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Research Article

Therapeutic Potential of Withaferin-A and Propolis Combinational Drug Therapy for Breast Cancer: An *In Vivo* Interpretation for Validating the Antiproliferative Efficacy and Ameliorative Potential in Benzo[a]pyrene-Induced Breast Metastasis

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Cancer is a serious health problem, with a rising trend in its occurrence documented globally. Female breast cancer is the most common type of cancer worldwide with treatment consisting of different strategies like mastectomy, chemotherapy, and radiotherapy. Anticancer property has been studied in withaferin-A (WA), a bioactive compound of *Withania somnifera*. Another natural substance derived from bees, propolis, has been investigated for many beneficial effects on human diseases. The current study aims to investigate the ameliorative efficacy and antiproliferative potential of combinational drug therapy of withaferin-A and propolis on breast cancer cells. By evaluating the levels of glycoproteins, nucleic acids, and the marker enzymes in benzo[a]pyrene-induced breast cancer-bearing female Wistar rats, the pharmacodynamic effects of withaferin-A and propolis drug combination were examined. Biochemical analysis of DNA, RNA, and protein levels in the liver demonstrated typical results after propolis therapy. Withaferin-A and propolis drug combination treatment significantly decreased nucleic acid synthesis, indicating that combination chemotherapy has increased breast tumoricidal efficiency. The pharmacological combination therapy exhibited the capacity to control glycoproteins associated with tumor growth (hexose, hexosamine, and sialic acid), with a considerable decrease in their levels detected. Histopathological analysis of the mammary glands demonstrated a decrease in hyperplasia and cell proliferation, indicating that the treatment has the ability to reverse architectural and morphological abnormalities associated with breast cancer. This study found that natural drug compounds in combination have shown regenerative and regulating effects when given to rats carrying the breast cancer gene.

1. Introduction

Cancer is a significant health condition, and an increasing trend of its incidence has been reported worldwide for the next couple of decades. Millions of the populations are affected by carcinoma every year, according to the GLOBACON-2020 statistics [1]. In India, approximately 12% of the population faces cancer risk, which is an alarming situation for the healthcare system in the country. Among various tumors, lung cancer was the leading cancer till recently due to exposure to ubiquitously distributed pollutants. However, currently, female breast cancer superseded lung cancer worldwide. Female breast cancer is reported to have a high incidence among all cancers, with 2.3 million newly diagnosed cases becoming the most reported malignancy. By the very end of this decade, breast cancer will become the most prevalent cancer worldwide [2]. In 2020, approximately 7 million people died worldwide, and 2.3 million women were spotted with breast cancer. Apart from familial aspects, predisposition factors like consumption of contraceptives and alcohol and lifestyle modifications contribute to breast cancer risk. Furthermore, late-stage diagnosis due to lack of awareness makes it difficult to treat the condition. Alluding to the Indian subcontinent, women are at risk of developing breast cancer from their early thirties, which further escalates with age. Although female breast cancer is quite common, cases of male breast cancer have also been reported [3].

Breast cancer treatment consists of different strategies like mastectomy, chemotherapy, and radiotherapy. The treatment strategy selection is mainly based on the spread of tumors in primary and metastatic tissues, and the combined approach is usually much preferred [4]. Chemotherapy is either given solely or can be used as an adjuvant or neoadjuvant modality. However, surgical breast removal poses cosmetic complaints to the hosting women. Debulking the breast tumor with chemotherapy and radiotherapy is preferred, especially in the early stages [5]. Among various approved drugs, anthracyclines, taxanes, 5-fluorouracil, and platinum compounds are routinely employed. For ages, herbs have been used for various health conditions in the traditional system of medicine due to their potential to cure many infectious and noncommunicable human diseases. This is because many bioactive substances are integral to the plant community, which play a protective role in disease conditions. Due to the rich source of bioactive compounds, researchers screen plants in various experimental models [6].

Solanaceae family member, *Withania somnifera* L. Dunal, is a perennial shrub widely known as ashwagandha in the Indian traditional system of medicine. The pharmacological activities of *Withania somnifera* are identified because of the presence of bioactive compounds like withaferin-A (WA) [7]. Structurally, the compound is related to the steroidal lactone. The anti-inflammatory property of WA is widely investigated. However, its potential to exhibit antiproliferative, antioxidant, anticoagulant, and antipyretic effects has also been elucidated. Chiefly, the anticancer activity of this herb is attributed to its ability to induce apoptosis by cytochrome C release, caspase activation, cell cycle (G2/M) arrest, and prevention of angiogenesis and cancer cell metastasis. Its anticancer properties have been studied *in vitro* and *in vivo* experimental breast cancer models [8].

