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Original Research Article

Induction of apoptosis and inhibitory potential of the methanol extract of *Caesalpinia bonduc* (L.) Roxb against breast cancer

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Abstract: *Caesalpinia bonduc (L.) Roxb* seed contains significant amounts of polyphenolic compounds that exhibit powerful antioxidant activity. The purpose of this study was to evaluate the cytotoxicity and apoptotic activity of *Caesalpinia bonduc (L.) Roxb* seed extract in a human breast cancer cell line (MCF-7) and antitumor activity against C3H (Jax) induced mammary cancer in C3H/Jax mice. Methanol extract of seeds of *Caesalpinia bonduc (L.) Roxb* was tested for cytotoxic properties on a human breast cancer cell line (MCF-7) by sulforhodamine B assay. Various doses of methanol extract of Caesalpinia *bonduc (L.) Roxb* (MECB) were further evaluated for induction of apoptosis in MCF-7 cells and evaluated for its inhibitory potential against C3H (Jax) induced mammary cancer in C3H/Jax mice. Methanol extract of *Caesalpinia bonduc (L.) Roxb* seed showed 78.4% growth inhibition against human breast cancer cells. The IC_{50} value of extract was 19 ± 1.4 µg/ml. Plant extract of *Caesalpinia bonduc (L.) Roxb* was observed to induce apoptosis of MCF-7 cells as evidenced by Sulforhodamine B dye (SRB) assay, cell-morphological changes and chromatin condensation. MECB produced significant tumor suppression during the entire duration of the study in C3H (Jax) induced tumor. Mean survival time and percentage life span also increased in MECB treated group as compared to control. Histopathology of mammary tumor showed suppressed expression of neovascularization in mammary tumor. MECB exert its chemo preventive activity may be due to antiangiogenic and apoptotic effect.

Keywords: MCF-7 cells, Breast cancer, Apoptosis, C3H (Jax) tumor

Introduction

Breast cancer is the second most prevalent cancer in the world today next to lung cancer and is a major public health problem in developing countries like India. Every year, 75,000 new cases of breast cancer are reported in India. In future, this figure may further increase due to factors like environmental pollution, food habits, consumption of genetically modified food stuffs among others. Breast cancer is becoming more common among urban women than in rural women and it might be due to the aging of the population and increase in age–specific incidents. Breast cancer is both genetically and histopathologically heterogeneous and the mechanism(s) underlying breast cancer development remains unclear. A major limitation with the present cancer chemotherapy is the serious deficiency of active drugs for the curative therapy of tumors. For thousands of years, natural products have played an important role throughout the world in the treatment and prevention of human diseases. Over 60% of the currently used anticancer agents are derived in one way or other from natural sources (1).

Apoptosis plays a vital role in controlling cell number in many physiological and developmental stages, tissue homeostasis, and regulation of immune system, while insufficient apoptosis is an integral part of cancer development. Morphologically, apoptosis is characterized by the appearance of membrane blebbing, cell shrinkage, chromatin condensation, DNA cleavage, and the fragmentation of the cell into membrane-bound apoptotic bodies. However, to the best of our knowledge, the effects of MECB on the growth of breast cancer cells and its underlying mechanisms have yet to be determined in detail. The objective of the present study was to explore the effect on apoptosis induction and mechanisms involved in MCF- 7 cells (2).

