels of total cholesterol (mg/dl)
(4b): The mean levels of triglycerides (mg/dl)
(4c): The mean levels of HDL (mg/dl).
(4d): The mean levels of LDL (mg/dl)
G1: Control untreated group; G2: DMBA treated group; G3: (DMBA + aqueous leave extract) treated group; G4: (DMBA + Chloroform leave extract) treated group; G5: (DMBA + Ethanol leave extract) treated group.

Discussion

Multiple investigations have been carried out in recent times to examine the properties of extracts from *C. procera* [22, 23]. Our results showed that the group exposed to DMBA developed carcinogenesis in all animals; however, rats given *C. procera* leaf extracts had a lower tumor occurrence and smaller tumor mass compared to the DMBA-only group. These results are in alignment with numerous research observations that provide evidence for the fact that *C. procera* possesses a significantly elevated antiproliferative activity due to the presence of its biologically active compounds. These findings further strengthen the notion that *C. procera's* antiproliferative activity can be attributed to its diverse array of biologically active compounds, thereby solidifying its potential as a valuable source for the development of novel therapeutic interventions [23]. The study conducted by Choedon *et al.* successfully showcased that three distinct cardenolides with anticancer properties, which were extracted from the plant species *C. procera*, exhibited an extraordinary level of cytotoxicity when tested against numerous cancer cell lines. The findings of this research provide compelling evidence for the potential therapeutic application of these cardenolides in the treatment of cancer. This study serves as a significant contribution to the field of oncology, as it sheds light on the promising role of natural compounds derived from plants in the development of effective anticancer therapies [24]. The research conducted by Hasballah et al. revealed that the chemopreventive activity of the methanolic extract of *C. procera* was observed both in vitro and in vivo in hepatocellular carcinoma. The study demonstrated that the application of the methanolic extract of *C. procera* effectively suppressed the growth and development of hepatocellular carcinoma cells in laboratory settings, indicating its potential as a preventive measure against this type of cancer. Furthermore, the chemopreventive properties of the extract were also observed in vivo, suggesting that it may have a similar effect in a living organism. These findings contribute to our understanding of the potential therapeutic applications of *C. procera* and emphasize the importance of further investigation into its mechanisms of action and potential clinical applications [25]; the capacity of a hemisynthetic cardenolide to impede the proliferation of cancer cells by eliciting apoptosis has been convincingly demonstrated.

A remarkable discrepancy in the statistical analysis of body weight and rates of survival was noted among the various experimental groups, where it was evident that the DMBA-only treated rats experienced a noteworthy reduction in body weight when compared to the animals in the normal

control group, despite the fact that there was no alteration in their food and water intake, thus corroborating the conclusions drawn by Gul et al. [26].

The significant decrease in body weight could potentially be attributed to the process by which normal cells undergo a transformation into a malignant state, which comprises of various distinguishable stages, including the initiation of DNA damage, as well as subsequent events that occur during the progression of tumors in animals [27]. Several mechanisms lead to a reduction in the weight of the host organism in cases where carcinomas take place.

In a study conducted by Ramar et al. [28], the antitumor activities of protein derived from the root bark of C. procera were thoroughly examined. The researchers discovered that when this protein was administered to rats with DMBA-induced breast cancer, either on its own or in conjunction with cyclophosphamide; there was a noteworthy reduction in tumor volume. Remarkably, this reduction in tumor size occurred without any adverse effects on the rats' body weight [28].

The extracts effectively reduced tumor appearance and size, with the ethanolic extract showing the highest efficacy in reducing tumor incidence. Cancer cells impede the process of programmed cell death by acquiring an assortment of mutations or alterations, thereby evading apoptosis. [29]. Plant leaf extracts have been discovered to possess the ability to induce apoptosis, a programmed cell death mechanism. Moreover, numerous scientific studies have highlighted the fact that C. procera, a specific plant species, has the capacity to trigger apoptosis in a select group of cell lines derived from tumors, particularly in breast cancer models, thereby implying that other types of malignancies might also be vulnerable to apoptosis induction in living organisms [30].

Malignant neoplastic change is a complex process characterized by increased cell proliferation, dysplasia, and infrequent apoptosis [31]. The utilization of naturally derived substances, with their inherent chemical compounds, in the chemical prevention of cancer, represents a highly promising and auspicious strategy for the prevention and mitigation of the occurrence of diverse cancer types, as well as for hindering, delaying, or even treating the disease [32].

Elevated plasma lipids and lipoproteins were observed in groups given DMBA, while HDL decreased; however, administration of leaf extract significantly improved the results to nearly normal levels compared to the control rat.

Previous research has shown that the proliferation of cells in breast tumors is often accompanied by changes in plasma lipids and lipoproteins, indicating a potential link between lipids and the risk of breast cancer; recent studies have also suggested that increased lipid levels in tumor cell membranes may contribute to the progression and multiplication of tumor cells [33].

Limitations

In this study, *C. procera* leaf extracts were used without identifying the specific components of this extract. We recognize that as a limitation and strongly recommend that further studies be conducted in the future to identify the exact components of this extract.

We also did not study the mechanism by which the *C. procera* leaf extracts works to better understand and clarify the signaling mechanisms involved so we recomrndes further research should be conducted to investigate in details the mechanism by which the *C. procera* leaf extracts works.

3. Conclusion and future scope

The protective effect of aqueous, chloroform, and ethanolic *C. procera* leaf extracts in preventing or reducing DMBA-induced breast carcinoma was demonstrated in our study. These findings suggest the Potential of using plant extracts as alternative medicines for treating breast cancer, and further research is warranted to explore their pharmacological significance.

Conflict of interests

The authors declare that there are no conflicts of interests.

Acknowledgement

The authors extend their appreciation to the deanship of scientific research at Shaqra University for funding this research work through the project number (SU-ANN-2023037).

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