Another natural substance, a resin-like material derived from bees is propolis, has been investigated for many beneficial effects on human diseases. Bees collect several substances from plants and secret them along with saliva as propolis. Its useful role in wound healing, cosmetic benefit, and dermatological disorders has gained the popularity of propolis. However, propolis activity may vary depending on its composition and the region of its origin [9]. Investigations on anticancer activity on respiratory, gastrointestinal, and female reproductive cancer cell lines, irrespective of geographical location and season of collection, proved its effectiveness. Ingredients like chrysin, artepillin C, and caffeic acid phenethyl ester (CAPE) are responsible for the biological activity of propolis [10]. The anticancer activity of propolis is multifaceted by affecting cancer cell generation, propagation, survival, and apoptosis by inhibiting signaling pathways and inducing apoptosis in tumor cells. At the molecular level, it manifests the hangup of mitogen-activated protein kinase (MAPK) phosphoinositide 3-kinases (PI3K)/Akt, VEGF, JAK-STAT, TLR4, and NFκB pathways [11].

The investigational compounds, withaferin-A and propolis, are nonidentical in nature. However, both compounds exert distinct mechanisms for their antiproliferative activity in cancer cell lines. The present study examined the combinational action of both drug compounds in benzo[a] pyrene-induced breast cancer in female Wistar rats, a first of its kind.

2. Materials and Methods

2.1. Preparation of Propolis Ethanolic Extract. Propolis was drawn out in 95% v/v ethanol over the course of four days at 37°C with sporadic shaking. The ethanolic extract was next filtered using Whatman filter paper No. 1 before being heated to 60°C and evaporated in a rotary evaporator with decreased pressure.

2.2. Ethical Clearance and Animal Maintenance. The Wistar rat studies were conducted at the Central Research Laboratory of Meenakshi Medical College & Research Institute located in Enathur, Kanchipuram, Tamilnadu, India. The research was done between October 2019 and June 2021. Institutional Animal Ethical Clearance (IAEC No. 003/2019) was acquired on March 7, 2019. The National Institute of Nutrition situated in Hyderabad, India, provided us with 36 female Wistar rats that weighed 150–200 g that were sheltered in stainless steel lids covered polypropylene cages and acclimatized for 7 days. The animals were maintained in an air-circulated environment with the arrangement of standard 12 : 12 h light : dark cycles. They were fed with

commercial rodent pelleted foods (Gold Mohr rat feed, Ms. Hindustan Lever Ltd., Mumbai) and drinking water *ad libitum* in standard intervals [12].

2.3. Experimental Design. The suppliers for benzo[a]pyrene, propolis, and withaferin-A were Aldrich Sigma Chemical, Mumbai, and SRL Chemicals, Chennai, provided the rest of the chemical compounds. The levels of breast cancer marker enzymes such as gamma-glutamyl transferase (γ-GT), aryl hydrocarbon hydroxylase, lactate dehydrogenase (LDH), glycoprotein (hexoses), and nucleic levels (DNA and RNA) were monitored in the experimental rodents in intervals followed by tissue histopathological study.

The antiproliferative effect of the withaferin-A and propolis on benzo[a]pyrene -induced mammary tumors was investigated following the method of Meghalatha et al. as published in our earlier work [13]. Invasive ductal carcinoma was induced in the right breast of rats using benzo[a] pyrene in animal Group II, Group III, Group IV, and Group V.

The female Wistar rats were randomized into six groups, with six animals in every group. The experimental groups of animals were treated as follows:

(1) Animals in Group I were administered with regular saline (normal control).

(2) Animals in Group II were administered benzo[a] pyrene (20 mg mixed in 0.5 ml each of saline and sunflower oil and injected into mammary pads using the "air pouch technique") was supplied twice weekly for three months to the animals in the interventional group (negative control).

(3) Animals in Group III induced with breast cancer were provided with oral administration of Withaferin A (30 mg/kg body weight) once a week for a period of 30 days

(4) Animals in Group IV induced with breast cancer were given an oral dosage of an ethanolic propolis preparation (50 mg/kg body weight) for 30 days.

(5) Animals in Group V induced with breast cancer were given both withaferin-A (30 mg/kg body weight) and an ethanolic propolis mixture (50 mg/kg body weight; daily) for 30 days through oral administration.

(6) Animals in the Group VI naïve group were given both Withaferin A and an ethanolic propolis mixture for 30 days through oral administration.

2.4. Air Pouch Generation, Carcinogen Ingestion, and Monitoring in Animals. The technique used by Meghalatha et al. was followed to create an air pouch in female Wistar rats. There is about 2 ml of air in the 5 ml syringe. It was sealed autoclaved for 20 minutes at 15 psi. The sterile air from a syringe was subcutaneously injected just behind the breast fat pad to generate a sterile air pouch. The air that was in the bag had time to stabilize for a day prior to the carcinogen's administration [13].

The resulting solid tumor was approximated as a prolate ellipsoid, with one major dimension (D) and one minor dimension (d). These tumor dimensions were measured using a Vernier caliper, and the tumor volume was calculated using the following formula [14]:

$$\text{Tumor volume } (V) = D \text{ x } d \langle \text{sup} \rangle^2 \text{ x } \frac{\pi}{6}, \quad (1)$$

where V represents the tumor volume, D is the largest dimension, and d is the smallest.

