ORIGINAL ARTICLE APMC – 350

Effect of Berberine on Prevention of 7, 12 Dimethylbenz (α) Anthracene Induced Mammary Carcinogenesis in Rats

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ABSTRACT

Objective: Berberine is a compound that has in vitro anticancer activity. This study was planned to assess chemopreventive potential of berberine on 7, 12 dimethylbenz (a) anthracene (DMBA) induced mammary carcinogenesis in rats. **Study Design:** Experimental study. **Settings:** Pharmacology Department, Post Graduate Medical Institute, Lahore and Departments of Pathology, Independent Medical College Faisalabad and Punjab Medical College Faisalabad **Period:** September 2013 to August 2014. **Methods:** A total of 30 female Sprague-Dawley rats were divided by simple random sampling into 3 groups. Group A was kept as negative control. A single dose of 20 mg of DMBA was given to both group B and C to develop mammary cancer. Berberine 75 mg/ 2ml /kg body weight was given to group C orally throughout the study period of 24 weeks. Chemopreventive potential of berberine was assessed by tumor latency, tumor incidence, tumor burden, tumor size as well as histopathologic characteristics of tumors. **Results:** Tumor incidence was decreased from 100% in DMBA group to 50% in berberine treated group. Tumor latency was prolonged from 19 week in DMBA group to 21week in berberine treated group. Tumor burden was reduced by 83.87 % while tumor size was not reduced in berberine treated rats. In group B out of 31 tumors 28(90.3%) were grade II and 3(9.7%) were grade III adenocarcinomas. In group C all tumors were grade I adenocarcinomas according to Scarff Bloom Richardson Scheme. **Conclusion:** Berberine possesses anticancer potential in DMBA induced mammary carcinogenesis.

Keywords: Berberine, Chemoprevention, DMBA, Mammary carcinogenesis

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Submitted for Publication: 10-01-2017 *Accepted for Publication: 01-02-2017*

Article Citation: Anwar A, Baig K, Shamsi FB, Chiragh S. Effect of Berberine on Prevention of 7, 12 Dimethylbenz (α) Anthracene Induced Mammary Carcinogenesis in Rats. APMC 2017;11(1):38-42.

INTRODUCTION

Breast cancer is second most common diagnosed cancer[1] and approximately 1.4 million women are diagnosed with it every year worldwide. In United States its mortality was 39,000 in year 2012 and incidence is increasing[2].

Since the incidence of breast cancer is increasing in almost every part of the world, there is need to develop therapeutic measures for breast cancer control. To fulfill this need, chemoprevention is a novel approach. Tamoxifen is being used for chemoprevention[3]. As tamoxifen have certain side effects like hot flushes, osteoporosis[4], there is need to develop a new compound with fewer side effects. Berberine is an isoquinoline derivative alkaloid. It has been derived from stem bark, roots and rhizome of different plant species, including Berberis aquifolium, Berberis vulgaris, Arcangeliesia flava, hydrastis Canadensis , Berberis aristata and Coptis chinensi [5].

In Ayurvedic medicine, Berberis Aristata and berberine is being used for prevention and treatment of cancer including breast cancer[6]. Recent researches have also evaluated that berberine possesses anticancer activities[7]. Its anticancer effect has been studied on various cancer cell lines including colon[8] and lung[9].

Anticancer effect of berberine has also been studied on human breast cancer cells in vitro[10]. Therefore there is need to conduct in vivo studies to evaluate the effect of berberine on breast carcinogenesis. Keeping all these observations in mind, this study was designed to see in vivo effect of berberine on rat mammary cancer induced by DMBA.

METHODOLOGY

Study Design: Experimental study.

Settings: Pharmacology Department, Post Graduate Medical Institute, Lahore and Departments of Pathology, Independent Medical

College Faisalabad and Punjab Medical College Faisalabad

Period: September 2013 to August 2014.

Methods: Healthy female Sprague-Dawley rats, of 6 weeks age were purchased from University of Veterinary and Animal Sciences, Lahore and kept in animal house at Post Graduate Medical Institute, Lahore in iron cages under hygienic conditions. The room temperature was maintained at 25± 2 ºC under a day night cycle and rats were fed with rat chow and water ad libitum. They received humane care according to criteria outlined in the Guide for the care and use of laboratory animals. Rats were divided by simple random sampling into 3 groups (A, B, C) each containing 10 rats. They were kept for acclimatization for one week.