Caesalpinia bonducella (L) Fleming (synonym: *Caesalpinia crista L., C. bonduc*

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(L.) Roxb belongs to family Caesalpiniaceae and is commonly known as Natakaranja in Hindi (3). The seeds of the plant are almost globular in shape, grey, hard with a smooth shiny surface (4). The seeds of the plant contain bonducin, proteins, saponin, starch, sucrose, an enzyme, two phytosterols namely sitosterol and hepatsane, fatty acids such as palmitic acid , stearic acid, lognoceric, oleic, linolenic acid. The seed kernals of the plant contain furano diterpenes- α caesalpin, β caesalpin, γ caesalpin, δ caesalpin, ε caesalpin, and F-caesalpin(5). The twigs and young leaves of *Caesalpinia bonduc (L.) Roxb* are tradionally used for tumors, inflammation and liver disorders. In addition, various parts of this plant has been reported to possess multiple therapeutic properties like adaptogenic (6), antimicrobial(7), antiproliferatve(8), antidiabetic(9), antifilarial(10), contractility on uterus(11), hepatprotective(12), antitumor and antioxidant activities(13).The present study aimed to evaluate the cytotoxicity and anticancer effects of methanol extract of seeds of *Caesalpinia bonduc (L.) Roxb* (MECB). This study sheds light on the mechanism of induction of apoptotic activity induced by the active principles of *Caesalpinia bonduc (L.) Roxb* in human breast cancer cells MCF-7.

Materials and methods

Plant Material:

Caesalpinia bonduc (L.) Roxb seeds were collected from the local market at Mumbai. It was authenticated by Dr. (Mrs) A.S. Upadhyae, Botany group, Plant division, Agarkar Research Institute, Pune. A voucher specimen was deposited in GITAM Institute of Pharmacy, Andhra Pradesh, India.

Preparation of extract:

The seeds of *Caesalpinia bonducella* were shade dried and subjected to soxhlet extraction with 95% methanol. The extract was concentrated in a rotary flash evaporator, residue was dried in desiccator over sodium sulfite (14,15).

Chemicals and reagents:

Human breast cancer line, MCF-7 and murine C3H (Jax) cell line were obtained from National Cancer Institute (NCI), USA. RPMI-1640 medium and fetal bovine serum (FBS) was purchased from Gibco Corp. (NJ). Sulforhodamine B dye was bought from Sigma Chemical Co. All sterile plastic-wares were procured from Nunc Inc. (Denmark). All other reagents used were of analytical grade. Cells-cDNA kit was purchased from Ambion Inc (Austin Tex, USA).

In vitro cytotoxicity studies:

MCF-7 cells were cultured in RPMI-1640 medium supplemented with FBS (10%) at 37°C and maintained in a CO2 incubator in Nunc Tissue culture flasks. Cultures (70% confluent) were used to determine the cytotoxic effects of MECB extract. MCF-7 cells were seeded in 96-well microtitre-plates (at a concentration of 1×10^4 cells/well) and incubated for 24 h and MECB were added in triplicate in different lanes of the wells. Cell cultures were fixed by slow addition of cold 50% trichloroacetic acid (TCA) and SRB solution (0.4% w/v in 1% acetic acid) was added to each of the wells and the plate was kept at room temperature for 20 min. The unbound dye was removed by washing with 1% acetic acid and bound dye was then extracted by addition of 10mM Tris base (pH 10.5) to each of the wells. Optical density was measured at 540 nm on an ELISA microplate reader (16, 17).

Effect on the apoptosis of MCF-7 cells Morphological and nuclei changes:

MCF-7 cells were cultured in 96 -well plates in the presence of 50,100 µg/ml of MECB at 37^0C for 24 hrs. 60µl of the medium was removed from the wells and the same amount of the diluted dye was added. The cells were then incubated at 37ºc for 10 minutes in a 5% CO_2 incubator. 60µl of the medium was removed from the wells and observed under Nikon inverted fluorescent microscope and fluorescent images were captured. Apoptotic nucleus with condensed chromatin was scored in percentage from 200 - 300 cells/sample (18, 19).

Tumor growth inhibitory activity using C3H (Jax) mammary tumor model:

Murine C3H (Jax) cells were injected subcutaneously on the dorsal site of C3H/Jax mice. After 10-12 days of observation, a mammary tumor mass was observed in mice inoculated with murine C3H (Jax) cells. The tumor mass is removed and cut the tumor in to small pieces of approximately 2 mm^3 with the help of scalpel blade. Tumor pieces were xenografted in to the right hand limb area of new mice. From the day tumor volume reached 100 mm^3, mice xenografted with a tumor fragment were orally administered

MECB extract daily for 29 days. The untreated control group was given orally vehicle (n=12, tumor Control, Group-I), while the test groups were administered orally with MECB (n=12, 200, 400 mg/kg b.wt, Group -2&3) once daily for 29 days and intraperitoneally with standard adiramycin (n=12, 2.5 mg/kg b.wt, Group-4) on 1st, 5th, 9th day of treatment period (i.e. total 3 injections). Tumor diameter is measured every 4 day with a digital caliper and tumor volume is calculated (19).