The following ingredients were added to a sterile vial: 20 mg of benzo[a]pyrene, 0.5 ml of sterile saline, and 0.5 ml of sunflower oil. The vial was aggressively vortexed after stoppered to produce an emulsion for even spread. The air pouch received a single injection of benzo[a]pyrene. The tumor was closely monitored up to the 90^{th} day, after which it attained its maximum size. The daily monitoring of the experimental animal's body weight was conducted throughout the study. At the conclusion of the experimental period, data on tumor volume and the mean survival period were recorded.

2.5. Collection of Blood and Organs. Every single one of the test rats was decapitated in the neck region after the experiment. To distinguish between plasma and serum and measure blood parameters, the blood was drawn both with and without the use of ethylene diamine tetraacetic acid (EDTA). The breast and liver tissues have been homogenized with a motor-driven Teflon-coated homogenizer in icy cold water to obtain 10% homogenate (Tris-HCl buffer, 0.1 M, pH 7.4). After the plasma was removed, packed cells that were still present were rinsed in isotonic saline to get rid of the buffy coat. Four milliliters of packed cells were cleaned three times with isotonic buffered Tris-HCl at 0.1 M pH. Hemolysis was carried out by pipetting the cleansed red blood cell solution into centrifuge tubes made of polypropylene containing hypotonic buffer (Tris-HCl buffer, 0.015 M, pH 7.2). The sedimentation of erythrocyte ghosts was carried out in a high-speed refrigerator centrifuged at $20,000 \times g$ for 40 min.

2.6. Tissue Sample Collection for Histopathology and Biochemical Analysis. At the conclusion of experiment, all of the animals remained given a light ether anesthesia. After the animals were sacrificed via cervical decapitation, the breast and liver tissue were rapidly removed. The blood clot and other tissue components were removed from the tissues by washing them in physiological saline before they were blotted dry.

Breast and liver tissues weighing about 100 mg were homogenized at 4°C in Tris-HCl solution 3 hours after the sacrifice (0.01 M; pH −7.4). The tissue homogenates were then centrifuged for 30 minutes at 2500 rpm. Until further biochemical investigation, the produced supernatants were stored at low temperatures (12 to 15°C). Within 48 hours after sample collection, every test procedure was completed [17].

2.7. Statistical Evaluation. Using the statistical software SPSS (Statistical Package for Social Science), one-way analysis of variance (ANOVA) and Tukey's multiple comparison test were used to regulate the implication of the mean variances between the various treatment groups (version 17) [18].

3. Results

3.1. Activity of Withaferin-A along with Propolis on the Serum Marker Enzyme Activities. The pharmacodynamic effect of withaferin-A along with propolis on the serum marker enzyme activities in the naïve and interventional group rats is shown in Figure 1. The marker enzymes such as gamma-glutamyl transferase (γ-GT), aryl hydrocarbon hydroxylase (AHH), and lactate dehydrogenase (LDH) activities were noticed to increase significantly ($p < 0.001$) in Group II compared with Group I. Groups III and IV instigated with a great ($p < 0.05$; $p < 0.001$) reduction in these enzyme quantities compared with Group II rats. When compared to the rats of Group II, the Group V rats who received a mixture therapy of both withaferin-A and propolis also obtained a substantial ($p < 0.001$) decline in their quantity. The activity of these marker enzymes did not, however, differ significantly among the Group I and Group VI rats.

3.2. Activity of Withaferin-A along with Propolis on the Glycoprotein Levels in Plasma and Breast Tissues. The pharmacodynamic effect of withaferin-A along with propolis on the glycoprotein levels in plasma and breast tissues, respectively, of naïve and interventional groups are shown in Tables 1 and 2. All the three glycoproteins—hexose, sialic acid, and hexosamine—were analyzed and are considerably ($p < 0.001$) improved in the Group II rats compared with Group I rats. Rats of Group III resulted a large ($p < 0.01$; $p < 0.001$) reduction in their levels against the rats of Group II. Rats of Group IV also produced a significant ($p < 0.05$; $p < 0.01$) reduction in glycoprotein functions, but the combination of both withaferin-A and propolis in Group V rats caused a very much noticeable ($p < 0.01$; $p < 0.001$) declined glycoprotein activity compared with the Group II rats. The levels of glycoproteins in Group I and VI (administered with the combination of withaferin-A and propolis) rats were not significantly different.

3.3. Activity of Withaferin-A with Propolis on Glycoprotein Levels in the Hepatocytes. The pharmacodynamic effect of withaferin-A with propolis on glycoprotein levels in the hepatocytes of naïve and interventional group rats are shown in Figure 2. Considerably higher ($p < 0.001$ and $p < 0.01$) glycoprotein quantity was shown by the rats of Group II against the rats of Group I. In comparison with Group II rats, the quantities of these glycoproteins were observed to be significantly ($p < 0.001$; $p < 0.05$) decreased in Groups III and IV. Combined therapy of withaferin-A and propolis in Group V produced a highly noticeable ($p < 0.001$; $p < 0.05$) declines in glycoprotein quantity compared with the rats of Group II. The rats of Groups I and VI (administered with the mixture of withaferin-A and propolis) did not significantly differ in their levels of glycoprotein.

