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EFFECT OF HYDRO ALCOHOLIC EXTRACT OF *Vitis vinifera* linn AGAINST DIMETHYL BENZ [A] ANTHRACENE INDUCED MAMMARY GLAND TUMOUR IN FEMALE SPARGUE-DAWLEY RATS

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ABSTRACT

The present study aimed that investigate the antitumor potential of hydro alcoholic extract of *Vitis vinifera* linn for 45 days against 7, 12-dimethyl Benz[a]anthracene (DMBA- 7.5mg/Kg) induced breast cancer in female Sprague-dawley rats. A treatment protocol started after DMBA administration. Results obtained displayed that there was a significant elevation in the terms of tumour incident and tumour multiplicity in DMBA injected rats. The potential reduction in tumour volume was observed in treatment groups whereas treated with HAEVV reverse these changes. The activities of superoxide dismutase, catalase, and glutathione peroxidase (free radical scavengers) were found to be escalated in treatment groups when compared to toxic control. Histopathological examination revealed the formation of tumour in DMBA induced rats. Whereas treatment with extract significantly decreased the proliferation and replacement of normal ductular and alveolar structure of mammary gland. Therefore it can be concluded that the HAEVV was provided antioxidant defense, with strong anti-tumour activity against DMBA-induced mammary tumours.

KEYWORDS: Breast cancer, DMBA, *Vitis vinifera* linn, HAEVV.

INTRODUCTION

Cancer is a major public health problem in the world. Breast cancer is the second leading cause of cancer deaths that usually arises from breast cells. Breast cancer usually starts off in the inner lining of milk ducts or the lobules that supply them with milk. A malignant tumor can spread to other parts of the body. Although men can also breast cancer, in cases of male breast cancer account for less than 0.05% of all diagnosed.[1] Breast cancer will initially develop in breast tissue, normally in the glands and milk ducts. This type of cancer is still considered to be breast cancer, even if it is discovered after travelling to other areas of the body, like the liver, lungs, and bones. Breast cancer is related to age with only 5% of all breast cancers occur in women under 40 years old.[2]

Reactive oxygen species (ROS) are involved in a variety of important pathophysiological conditions including mutagenesis and carcinogenesis. Oxidative stress has the potential to cause cellular DNA damage, lipid peroxidation, and membrane disruption. Human body is equipped with various antioxidants such as superoxide dismutase (SOD), glutathione peroxidise (GPx), catalase (CAT), glutathione (GSH), ascorbic acid (vitamin C), a-tocopherol (vitamin E), and so on, which can counteract the deleterious action of ROS and prevent from cellular and molecular damage. Bioactive compounds from plant origin have the potential to subside the biochemical imbalances induced by various toxins associated with free radicals.

Treatment includes such as Radiation therapy, Surgery, Biological therapy, Hormone therapy, Chemotherapy.[3] Though treatment causes substantial cost and side effects, it has led a way to search plant based treatments for breast cancer. Herbal plants have been used since centuries to prevent and/or reduce the oxidative stress and DNA damage.

Vitis vinifera linn is a herbal plant with various health benefits which are attributed by is Various parts including shoot, leaves, fruit, seed, skin, and flower contain diverse phytochemicals, such as Linoleic acid, α-Terpineol, Proline, Procyanidin, Quercetin, Valencene. Some of the compounds are under preliminary research to determine their potential biological properties.[4]

In vivo mammary gland cancer model development was done by using various chemical methods, of which 7, 12-dimethyl Benz[a]anthracene (DMBA) is a chemical carcinogen, commonly used to induce breast carcinogenesis by raising oxidative stress and mammary gland ducts damage.[5] Hence In the present study, DMBA induced Breast cancer in female Sprague dawley

rats was used to evaluate the hydro alcoholic extract of *Vitis vinifera* linn . Histological, biochemical, antioxidant enzyme status, as well as the related cytokines profiles was studied.

MATERIALS AND METHODS

Experimental Animals

Thirty female Sprague–Dawley rats weighing (190 g) were housed in well ventilated large spacious polypropylene cages and had 12±1 h light and dark cycle throughout the experimental period. The animals received a balanced diet of pellet rat feed and water ad libitum. Before the commencement of the experiment the animals are segregated based on their gender and quarantined for 15 days.[6][7]

Induction of Breast cancer

There are various techniques for including breast cancer in animals. Chemically induced models such as 1-methyl-1-nitrsourea (MNU), 7,12-Dimethyl benz(a)anthracene (DMBA), dimethyl nitrosamine(DEN) or azoxymethane (AOM),medroxy progesterone(MPA), Diethyl stibestrol. have been used in experimental studies of breast cancer.[8]

In the present study, DMBA induced Breast cancer in rats was used to evaluate the activity against Breast cancer.