At the end of the study six mice from each group were sacrificed for the study of antitumor activity, hematological, biochemical and histopathological analysis. The tumor bearing mice were sacrificed by cervical dislocation and the tumors were excised, appropriately photographed and fixed in 10 % phosphate buffered formalin until further evaluation. The tumor development study was lasted 29 days. The rest of the animal groups were kept to check the survival time (MST) of tumor bearing hosts for 6 weeks. MST and percent increase in life span (ILS) was calculated (13).

Statistical analysis:

Data were presented as mean± SD. Differences between the mean of control and treatment groups were analyzed using one-way analysis of variance followed by Dunnet test and Tukey's Kramer test.

Results

Thin layer chromatography

MECB showed the presence of flavonoids, terpenoids, sterols, alkaloid and tannins. The Chromatography was performed by Thin Layer Chromatography (TLC) using different solvent systems (Figure 1). TLC of flavonoid showed the presence of one spot corresponding to compound with flavonoid behavior (yellow-brown fluorescensce, after observed under UV), Standard (std) Quercetin (Rf = 0.87), MECB (Rf =0.87). The Rf value of standard quercetin was found to be 0.87 in all tracks. The chromatogram obtained from the extract was very similar to the std quercetin. TLC of MECB using Toluene: Ethyl acetate (93:7) showed 8 spots at different Rf value, TLC of MECB using Toluene: Ethyl acetate: Methanol (15:3:1.5) showed bands at different Rf 0.62, 0.57, 0.48, 0.42, 0.35, 0.28 0.24 (Figure1).

Ethyl Acetate: Formic acid: Glacial Acetic: Toluene: Ethyl acetate Methanol: Toluene: Ethyl acetate Acid: Water

Quercetine MECB MECB MECB

Fig.1: Thin Layer Chromatography of MECB

***In-vitro* cell proliferation:**

Both MECB and std Adiramycin (Adr) showed very good anti-proliferative activity (>50% cell growth inhibition) towards human breast cancer cells tested, in-vitro. The activity of extract was concentration dependent, maximum activity being obtained at the highest concentration tested (80µg/ml), whereas standard drug Adr showed cytotoxic effect even at lower concentration. The IC50 values of MECB and Adiramycin on MCF-7cells was found to be 19 ± 1.4µg/ml and < 10 µg/ml respectively. However, in the case of MCF-7 cancer cells, the anti-proliferatory effect of MECB was found to be improved but statistically significant (two way analysis of variance, *P* <0.05) as compared with std Adr (Figure 2).

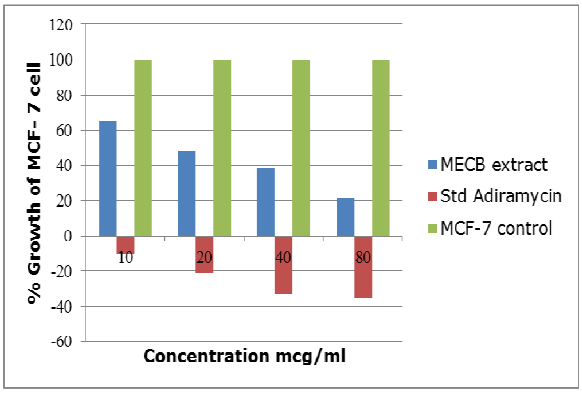
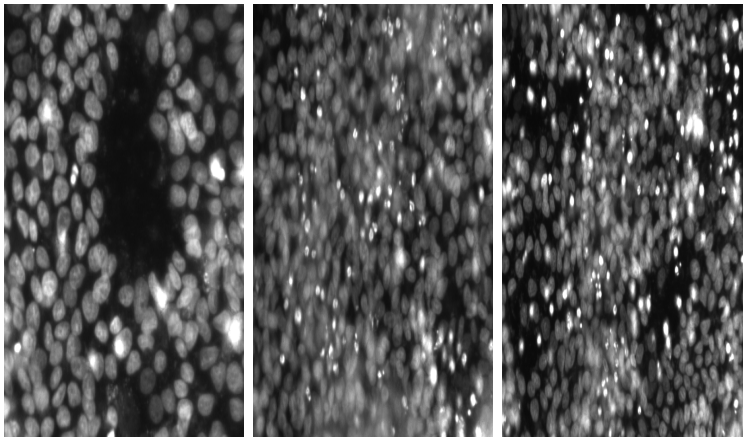