3.4. Activity Withaferin-A along with Propolis on the Nucleic Acids (DNA and RNA) Quantities in the Breast Tissues and Hepatocytes. The pharmacodynamic effect of withaferin-A along with propolis on the nucleic acids (DNA and RNA) quantities in the breast tissues and hepatocytes of naïve group and interventional groups, respectively (Figures 3 and 4). When compared to Group I, it was observed that the activities of both nucleic acids—DNA and RNA—were considerably ($p < 0.001$) higher in the Group II. On matching with Group II rats, a substantial ($p < 0.001$; $p < 0.01$) declined activities of DNA and RNA were found in both Group III and Group IV of rats. Reduced significant ($p < 0.001$) levels of these nucleic acid activities were identified in Group V rats in contrast to Group II rats. However, significant variation was not observed within nucleic acid activities between the Group I rats and Group VI rats.

3.5. Histopathological Studies. The histopathological changes in the mammary glands of rats of the naïve group and interventional group are represented in Figure 5. The Group I includes the normal control breast cells were marked with hematoxylin and eosin and showed typical cellular architecture and nuclear size. The Group II includes the breast cancer-bearing animals showed marked hyperplasia with atypical hyperchromatic cells (H&E X 10). The Group III includes the breast cancer of withaferin-A-treated animals indicated significantly reduced hyperplasia or cell proliferation. The Group IV includes breast cancer of propolis-treated animals, which showed perceptible recovery in the breast architecture with normalizing of central vein and nuclear size. The Group V includes the breast of withaferin-A and propolis combination-treated animals showed typical morphological architecture and normal central vein. The Group VI includes the control of withaferin-A and propolis combination-treated animals showed normal architecture and no changes in the central vein and nucleus.

4. Discussion

Both the allopathic and Indian schools of medicine have employed medicinal plants to treat various ailments for more than a few hundred years. Alkaloids, saponins, glycosides, flavonoids, and tannins are only a few of the active chemical components that plants have; therefore, scientists are continually trying to extract and find new, stronger plant principles. It is believed that isolating the active ingredients from medicinal plants would result in more accurate, safe, and effective drugs [19]. A serious health problem affecting people everywhere is cancer. Between industrialized and underdeveloped nations, there are differences in the incidence of cancer in a particular organ. Each year, over 10 million new cases of cancer are discovered worldwide. Ten

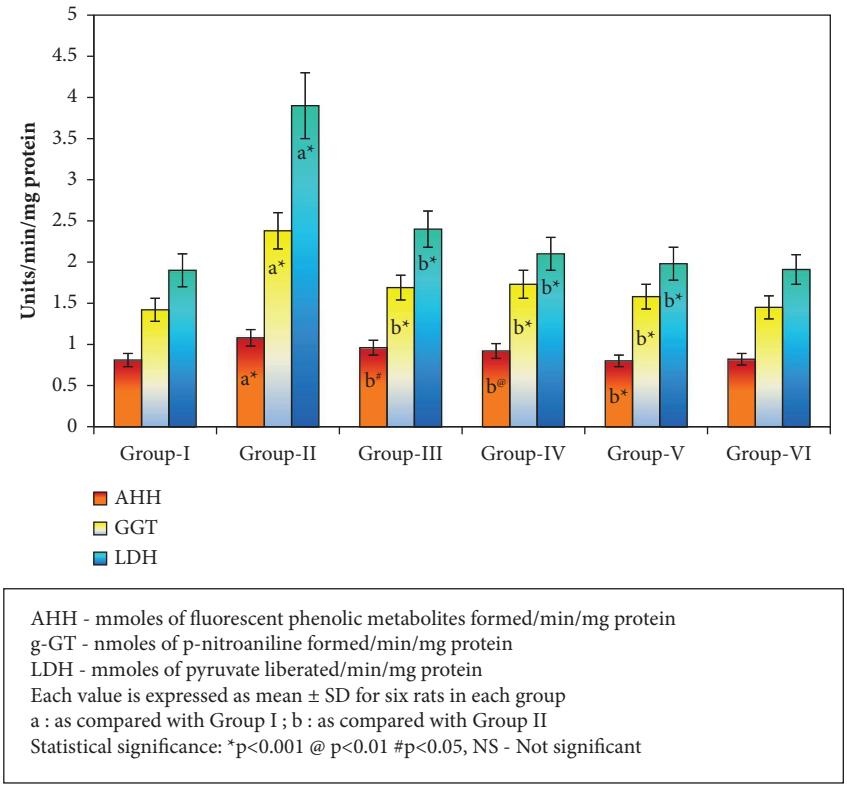

Figure 1: Pharmacodynamic effect of Withaferin-A along with propolis on the activity of the marker enzymes in the serum of naïve and interventional rats.

million people are expected to die from cancer by 2025 [20]. As a result, the development of cancer depends on elements related to the breast, such as ductal carcinoma and abnormal gene and protein expression. The rat breast experiences cancer in a variety of phases and progressions. The chemical carcinogen employed in the current investigation was benzo [a]pyrene. This chemical influences the carcinogenesis stage known as initiation when mammary cell proliferation is elevated and causes breast cancer in rats [21].