Mammary gland tumor was induced by a single dose of 20 mg of DMBA dissolved in 1 ml of soy oil given by gavage at the age of 7 weeks[11] to both group B(positive control) and group C(experimental) while group A (negative control) was given 1ml of soy oil. Berberine 75 mg/ 2 ml/ kg suspended in distilled water[12] was given daily by gavage as a single morning dose throughout the study period of 24 weeks to group C, while group A and B were given 2 ml/ kg of distilled water by same route.

Body weight was measured in grams at the start of experiment and afterwards weekly till 24 weeks. Physical examination was performed weekly. Mammary glands were checked by inspection and palpation. Number of tumors was checked. Post injection time before the appearance of first breast mass was observed. At 24 weeks rats were sacrificed and all mammary glands with tumors were removed and fixed in 10% formalin solution for 24 hours. After processing, hematoxylin and eosin stained sections were prepared. Tumor size was measured in mm by ocular micrometer.

Histological grading was done by using 3 morphologic features namely, tubule formation (10x), (20x), nuclear pleomorphism (40x) and number of mitoses (40x), according to modified Scarf-Bloom-Richardson scheme. Tubule formation or pattern grade was classified in 3 grades (I, II, III), depending on the extent of solid areas. Grade I: Carcinomas with less than 25% of solid areas. Grade II: Carcinomas with 25% to 75% of solid areas. Grade III: Carcinomas with more than 75% of solid areas. Nuclear pleomorphism was classified into 3 categories (I, II, III) depending upon the size and shape of nuclei, chromatin pattern and the presence or absence of nucleoli. Tumor was classified according to the number of mitoses in 10 high-power fields into 3 grades. Grade I: Carcinomas with less than 10 mitoses / 10 HPF. Grade II: Carcinomas with 10 to 19 mitoses / 10 HPF. Grade III: Carcinomas with 20 or more mitoses / 10 HPF. Final grading was done after analyzing these three parameters as follows: Grade I carcinoma: III-V score. Grade II carcinoma: VI-VII score. Grade III carcinoma: VIII-IX score[13].

Parameters were body weight, tumor burden, tumor latency, tumor size and histopathological characteristics.

STATISTICAL ANALYSIS

Data was analyzed using statistical package for social sciences (SPSS) version 20. Quantitative data (body weight, tumor latency, tumor burden, tumor size) was presented in form of Mean ± S.E. Independent sample two tailed t-test and ANOVA were used. Qualitative data (microscopic characteristics of tumor) was presented in form of frequency and percentage and was analyzed by Chi-square test. P-value of ≤ 0.05 was considered significant.

RESULTS

This study contained 30 rats of weight between 102-109 grams. In group A body weight increased from 105.23 ± 5.37g to 298.75 ± 10.24g. In group B body weight increased from 109.13 ± 5.68g to 299.54 ± 9.49g and in group C this increase was from 102.96 ± 5.97g to 293.66 ± 6.04g. When comparison was made between groups at 0 week and 24 week by ANOVA, it was observed that difference between groups was insignificant.

There was no rat with tumor in group A. In group B all rats developed tumors. In group C, 5 out of 10 rats developed tumors. There were total 31 tumors in group B and in group C there were 5 tumors. Up to 18th week no rat developed tumor. At 19th week, there were 21 tumors in group B and no tumor in group C. At 20th week there were 31 tumors in group B versus no tumor in group C. At 21st week there were 3 tumors in group C. At 22nd week, there were 5 tumors in group C. Tumor latency was 19.7 ± 0.09 weeks in group B and 21.4 ± 0.24 weeks in group C. Difference was significant with *p-value* 0.000. Tumor burden (tumor number/rat) in group B was 3.10 ±0.27 and in group C and 0.50 ± 0.0. Reduction in tumor burden was by 83.87%. Difference between two groups was statistically significant with *p-value* 0.000.

In group B mean tumor size was 12.87 ± 0.59 mm while in group C it was 12.00±1.64 mm. Difference between two groups was not statistically significant with *p-value* 0.596.

All tumors in group B and group C were adenocarcinomas. Their grading is given in table 1, while figures 1-3 show morphological features of the tumors.