Preparation of Extract

A voucher specimen of *Vitis vinifera* linn has been deposited at the herbarium, in the Department of Pharmacognosy, K.M. College of Pharmacy, (No. OI-2019-36: KMCP). Powders of *Vitis vinifera* linn extracts are light brown colour.

Hydro alcoholic extracts of *Vitis vinifera* linn (yield = 7.5%) were prepared by rotary vacuum evaporator. 500gm of coarsely powdered and dried skin of *Vitis vinifera* linn with 2 litres of 70% Ethanol at 70^0C temperature, for 1 hour in a round bottom flask with condenser attached. Filter and collect the extract. Repeat extraction with 2 litres of 70% Ethanol. Filter and collect the extract. Extract the marc with 2 litres of water. Filter and combine the extracts. The combined extract was evaporated to dryness under reduced pressure in a Buchi Rotary Evaporator (Switzerland) at 65°C, to obtain a brownish colour residue. This extract was used for the experimentation.

Vitis vinifera linn extracts were stored in a refrigerator at -20^0C to prevent from light and degeneration.

Experimental Design

Female Sprague – Dawley rats (n =30), were acclimatized for 2 weeks before to start of experiments. Rats were randomly categorized into five experimental groups (n = 6). Mammary tumour was induced by a single dose of 7, 12-Dimethyl benzanthracene [7.5mg] was dissolved in an emulsion of sunflower oil (0.5 mL) and physiological saline (0.25ml) just prior to use. [9] [10] All the animals in four groups were given DMBA by subcutaneously except the normal control. **G1** (normal control), **G2** served as toxic control receives normal diet and water. **G3** served as the positive control treated with injection of Vinblastin at 0.5 mg/kg body weight were given by Intra peritoneally. **G4** & **G5** served as a treatment control, treated with 200 & 400mg/kg body weight of Hydro alcoholic extract of *Vitis vinifera* linn (HAEVV). After 45 days, all the experimental animals from every group and were anaesthetized with diethyl ether and sacrificed by euthanasia method. The breast tissues were surgically dissected and used for measurement of tumour volume (mm in diameter) and histopathological examination. The tissue homogenates were used for the measurement of biochemical parameters.[11]

Evaluation of Biochemical Parameters

Effects of Hydro alcoholic extract of *Vitis vinifera* linn on activities of superoxide dismutase, catalase, glutathione peroxidase and lipid peroxide were estimated in the breasts of treated, as well as untreated, rats.

Histopathological Examination

Mammary tissues were fixed in 10% buffered formalin, embedded in paraffin using a conventional automated system. Tissue fragments were fixed in formalin and 5µm section was obtained from the paraffin block and stained with haematoxylin and eosin for histologic examination. Breast tissue pathology and histologic type were evaluated by application of the same pathologic criteria used for the classification of human tumours. Serial paraffin sections of each tissue image were observed by light microscopy.

Statistical Analysis

Statistical comparisons between control and treatment mean values of two parameters were analysed using the Student's t-test. Multiple comparisons were done using ANOVA. The differences were statistically significant at $P < 0.01$; $P < 0.05$.

RESULTS

Anti-tumor activity

Animals in the breast cancer control group II attained a promotional stage tumor after 45 days. At the end of the experiment in non-treated rats, DMBA-induced breast tumors increased to the maximum in terms of tumor incidence (100%), tumor multiplicity per rat, compared to the normal control rats. A Significant reduction in Tumor volume was observed in treatment groups. While the animals administered vinblastin (0.5 mg/kg) individually achieved (41.21%) of tumor reduction after 45 days treatment. The combination DMBA (7.5 mg/kg, S.C) + Hydro alcoholic extract of *Vitis vinifera* linn (200 mg/kg, orally) treated animals achieved a significant decrease (25.83%) in the mammary tumor size with change in the total body weight of the animals (G2). However, Hydro alcoholic extract of *Vitis vinifera* linn at

a dose of 400mg/kg treated group achieved 33.26% breast tumor reduction after 45 days as shown in Table no.1

Antioxidant activity

Free radical scavengers such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and lipid peroxide (LPx) levels were recorded (Tables.No.2). The level and activity of SOD, CAT was found to be very high in the breasts of cancer-bearing rats treated with vinblastin and both doses of Hydro alcoholic extract of *Vitis vinifera* linn treated rats. The elevated level was close to the normal control rats versus the other groups. The GPx level was equally increased in vinblastin and both doses of Hydro alcoholic extract of *Vitis vinifera* linn therapy treated groups versus the cancer control rats. LPx levels were very much influenced by the chemical carcinogen in the control animals, however, the significant reduction of LPx was observed in the vinblastin and both doses of Hydro alcoholic extract of *Vitis vinifera* linn treated group and it was near to the normal discovered in the control group.