Withaferin A is a most effective anticancer medicine for breast cancer. Grouping medications perform better than standalone medications. Combining the medications has distinct results. The rat can obtain it according to their maximum dose that is justifiable [22]. One of the most effective antineoplastic drugs used to treat breast cancer, Withaferin A, has undergone extensive study to lessen its dose-limiting toxicity. The search for methods to promote Withaferin A's harmful manifestation is still continuing. The Indian medical system has advocated a wide range of pharmaceuticals for treating different human ailments and other unfavorable situations, whether they have mineral or botanical roots. Ayurveda, one of India's oldest medical systems, has been practiced there since 6000 B.C. [23]. Propolis is a bioactive chemical produced from honey bee wax. Other pharmacological properties of propolis, such as its anti-inflammatory, antitumor, antigenotoxic, and antioxidant properties, have also been related to it. Recent studies have shown that propolis induces apoptosis, which reduces the development of cancer cells in people [24].

Withaferin A and propolis, both recognized chemotherapeutic agents, significantly enhance the immune response, particularly in terms of IgG and IgM, within the breast tumor microenvironment [25, 26]. In the current study, this phenomenon was observed in a rat model of breast tumors, showcasing an improvement in therapeutic targets specific to breast cancer. The antitumor efficacy of withaferin-A and propolis on benzo[a]pyrene-induced ductal carcinoma (in situ type of breast cancer) was observed in Wistar rats. Ductal carcinoma in situ is a noninvasive cancer where abnormal cells have been found in the lining of the breast milk duct. The atypical cells have not spread outside of the ducts into the surrounding breast tissue. Ductal carcinoma in situ is very early cancer that is highly treatable, but if it is left untreated or undetected, it may spread into the surrounding breast tissue [27].

A well-known marker for apoptotic balance and cell detoxification is gamma-glutamyl transferase (GGT) [28]. It simplifies the transfer of an amino acids and peptide's glutamyl moiety. The activity of the cell surface enzyme γ-GT, which cleaves extracellular glutathione to produce the building blocks for enhanced intracellular glutathione synthesis, acts as a particular marker to predict the carcinogenic formation. Elevated level of γ-GT was noticed in the carcinoma tissues [29, 30]. Chemical carcinogens affecting the breast may start some systemic action that induces γ-GT synthesis [28, 31]. When compared to the cancer-bearing (G-II) mice, the animals treated with Withaferin-A and propolis had lower levels of γ-GT.

Table 1: Pharmacodynamic effect of Withaferin-A along with propolis on the level of glycoproteins in the plasma of naïve and interventional rats.

Particulars	Group I (control)	Group II (benzo[a]pyrene-induced)	Group III (withaferin-A-treated)	Group IV (propolis-treated)	Group V (both withaferin-A- and propolis-treated)	Group VI (control rats treated with withaferin-A- and propolis-treated)
Hexose	193.65 ± 17.76	257.49 ± 23.09a$^{\#}$	219.66 ± 20.12b$^{@}$	216.22 ± 20.846b*	193.65 ± 17.76	193.88 ± 20.68
Hexosamine	30.76 ± 2.81	45.71 ± 4.10a$^{\#}$	38.45 ± 3.59b$^{@}$	41.41 ± 4.01b*	30.76 ± 2.81	31.01 ± 2.86
Sialic acid	42.61 ± 4.02	67.72 ± 6.26a$^{\#}$	53.31 ± 5.12b$^{\#}$	58.61 ± 5.67b$^{@}$	44.61 ± 4.02	43.12 ± 4.45

Each value is expressed as mean ± SD for six rats in each group. Units: mg/dl. a: as compared with Group I. b: as compared with Group II. Statistical significance: $^{*}p < 0.001$; $^{@}p < 0.01$; $^{\#}p < 0.05$.

Table 2: Pharmacodynamic effect of withaferin-A along with propolis on the level of glycoproteins in the breast of naïve and interventional rats.

Particulars	Group I (control)	Group II (benzo[a] pyrene-induced)	Group III (withaferin-A-treated)	Group IV (propolis-treated)	Group V (both withaferin-A- and propolis-treated)	Group VI (control rats treated with withaferin-A- and propolis-treated)
Hexose	1.12 ± 0.12	$3.35 \pm 0.33^{a*}$	$1.85 \pm 0.14^{b*}$	$1.52 \pm 0.17^{b*}$	$1.3 \pm 0.16^{b*}$	1.31 ± 0.16
Hexosamine	0.35 ± 0.05	$0.99 \pm 0.07^{a*}$	$0.68 \pm 0.06^{b*}$	$0.66 \pm 0.06^{b*}$	$0.42 \pm 0.05^{b*}$	0.40 ± 0.05
Sialic acid	0.31 ± 0.04	$0.59 \pm 0.04^{a*}$	$0.43 \pm 0.01^{b*}$	$0.39 \pm 0.03^{b*}$	$0.31 \pm 0.05^{b*}$	0.31 ± 0.05

Each value is expressed as mean ± SD for six rats in each group. Units: mg/g of defatted tissue. [a]: as compared with Group I. [b]: as compared with Group II. Statistical significance: $^{*}p < 0.001$; $^{@}p < 0.01$; $^{\#}p < 0.05$.