Table 1: Analysis of histopathologic grades between positive control (B) and experimental group (C) (n=10)

Parameter	Grade	Group B DMBA Treated n (%)	Group C DMBA+Berberine treated n (%)	p-value (chisquare)
Tubule formation	I II III	1(3.2%) 29(93.5%) 1(3.2%)	3(60.0%) 2(40.0%) 0(0%)	0.001*** (14.085)
Nuclear pleomorphism	I II III	0(0%) 25(80.6%) 6(19.4%)	2(40.0%) 3(60.0%) 0(0%)	0.001*** (13.604)
No of mitosis/10 HPF	I II III	0(0%) 25(80.6%) 6(19.4%)	3(60.0%) 2(40.0%) 0(0%)	0.000*** (57.577)
Histological grading Scarfbloom Richardson scheme	I II III	0(0%) 28(90.3%) 3(9.7%)	5(100%) 0(0%) 0(0%)	0.000***

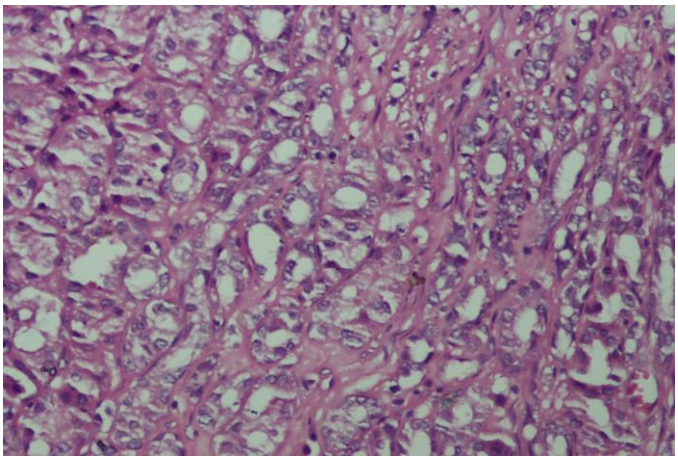

Figure 1: Microphotograph of mammary tissue from experimental group showing more than 75% of tubule formation (20x, H&E)

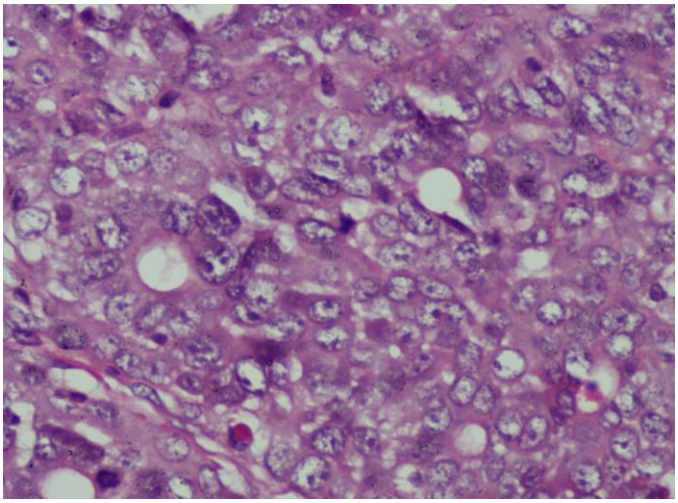

Figure 2: Microphotograph of mammary tissue from experimental group showing nuclear pleomorphism (40x, H&E)

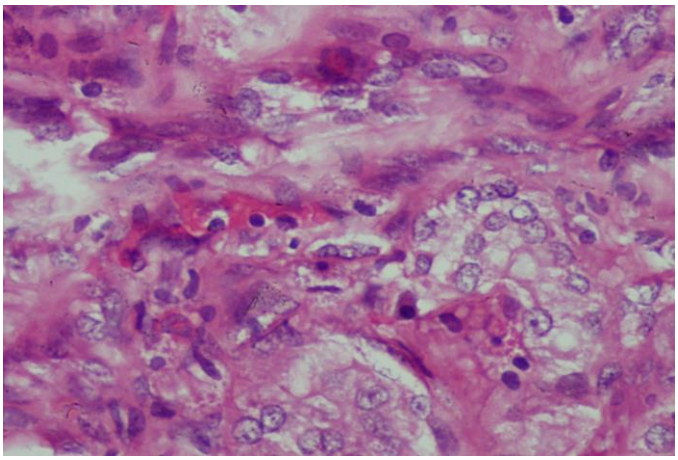

Figure 3: Microphotograph of mammary tissues from experimental group showing mitotic activity (40x, H&E)