Histopathological examination

Histopathology revealed that most carcinomas exhibited an identical nuclear pattern. Most tumors were predominantly epithelial with fibrous tissue surrounding the mammary ducts. Most carcinomas exhibited a mixed structural pattern with invasion of neighbouring tissues and intense stromal desmoplastic reaction. In vivo, the treated groups with vinblastin and both doses of Hydro alcoholic extract of *Vitis vinifera* linn showed tumor tubules and formation of intra-tumor vascularization. The vast majority of the lesions that developed in the rat mammary glands were mostly carcinomas. Treatment with Hydro alcoholic extract of *Vitis vinifera* linn showed reduced proliferation and replacement of normal ductular and alveolar structure of mammary tissue.

Table No. 1: The Effect of Hydro Alcoholic Extract of *Vitis vinifera* linn on Antitumor Activity.

Group	Body weight	Tumour volume(mm)	Reduction of tumour percentage (%)
G1	214.8±4.45	-	-
G2	157.0±3.75^{**a}	47.80±1.90^{**a}	-
G3	185.35±3.55^{**b}	28.10±1.50^{**b}	41.21^{**b}
G4	169.0±3.30^{**b}	35.45±1.65^{**b}	25.83^{**b}
G5	179.00±3.40^{**b}	31.90±1.55^{**b}	33.26^{**b}

G1-NORMAL, G2-TOXIC, G3-STANDARD, G4-LOW DOSE (200mg/kg), G5-HIGH DOSE (400mg/kg)

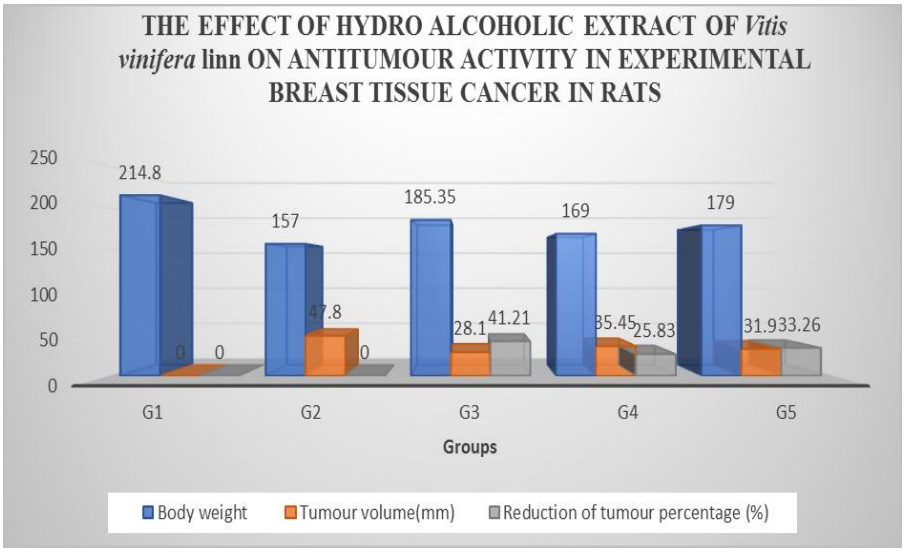
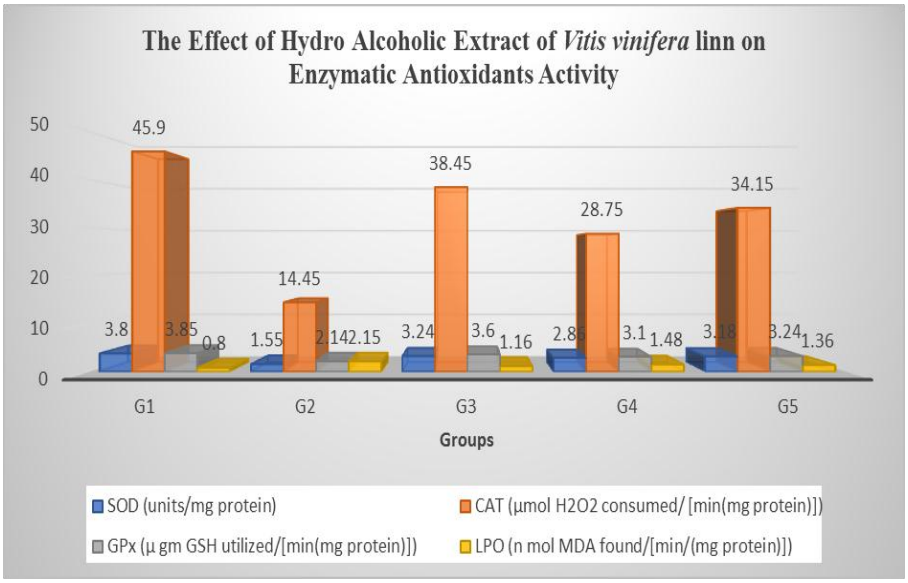