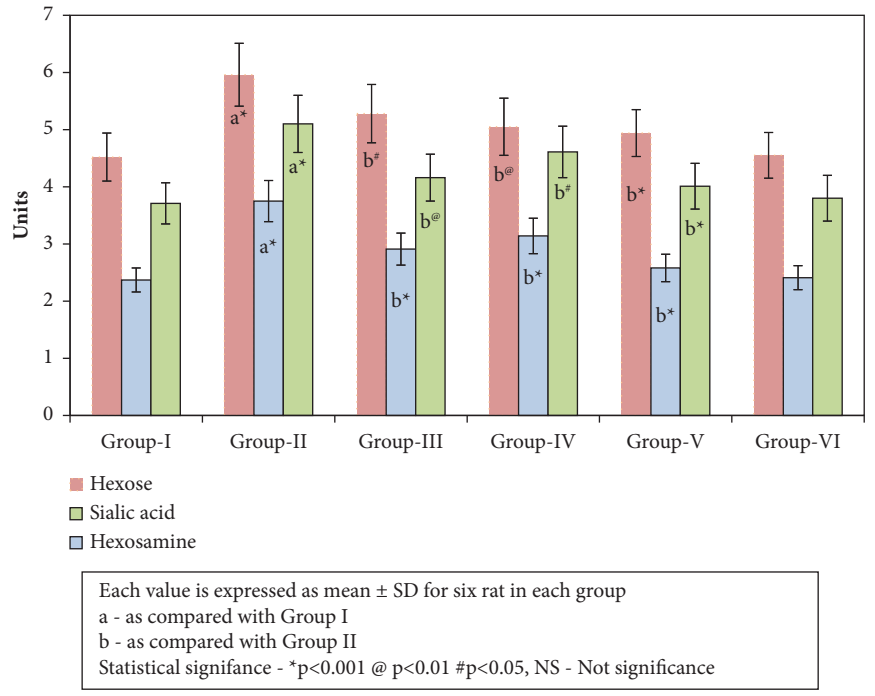

Figure 2: Pharmacodynamic effect of Withaferin-A along with propolis on the levels of glycoprotein in the liver of naïve and interventional rats.

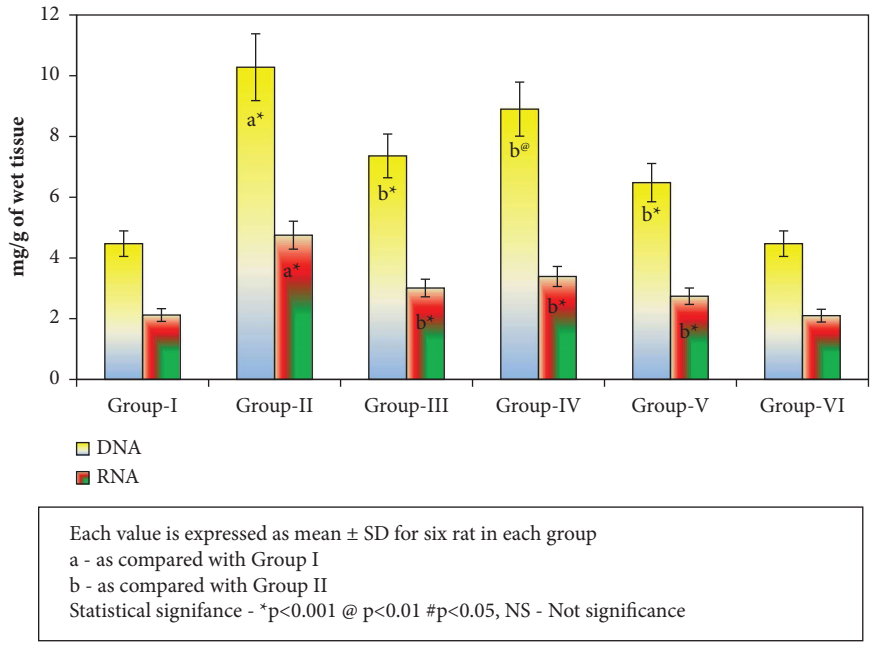

Figure 3: Pharmacodynamic activity of Withaferin-A along with propolis on the level of nucleic acids (DNA and RNA) in breast of naïve and interventional rats.

The most often used clinical enzyme in people with cancer for prognostic purposes is lactate dehydrogenase (LDH). It is crucial for the functioning of germ cells and can forecast chemotherapeutic reactions and the likelihood of recovery [32]. Glycolytic enzyme and LDH activities are typically 2-3 times higher in human cancer tissues. According to Sandhya Mishra et al., breast cancer patients had elevated levels of LDH [31].

