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EFFECT OF HYDRO ALCOHOLIC EXTRACT OF *Oroxylum indicum* 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 *Oroxylum indicum* linn for 45 days against 7, 12-dimethyl Benz[a]anthracene (DMBA- 7.5mg/Kg) induced oxidative stress and mammary carcinogenesis in female spargue-dawley rats. The treatment protocol started from the day immediately after DMBA administration. Results obtained indicated 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 HAEOI reverse these changes. The activities of superoxide dismutase, catalase, and glutathione peroxidise (free radical scavengers) were found to be potentially higher in treatment groups when compared to toxic control. Histopathological examination revealed the formation of tumour in DMBA induced rats. Whereas treatment with extract significantly reduced the proliferation and replacement of normal ductular and alveolar structure of mammary gland. Therefore it can be concluded that the HAEOI was provided antioxidant defence, with strong anti-tumour activity against DMBA- induced mammary tumours.

KEYWORDS: Breast cancer, *Oroxylum indicum* linn, DMBA.

INTRODUCTION

Breast cancer is a kind of cancer that develops from breast cells. Breast cancer usually starts off in the inner lining of milk ducts or the lobules. Although men can also breast cancer, cases of male breast cancer account for less than 0.05% of all diagnosed.[1] Breast cancer is caused when abnormal tissue in the breast begins to multiply uncontrollably. These cancerous cells can travel to other locations in the body and cause further damage. Breast cancer is represents 16% of all cancers in women.[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 protect 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.

Therapeutic interventions include Radiation therapy (radiotherapy), Surgery, Biological therapy (targeted drug therapy), Hormone therapy, Chemotherapy.[3] Though such treatment is associated with substantial cost and numerous 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.

Oroxylum indicum linn is a herbal plant with multiple health benefits which are attributed by is Various segments including leaves, root bark, heartwood, and seeds, contain diverse phytochemicals, such as prunetin, sitosterol, oroxindin, oroxylin-A, biochanin-A, ellagic acid, tetuin, anthraquinone, and emodin. Several of the compounds are under preliminary research to identify 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 utilized to induce breast carcinogenesis by increasing oxidative stress and mammary gland ducts damage.[5] Hence in the present study, we used the DMBA -induced mammary gland carcinogenesis model in female spargue-dawley rats and evaluated the carcinogenesis inhibition effects of the

hydro alcoholic extract of *Oroxylum indicum* 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 (6-8 weeks old) that weighed around 190g were housed in micro nylon boxes under standard animal housing conditions of 25°C ± 3°C and 12 ± 1 hrs day/night cycle, and had access to standard rat pellet and water ad libitum. The study is conducted after obtaining institutional animal ethical committee clearance.

Induction of Mammary Gland Carcinogenesis

There are various techniques for including mammary gland carcinogenesis 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 stilbestrol have been used in experimental studies of mammary gland carcinogenesis.[6]

In the present study, DMBA induced mammary gland tumour in rats was used to evaluate the activity against mammary gland tumour.

Preparation of Extract

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

Hydro alcoholic extracts of *Oroxylum Indicum* linn (yield = 7.5%) were prepared by soxhlet apparatus. 500gm of coarsely powdered and dried *Oroxylum Indicum* linn were extracted with 2 litres of petroleum ether, Chloroform at 40°C temperature, for 1 hour in a soxhlet apparatus. Filter and collect the extract. Repeat extraction with 2 litres of 70% and 30% Ethanol and water. Filter and collect the extract. The 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.

Oroxylum Indicum linn extracts were stored in a refrigerator at −20°C to protect from light and degeneration.

EXPERIMENTAL DESIGN

Female Sprague Dawley (n = 30) were acclimatized for 2 weeks prior to start of experiments. Rats were randomly divided into five experimental groups (n = 6). Mammary gland tumour induction was done using single dose of 7, 12-Dimethyl Benz (a) anthracene (DMBA) (7.5mg/kg) dissolved in sunflower oil (0.5mL) and physiological saline (0.25mL) just prior to use. Except normal control group, all the animals in four groups were injected with DMBA by subcutaneously. **G_1** (normal control), **G_2** served as the toxic control (mammary gland tumour) receives normal diet and Water. **G_3** (positive control) treated with injection of Vinblastin at 0.5 mg/kg body weight, Intra peritoneally. **G_4 & G_5** served as a treatment control, treated with 200 & 400mg/kg body weight of Hydro alcoholic extract of *Oroxylum Indicum* linn (HAEOI). After 45th day, all the experimental animals from each group (Control and mammary gland tumour) were sacrificed by an euthanasia method (diethyl ether). The breast tissues were surgically removed and used for measurement of tumour volume (mm in diameter) and histopathological examination. The tissue homogenates were used for the measurement of biochemical parameters.[7-9]

Evaluation of Biochemical Parameters

Effects of Hydro alcoholic extract of *Oroxylum Indicum* 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 captured by light microscopy.