Table No. 2: The Effect of Hydro Alcoholic Extract of *Vitis vinifera* linn on Enzymatic Antioxidants Activity.

GROUPS	SOD (units/mg protein)	CAT (µmol H_2O_2 consumed/ [min(mg protein)])	GPx (µ gm GSH utilized/[min(mg protein)])	LPO (n mol MDA found/[min/(mg protein)])
G1	3.80±0.12	45.90±2.50	3.85±0.14	0.80±0.04
G2	1.55±0.08^{**a}	14.45±1.12^{**a}	2.14±0.08^{**a}	2.15±0.09^{**a}
G3	3.24±0.10^{**b}	38.45±1.80^{**b}	3.60±0.12^{**b}	1.16±0.06^{**b}
G4	2.86±0.09^{**b}	28.75±1.40^{**b}	3.10±0.09^{**b}	1.48±0.08^{**b}
G5	3.18±0.10^{**b}	34.15±1.70^{**b}	3.24±0.10^{**b}	1.36±0.07^{**b}

G1-NORMAL, G2-TOXIC, G3-STANDARD, G4-LOW DOSE (200mg/kg), G5-HIGH DOSE (400mg/kg)



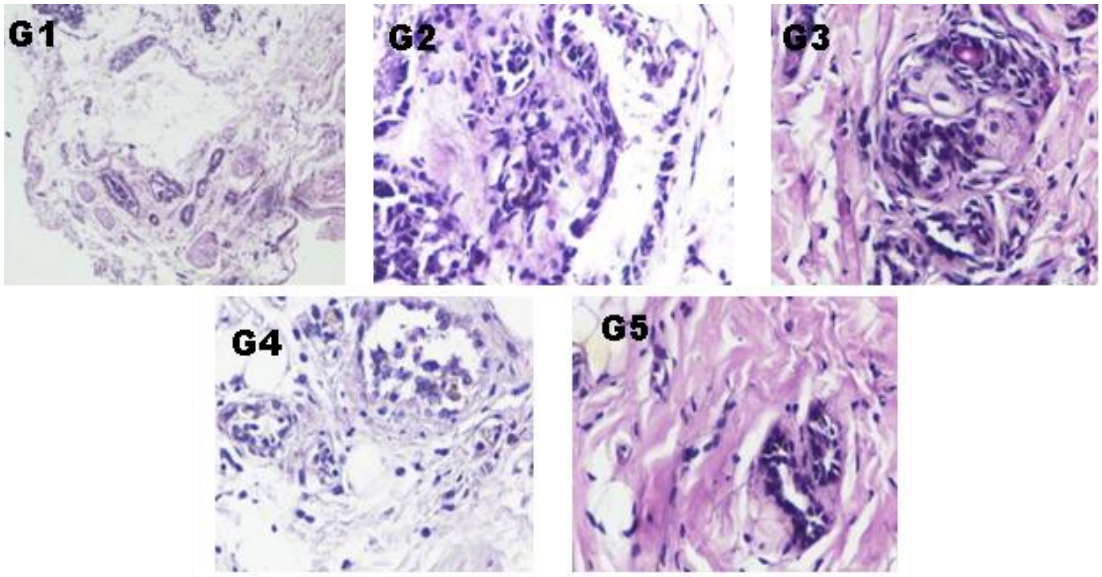


Figure 1: Histology of mammary gland in experimental rats after 45 days of treatment G1 - Normal control showed normal ductules, ducts and fibrous stroma and alveolar structure G2 - Toxic control showed breast parenchyma with markedly proliferated ductules and abundant fibrous stroma shows invasive ductal carcinoma of hyperchromatic tumour and clumping of Chromatids. G3–Mammary gland tumour + vinblastin (0.5 mg/kg) showed mild ductular proliferation with focal epithelial hyperplasia breast parenchyma with ductules, ducts and fibrous stroma G4 & G5 – Mammary gland tumour + HAEVV (200mg/kg, 400 mg/kg) showed reduced proliferation, breast parenchyma with ductules, ducts and fibrous stroma.