Fig.2: *In vitro* % cell growth of MCF-7, human mammary cancer cells

Morphological and nuclei changes:

In order to show the cytotoxicity of MECB is mediated by the induction of apoptosis, chromatin condensation was

analyzed in MCF-7 cells by Hoechst staining. Cells treated with 50, 100 µg/mL of MECB-showed evident morphological changes including rounding and blebbing as well as the presence of apoptotic bodies. Such morphological changes were not seen with control cells (without the extract treatment). Chromatin condensation and destructive fragmentation of the nucleus with intact cell membrane was seen with MCF-7 cells which had been treated with 50, 100 µg/ml of MECB indicated late apoptotic event. Apoptotic cells showed clear difference from non-apoptotic cells as the former are bright and their nuclei are condensed. Percentage of apoptosis was increasing with increase in concentration of extract (Figure 3 & 4).
with std Adr.



(A) (B) (C)

Fig. 3: Effect of MECB on the nuclear condensation of MCF-7 cells. (A): Hoescht stained untreated control cells, (B &C): Hoescht stained cells were examined for nuclei morphology in the presence of 50, 100 µg/ml of *Caesalpinia bonduc (L.) Roxb*

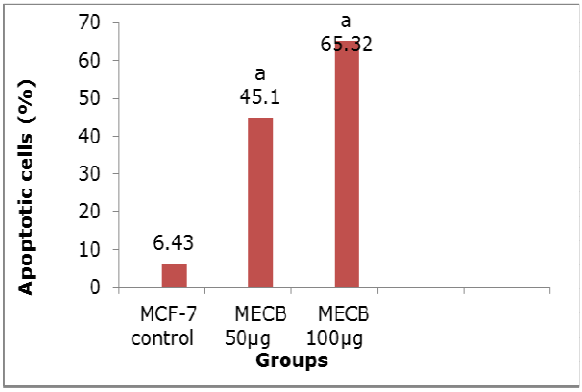


Fig.4: Percentage of apoptosis in cells treated with 50 and 100µg/ml of MECB extracts induced late apoptotic event in a concentration dependent manner. The values are expressed as mean ± SD; [a] P < 0.05

Tumor growth inhibitory activity using C3H (Jax) mammary tumor model

The mean tumor volume in orally treated group of MECB (200, 400 mg/kg) was 0.4032 cm^3, 0.406 cm^3 (One Way Anova followed by Dunnet test and Tukey's Kramer test P<0.01) were lower than that of the control group throughout the experiment period with a value of 1.085 cm^3 (P<0.001). Reduction in tumor volume in MECB treated groups indicates the tumor growth delay property. However, tumor volume in treated groups has not significantly reduced when compared to std Adr intraperitoneally with a value of 0.283cm^3(Figure 5).