Statistical Analysis

Statistical comparisons between control and treatment mean values of two parameters were analyzed 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

At the end of the experiment in non-treated rats, DMBA-induced breast tumours increased to the maximum in terms of tumour incidence (100%), tumour multiplicity per rat, compared to the normal control rats. A Significant reduction in Tumour volume was observed in treatment groups. While the animals administered vinblastin (0.5 mg/kg) individually achieved (41.21%) of tumour reduction after 45 days treatment. The combination DMBA (7.5 mg/kg, S.C) + Hydro alcoholic extract of *Oroxylum Indicum* linn (200 mg/kg, orally) treated animals achieved a significant decrease (25.83%) in the mammary tumour size with change in the total body weight of the animals (G2). However, Hydro alcoholic extract of *Oroxylum Indicum* linn at a dose of 400mg/kg treated group achieved 33.26% breast tumour 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 2**). Results in **Table 2** showed a significant reduction in the activities of Antioxidant enzymes like SOD, CAT, and GPX in DMBA induced Breast cancer in Sprague-Dawley rats whereas the values were significantly elevated after treatment with HAEOI (200mg/kg & 400mg/kg). Sprague-Dawley rats treated with standard drug vinblastin **G3** showed similar changes. The GPx level was equally increased in vinblastin and both doses of Hydro alcoholic extract of *Oroxylum indicum* linn therapy treated groups versus the cancer control rats. The level of LPx rises in DMBA induced breast cancer animals which are much influenced by the chemical carcinogen in the control animals, whereas significant reduction was observed in the Sprague-Dawley rats treated with vinblastin and both doses of Hydro alcoholic extract of *Oroxylum Indicum* linn.

Histopathological Examination

Histopathology revealed that most carcinomas exhibited an identical nuclear pattern. Most tumours were predominantly epithelial with fibrous tissue surrounding the mammary ducts. Most carcinomas exhibited a mixed structural pattern, with invasion of neighboring tissues and intense stromal desmoplastic reaction. *In vivo*, the treated groups with vinblastin and both doses of Hydro alcoholic extract of *Oroxylum Indicum* linn showed tumour tubules and formation of intra-tumour vascularization. The vast majority of the lesions that developed in the rat mammary glands were mostly carcinomas. Treatment with Hydro alcoholic extract of *Oroxylum Indicum* linn showed reduced proliferation and replacement of normal ductular and alveolar structure of mammary tissue. (Figure 1)

Table No. 1: The Effect of Hydro Alcoholic Extract of *Oroxylum Indicum* linn on Antitumour Activity.

Group	Body Weight(mg)	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)

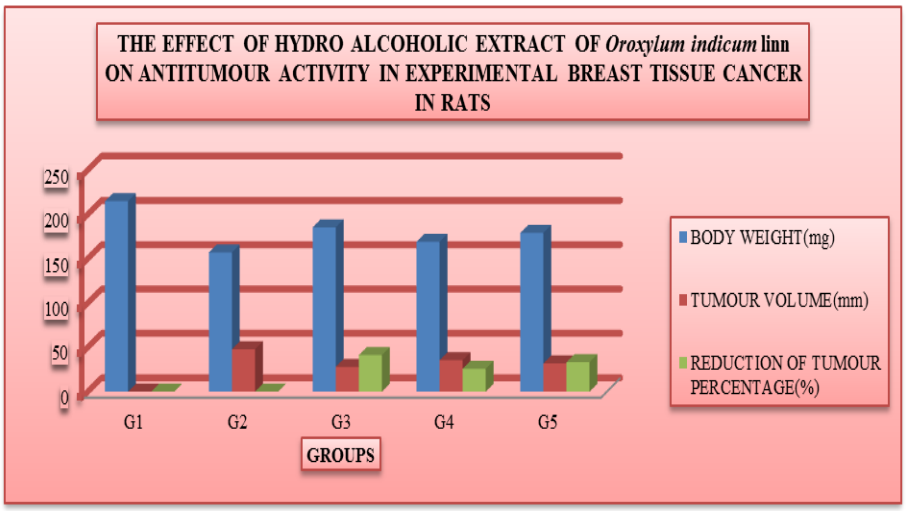
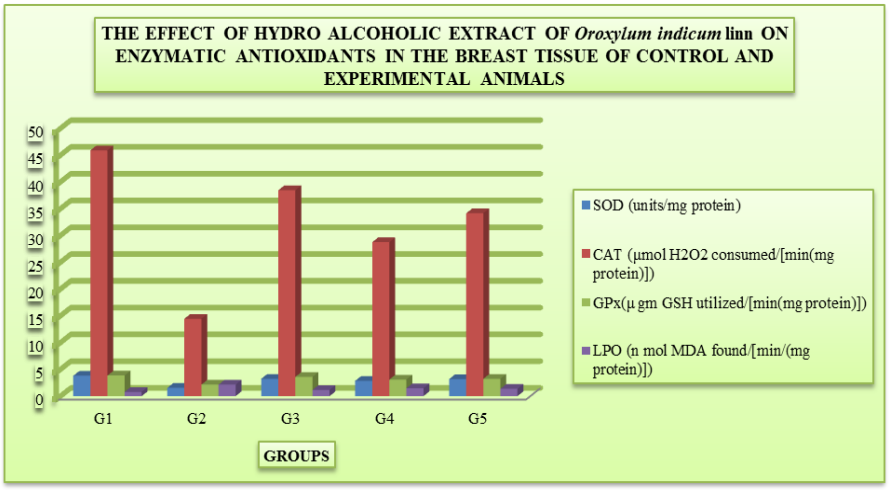