DISCUSSION

Mammary tumors arise spontaneously in dogs, rats and mice, therefore most of studies about experimental carcinogenesis are conducted with rodents. This study was on a rat model, which is the most commonly used, because the mammary tumors in rats arise in the epithelium of terminal end buds, which are analogous structures to the terminal ductal lobules in the human breast[14]. Carcinogenesis was induced by 7, 12 DMBA, which is organ specific carcinogen for several rat strains, mainly Sprague Dawley and Wister Furth[11]. There is a maximum chance of development of mammary carcinogenesis if DMBA is administered at ages between 45 to 60 days. Active breast organogenesis and high rate of proliferation of type 1and 2 lobules are characteristics of that period[15]. Keeping this in mind, DMBA was administered at 7 weeks of age.

In present study body weight of rats in three treatment groups were similar throughout the experiment. Same observation was made in most of other studies, where DMBA treatment did not affect the body weight significantly[16, 17]. In one study body weight in DMBA treated rats was significantly lower as compared to experimental group[18].

Tumor incidence, i.e., number of rats developing tumor was 100% in DMBA group. Other studies have shown same incidence with DMBA[16-18], while one study demonstrated 87% incidence[19]. It was reduced to 50% in berberine treated group in this study. Tumor latency, i.e., post injection time for appearance of first tumor was prolonged from 19 week in DMBA group to 21 week in berberine treated group. Earlier appearance of tumors with DMBA has been noticed where it was administered at an age of 50 days[17], which corresponds with 7 week age in this study. Tumor burden, i.e., number of tumor / rat was reduced by 83.87 % while tumor size was not reduced in berberine treated rats. Mammary tumors were induced in a study on Balb/C mice by injecting cancerous cells in mammary fat pad. Berberine administration led to decrease in tumor volume and weight[20].

All tumors in DMBA group as well in berberine treated group in this study were malignant. Studies using DMBA as inducing agent have reported all tumors to be malignant[16-19].

In this study, in DMBA group 90.3% tumors were of grade II and 9.7% were of grade III while in berberine treated rats, 100% tumors were found to be of grade I adenocarcinomas. Grading of tumors was based on three histopathological parameters i.e., tubule formation, nuclear pleomorphism, and no of mitosis/10 HPF. In positive control group 93.5% tumors had grade II tubule formation while in experimental group 60% had grade I and 40% grade II. Grade I tubule formation is a good prognostic factor[21]. In positive control group 80.6% of tumors showed grade II and 19.4% grade III nuclear pleomorphism while in experimental group 40% had grade I and 60% grade II. In control group 80.6% tumors showed grade II and 19.4% tumors showed grade III mitotic activity. In experimental group 60% had grade I and 40% grade II. Higher mitotic activity shows aggressiveness of tumor and grade I mitosis is related to good prognosis[21].

Present study did not show complete prevention. No study in vivo is available on role of berberine in prevention of rat mammary cancer induced with DMBA. Similar studies with other compounds also have not shown complete prevention[16-19]. Cell culture studies on breast cancer cell lines also show different sensitivities to berberine[10, 20, 22].

DMBA when used for induction of mammary carcinogenesis causes overproduction of reactive oxygen species during its metabolism. This initiates tissue injury by propagating lipid peroxidation, which causes damage to lipids, DNA and proteins[23]. Oxidative stress has been implicated in causation of many diseases like cancer and berberine has shown to reduce oxidative stress[24]. In this study berberine not only delayed development of tumor but also reduced the incidence and number along with better in histopathological grade.

CONCLUSION

Berberine delays and prevents development of DMBA induced mammary carcinogenesis in rats with betterment in histopathologic parameters.

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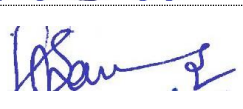
Dr. Farwa Batool Shamsi Assistant Professor, Pathology Punjab Medical College Faisalabad	Assisted the collection of data	
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