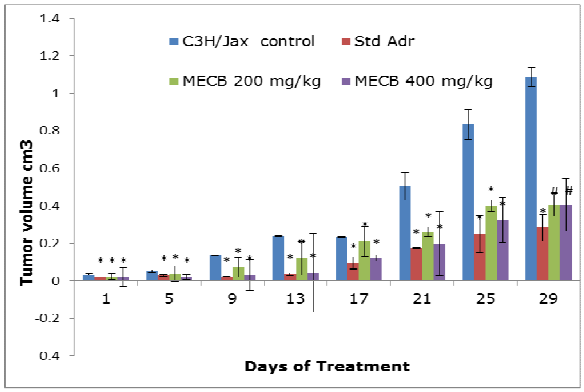


Fig.5: Inhibition of the growth of C3H (Jax) tumors in mice by treatment with methanol extract of MECB and std Adiramycin. Shown are the values of mean tumor volume (± SD, n=6). Mice treated with MECB and std adiramycin had significant smaller tumor volume compared with control mice at day 29. At day 29 the difference between the tumor volumes of MECB and treatment with Std Adiramycin was not significant. Statistical analysis was carried out by One Way Anova followed by Dunnet test and Tukey's Kramer test compared with control. * Significant Dunnet test at P< 0.01 and Tukey's Kramer test at P<0.001, # Significant Dunnet test at P< 0.01 and Tukey's Kramer test at P<0.01.

Inhibition of murine mammary carcinoma growth (%)

MECB showed good inhibition on C3H (Jax) tumor growth. MECB 200, 400 mg/kg showed inhibition of 62.8 & 62.85 % at day 29 and the maximum inhibition of 80.8 % were observed at day 13. However the inhibition rate of MECB was less than intraperitoneally administered Adr (82.14 at day 29). However at the end of the study all the treated groups showed significant retardation of tumor growth as compared to control (Table 2).

Table.2: Inhibition of C3H (Jax) tumor growth (%) of all treated groups

Groups	Days							
	01	05	09	13	17	21	25	29
Tumor Control	-	-	-	-	-	-	-	-
MECB 200 mg/kg	29.5	43.3	76.2	80.8	48.9	60	61.4	62.8
MECB 400 mg/kg	28.5	56.6	79.2	80.8	48	59	61.5	62.8
Std Adr	40	43.3	82.9	85	59.5	65.4	70	73.9

Effect on body weight of mammary tumor bearing animals

The body weight of C3H (Jax) control group was kept on increasing whereas in treated groups, slight increase in body weight was observed initially and maintained throughout the study period. The antitumor nature of tested drug was evidenced by the significant reduction in percent increase in body weight of animals treated with MECB and Adr when compared to C3H (Jax) tumor control (Figure 6). Increase in body weight in C3H (Jax) control group may be due to tumor growth whereas reduction in body weight in treated group evidenced the growth delay property of tested drug.

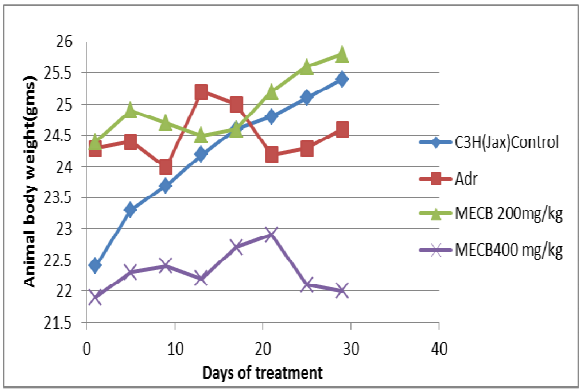


Fig.6: Body weight of experimental mice (gm) during tumor development study

Effect on the Survival Time and Increase in life span of mammary tumor bearing animals

To compare the life span of mice xenografted with C3H (Jax) tumor fragments, MST estimated from the day when the tumor volume reached at a size of 0.03- 0.1 cm^3. No mortality was observed in tumor treated groups throughout the study period. The MST of control group was 27±1.88 where as the MST of all treated groups were extended to 45 ± 0 with increase of life span by 66.6 % (Figure 7). The increase in life span of treated group was found to be significant when compared to control.