Table No. 2: The Effect of Hydro Alcoholic Extract of *Oroxylum Indicum* linn on Enzymatic Antioxidants Activity.

GROUP	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)



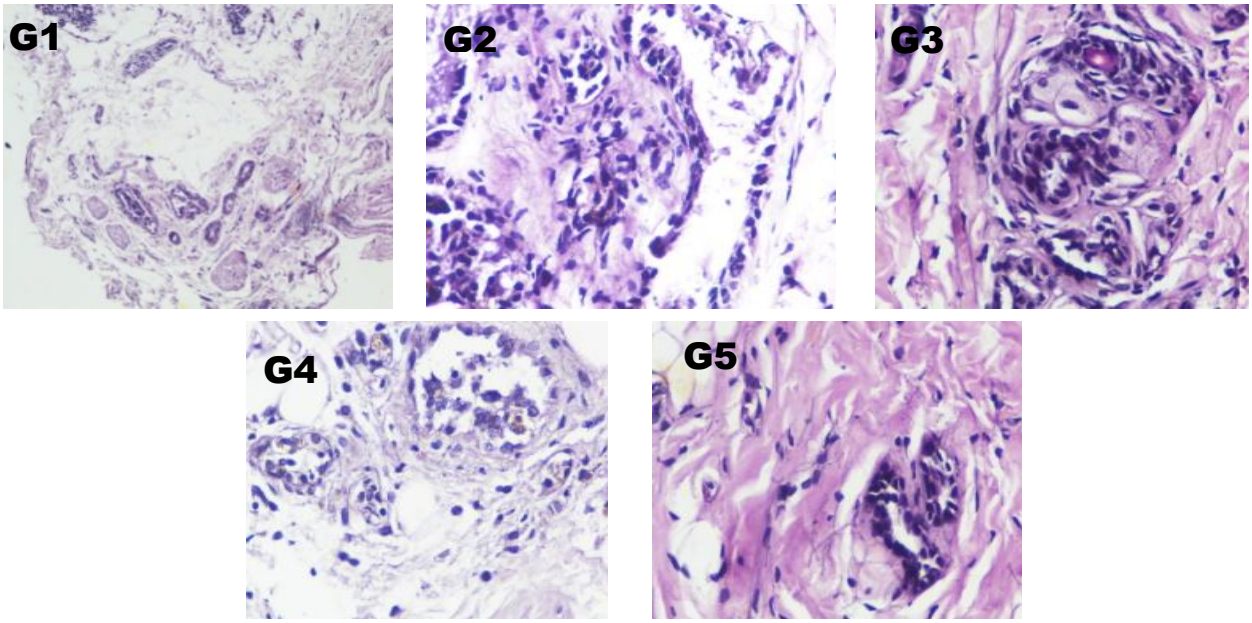


Figure 1: Histology of mammary gland in experimental rats after 45 days of treatment. G_1 - Normal control showed normal ductules, ducts and fibrous stroma and alveolar structure. **G_2 - Toxic control** showed breast parenchyma with markedly proliferated ductules and abundant fibrous stroma shows invasive ductal carcinoma of hyperchromatic tumour and clumping of chromatids. **G_3–Mammary gland tumour + vinblastin** (0.5 mg/kg) showed mild ductular proliferation with focal epithelial hyperplasia breast parenchyma with ductules, ducts and fibrous stroma. **G_4 & G_5 – Mammary gland tumour + HAEOI** (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.[10] In the present investigation, vinblastin and both doses of Hydro alcoholic extract of *Oroxylum Indicum* linn exhibited potential anticancer activity on DMBA-induced mammary tumours in rats. As a result, the body weight had also slightly increased, the tumour volume decreased, 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 *Oroxylum Indicum* 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 neighboring 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 *Oroxylum Indicum* 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.[11] The process of neovascularisation provides blood to support angiogenesis.[12]

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 *Oroxylum Indicum* 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 *Oroxylum Indicum* 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 *Oroxylum Indicum* 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 *Oroxylum Indicum* 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 *Oroxylum Indicum* 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 *Oroxylum Indicum* linn, which is known to quench the free radicals by maintaining antioxidants levels. Considering the antioxidant property of Hydro alcoholic extract of *Oroxylum Indicum* 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 *Oroxylum Indicum* linn remains to be studied in our laboratory.

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