The biological and pharmaceutical effects of Withaferin-A and propolis were linked to phenolic substances, particularly flavonoids and other molecules containing organosulfur acids. Due to their high flavonoid content, Withaferin-A and propolis have anticancer and antiproliferative properties that stabilize membrane permeability and inhibit LDH release [33]. Glycoproteins are frequently found in the lysosomes and mammalian cell

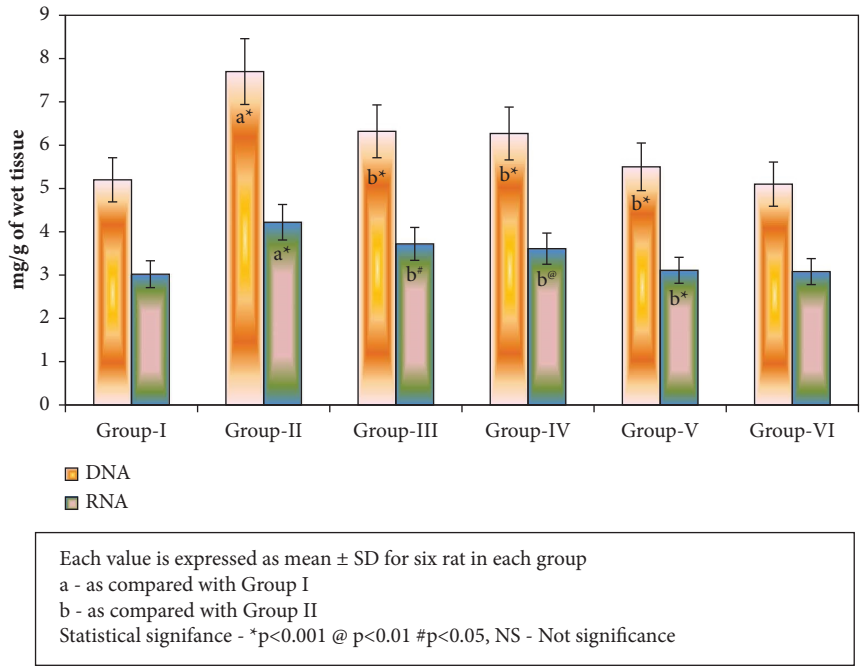

Figure 4: Pharmacodynamic effect of Withaferin-A along with propolis on the level of nucleic acids (DNA and RNA) in the hepatocytes of naïve and interventional rats.

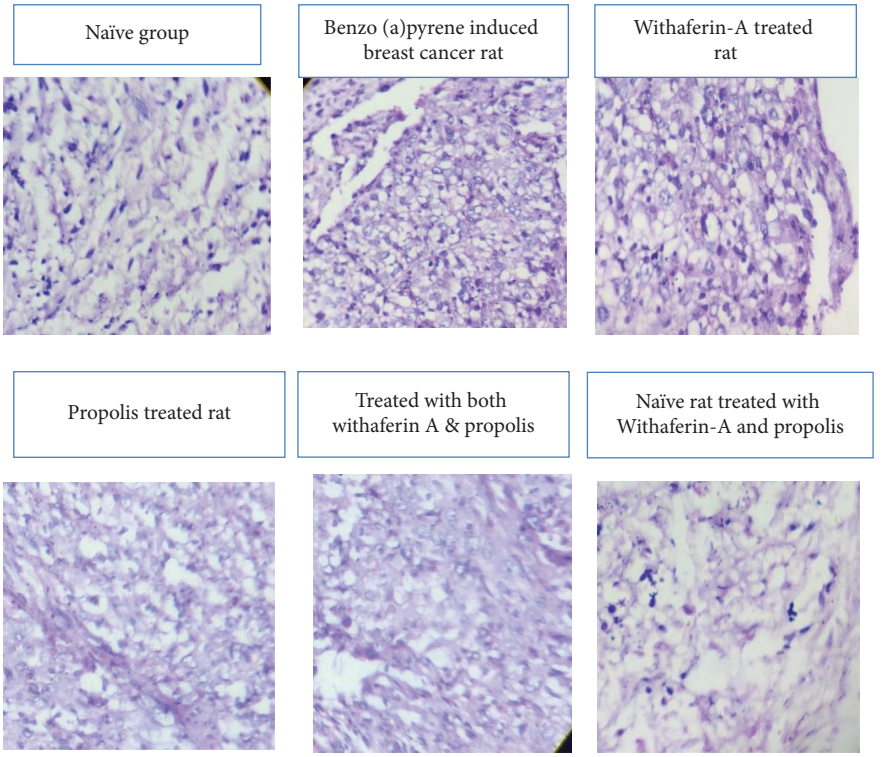

Figure 5: Histopathological changes in the mammary glands of rats of the naïve group and interventional group.

surfaces which contributes towards cell export [34, 35]. There is a clear link between glycoproteins and cancer, and the transport of metabolites via cellular membrane has also been linked to the carbohydrate moiety of glycoproteins [36, 37]. An increase of the glycoprotein elements is a well-known sign and marker of the development of tumor growth [38].