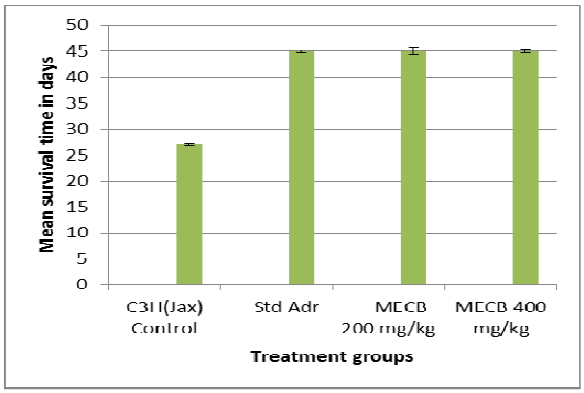


Fig.7: Mean survival time of murine C3H/Jax bearing mice

Effect on hematological parameters of mammary tumor bearing animals

C3H (Jax) tumor and tumor treated with MECB and Adr did not produce any change in PLT, MCV and MCHC. Hemoglobin content, RBC and WBC count in the tumor control group was significantly increased as compared to the treated group. PCV in tumor control was significantly increased as compared to the treated group. The total WBC and PCV was found to be decreased significantly in the treated groups when compared to the control group. Administration of MECB at the dose of 200, 400 mg/kg in C3H (Jax) bearing mice significantly reduced the WBC and PCV compared with the tumor control. Treatment with MECB and Adr changed these altered parameters more or less to the normal values.

Effect on Biochemical parameters of mammary tumor bearing animals

C3H (Jax) tumor and tumor treated with MECB and Adr did not produce any change in hepatic function parameters such as SGOT, SGPT. But a slight decrease was observed in the level of ALP in mice treated with 200 mg/kg b.wt of MECB and Adr treated group. The renal function markers such as blood urea nitrogen level was significantly decreased ($P< 0.05$) in MECB 200 mg/kg treated groups as compared to control.

Histopathology of mammary tumor

The slides were observed for evaluating the effect of test drugs on different parameters such as anaplasia, mitoses, neovasculization, fibroplasia, leuckocyte infiltration, apoptosis and necrosis.

Histopathology of MECB (200, 400 mg/kg) and std Adr treated tumor showed significant changes in mitoses, fibroplasias and anaplasia. Representative photomicrographs of hematoxylin– eosin stained tumor microsections showed minimal Anaplasis and leucocyte infiltration and mild mitoses in treated groups as compared to tumor control. Thus, control of the apoptotic mechanism may have significant therapeutic implications. Adr treated group showed moderate apoptosis as compared to tumor control (Table 3).

Table.3: Histopathology of solid tumor (mammary tumor)

Parameters	Group 1 C3H (Jax) control	2 MECB 200mg/ Kg b.wt	3 MECB 400mg/ Kg b.wt	4 Adr
Anaplasia	3	$1^{b,b'}$	$1^{c,b,b'}$	$1^{a,b,b'}$
Mitoses	3	$2^{c,b,b'}$	$2^{c,b,b'}$	$1^{a,b,b'}$
Neovascularization	3	$2^{c,b,b'}$	$1^{c,b,b'}$	$1^{a,b,b'}$
Fibroplasia	1	2^{c}	2^{c}	1^{c}
Leucocyte infiltration	3	$1^{a,b,b'}$	$1^{a,b,b'}$	$1^{a,b,b'}$
Apoptosis	1	$1^{a,c}$	$1^{a,c}$	$3^{a,b,b'}$

4(marked), 3(moderate), 2(mild), 1(minimal) and 0 (complete absence). Statistical analysis was carried out by One Way Anova followed by post hoc test. [a]significant (Kruskal-Wallis test, P< 0.05), [b,b']significant (Dunnet test, Tukey's Kramer test P< 05), [c] not significant.

Tumor angiogenesis inhibition study:

Representative photomicrographs of hematoxylin–eosin stained tumor microsections showed a dense vascularization in tumor of control group animals. Tumor treated with std Adr, MECB had significantly fewer microvessels compared with control. In fact, as evident from Table 3, the anti-angiogenesis effect was found to be significantly improved in the tumor of mice treated with std adr in comparison to MECB and control group (Dunnet test, Tukey's Kramer test P< 05). However the antiangiogenesis effect of MECB significantly improved in comparison to control group (Figure 8).