DISCUSSION

Animal experimental systems are particularly useful for the study of human mammary carcinogenesis. Since the rat mammary gland shows a high susceptibility to developing neoplasms which closely mimic human breast cancer, they have been selected in comparison to other animal models. [12] In the present investigation, vinblastin and both doses of Hydro alcoholic extract of *Vitis vinifera* linn exhibited potential anticancer activity on DMBA-induced mammary tumours in rats. As a result, the body weight had also slightly elevated, the tumour volume reduced, and the percentage of tumour inhibition was statistically significant ($P < 0.05$).

In our study, the anticancer drug vinblastin treated group significantly decreased tumour inhibition and tumour volume. However, there was a severe body weight loss observed in the toxic control group, versus the control rats. In the present investigation, vinblastin and both doses of Hydro alcoholic extract of *Vitis vinifera* linn treatment reduced the breast tumour by an average of 41, 25 and 33%.

The histopathological examination revealed that most carcinomas exhibited a mixed structural pattern such as nodular well, invasion of neighbouring tissues, with intense stromal desmoplastic reaction and necrosis. Upon correlating the histopathological examination, it was

evident that most carcinomas exhibited identical nuclear patterns. The tumours were predominantly epithelial and fibrous tissue surrounded the mammary ducts. However, the vinblastin and both doses of Hydro alcoholic extract of *Vitis vinifera* linn treated groups showed tumour tubules and formation of vascularization. The supply of blood to newly forming tissues and to tumours is a limiting factor that regulates growth.[12] The process of neovascularisation provides blood to support angiogenesis.[13]

This observation suggests that vascularization could be helpful in explaining the differential effects of cancer preventive agents on angiogenesis in the intra-tumoral region. The mechanistic actions of these compounds adhere and bind the tumour cells with help from the topoisomerase enzyme. The enzymatic action disturbs the spindle formation in the tumour cell. SOD acts as an anti-carcinogen inhibitor during initiation and promotion/transformation stages of carcinogenesis. CAT activity was significantly higher in the vinblastin and both doses of Hydro alcoholic extract of *Vitis vinifera* linn treated group versus the normal control animals. The control group showed lesser activity and formation of CAT compared to other groups. The LPx levels were increased more in the breast cancer-bearing animals. However, the potential reduction of lipid peroxides was recorded in the vinblastin and both doses of Hydro alcoholic extract of *Vitis vinifera* linn treated group and it was near to the normal in the control group. The LPx level was very much influenced by the chemical carcinogen (DMBA) in the breast cancer- bearing animals (breast cancer control).

The present findings include elevated ROS production with use of the chemical mutagen (DMBA), and the decreased level of antioxidants in breast cancer-bearing animals indicate oxidative stress, which may be the cause of lipid peroxidation-induced DNA damage, mutation and elevated level of LPO also play an important role for higher pathology of breast cancer in animals. The vinblastin and both doses of Hydro alcoholic extract of *Vitis vinifera* linn treatment kill the tumour cells. It did not promote tumour growth and metastasis by the incidence of lesser production of ROS formation due to the antioxidant enzymes such as SOD, CAT and GPx that can directly counter the oxidant attack and may protect cells against LPO and DNA damage.

CONCLUSION

The biochemical alterations observed in cancer bearing animals in the present study may be due to the induction of LPO and reduction of antioxidant level following carcinogen administration. However, administration of vinblastin and Hydro alcoholic extract of *Vitis vinifera* linn significantly reversed the alteration to near normal level in cancer-bearing animals. From the results it can be inferred that Hydro alcoholic extract of *Vitis vinifera* linn positively modulated the antioxidant activity by quenching and detoxifying the free radicals induced by DMBA. The attenuation of DMBA induced oxidative stress by the plant extract could be attributed to the antioxidants activity of Flavonoids, Terpenoids, Phenolic compounds present in the Hydro alcoholic extract of *Vitis vinifera* linn, which is known to quench the free radicals by maintaining antioxidants levels. Considering the antioxidant property of Hydro alcoholic extract of *Vitis vinifera* linn the bioactive compounds derived from this plant can be supplemented with anticancer medicines. Further investigation on the anticancer activity mechanisms of Hydro alcoholic extract of *Vitis vinifera* linn remains to be studied in our laboratory.

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