It is well known that throughout neoplastic processes, altered amounts of protein-bound carbohydrates affect cell membranes' stiffness, structure, and functioning [39, 40].

The current investigation discovered that animals with cancer caused by benzo[a]pyrene had significantly higher amounts of the glycoprotein's hexose, hexosamine, and sialic acid. The modification of surface carbohydrates throughout cell differentiation and neoplastic transition supports the role of surface carbohydrates in cell physiology and behavior [39]. Hexose and hexosamine levels were markedly elevated in breast cancer [41]. Sialic acids are acylated compounds of neuraminic acid that are primarily found in biological materials as nonreducing terminal residues of glycoprotein and ganglioside carbohydrate chains [42]. Serum sialic acids have been utilized as tumor markers since they are elevated in various malignancies, which include breast, brain, gastrointestinal, and gynecological cancers [43].

Enzymes called plasma sialyl transferases, which transfer sialic acid molecules from cysteine monophosphate were shown to be more active in several malignancies. According to Bernacki and Kim [44], animals with metastatic breast cancer exhibit increased sialyl transferase activity [45, 46]. Animals treated with Withaferin-A and propolis have significantly lower amounts of glycoproteins. This decrease in glycoprotein component levels suggests the power of medication to reduce malignancy by regulating the proliferation and transformation of cells.

Biochemical evaluation of DNA, RNA, and the protein contents in the liver also showed typical values in propolis treatment [47]. During neoplastic transformation, nucleic acids are crucial. With increasing malignancy in neoplastic disease, DNA aberrations rise. Since it has been established that benzo[a]pyrene binds to cellular DNA, strong evidence suggests that these B(a)P-DNA interactions are necessary for the start of the carcinogenic process [48]; as a result, the analysis of DNA is more significant in tumorous conditions.

Atypical DNA levels have also been found in breast carcinoma [49, 50], similar to the current findings. DNA and RNA levels are decreased by withaferin-A and propolis. The nucleic acid production was dramatically reduced by withaferin-A and propolis combination therapy, indicating combination chemotherapy's improved tumoricidal effectiveness. In the future, the withaferin-A and propolis combination therapy can be assessed for the presence of apoptotic indicators (p53, Bcl2, and caspases 3/9) through gene expression studies and western blot analysis.

The inherent toxicity of natural bioactive substances is frequently inevitable, and WA is no different in this regard. Nevertheless, the toxicity of WA has been a topic of contentious debate and significant apprehension [51]. WS extract, which is the primary bioactive compound, has been shown to be safe in all groups studied, as evidenced by many articles. A study on the toxicity of orally administered WA, conducted on both acute and subacute basis, produced consistent findings. The LD_{50} of WA in mice was determined to be greater than 2000 mg/kg body weight [52]. Conversely, additional research discovered that WA demonstrated specific poisonous adverse effects in mice, with a lethal dose (LD_{50}) of 54 mg/kg body weight [53]. Although WA has extensive anticancer effects, it is limited by potential toxicity, inadequate absorption when taken orally, and low yield [54].

5. Conclusion

The combination therapy of both withaferin-A and propolis drugs showed significant pharmacodynamic effect in a rat model of breast cancer induced by benzo[a]pyrene. It resulted in improvements in marker enzymes (γ-GT, AHH, and LDH) associated with breast cancer progression, indicating its potential in modulating cellular detoxification and apoptosis balance. The therapy also demonstrated the ability to regulate glycoproteins (hexose, hexosamine, and sialic acid) linked to tumor growth, with a significant decrease observed in their levels. This suggests its potential in controlling cell proliferation and transformation processes. Moreover, the combination drug therapy effectively reduced the elevated levels of DNA and RNA associated with neoplastic processes, highlighting its improved tumoricidal effectiveness. Histopathological examination revealed a reduction in hyperplasia and cell proliferation in the mammary glands, indicating the therapy's potential to reverse architectural and morphological changes related to breast cancer. This study provides evidence supporting the therapeutic potential of Withaferin-A and propolis combinational drug therapy in treating breast cancer. Furthermore, research is needed to explore the underlying mechanisms and evaluate its efficacy in human clinical trials.

Data Availability

The data used in this study are available within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this study for all the authors.

Authors' Contributions

Meghalatha TS, Arumugam Suresh, Natrajan Muninathan, and Perumal Asaithambi conceptualized the study. Meghalatha TS, Arumugam Suresh, Kuppusamy Baskaran, and Perumal Elumalai proposed the methodology. Kuppusamy Baskaran, Madhav E, and Shadab Kazmi performed formal analysis. Arumugam Suresh, Natrajan Muninathan, Kuppusamy Baskaran, Shobana Sampath, Mohammad Z. Ahmed, and Ali S. Alqahtani performed investigation. Meghalatha TS, Shobana Sampath, Perumal Elumalai, Madhav E, and Perumal Asaithambi wrote the original draft. Natrajan Muninathan and Shadab Kazmi reviewed and edited the article. Natrajan Muninathan and Perumal Asaithambi performed supervision. All authors have read and agreed to be the published version of the manuscript.

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