Photomicrographs of histology of mammary tumor, Murine C3H (Jax)

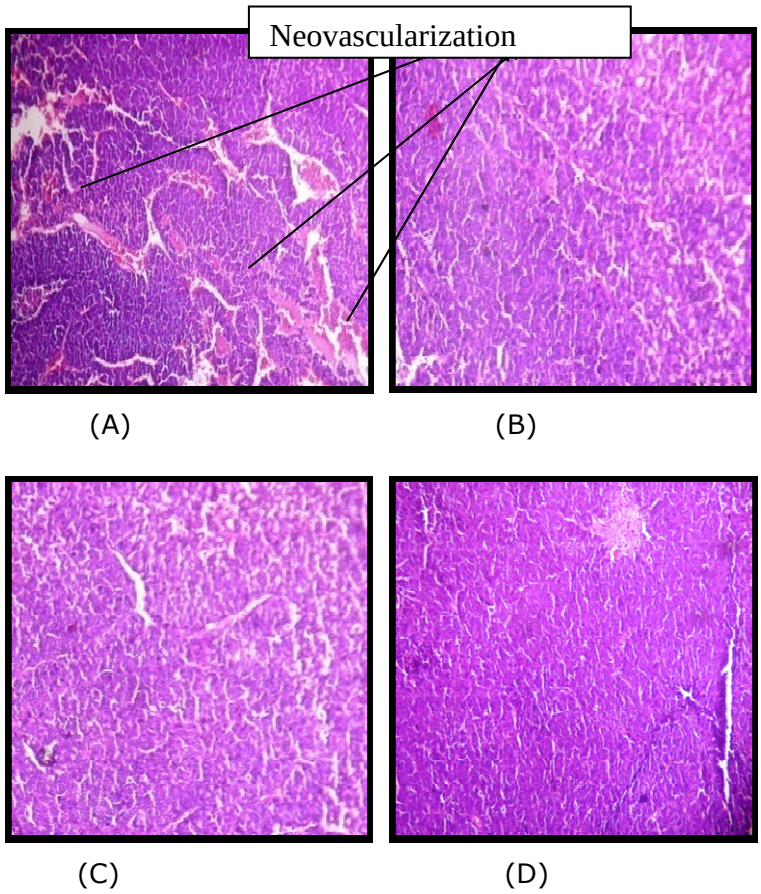


Fig. 8: Histology of mammary tumor. (A) Tumor control showed tumoral vascularization in mammary tumor at 29 day in C3H/Jax mice. A network of tumor feeding vessels was visible at the time of tumor excision in the subcutaneous tissue of control tumor (A) but has barely developed around tumor of drug treated mice (B, C & D). In control tumor dense microvasculature (red coloured patches) appeared throughout the section whereas, tumor microvessel density was significantly reduced in treated animal tumor. (B, C) Tumor treated with MECB 200, 400 mg/kg (D) Tumor treated with std Adr.

Discussion

Cancer is the second leading cause of death worldwide. Conventional therapies cause serious side effects and at best, merely extend the patient's lifespan by a few years. Cancer control may therefore benefit from the potential that resides in alternative therapies. There is thus, an increasing demand to use alternative concepts or approaches for the prevention of cancer. There is an increasing realization that chemotherapeutic agents act primarily by inducing cancer cell death through the mechanism of apoptosis (20).

Breast cancer is the most common type of cancer affecting women. It is the number 2 killer (after lung cancer) of women aged 35 – 54(21).

The result of present investigation reveals that *Caesalpinia bonduc (L) Roxb* with wide spectrum of medicinal properties have cytotoxicity against MCF-7 cell line. Cytotoxicity is one of the chemotherapeutic targets of anti-tumor activity. Most of the clinically used anti-tumor agents possess significant cytotoxicity activity in cell culture systems. Further studies confirmed that, the cytotoxicity potential is closely associated with chromatin condensation, one of the well known markers for apoptosis. 100 µg/ml of methanol extract significantly increased percentage of cells with condensed nuclei compared to control. The loss of chromatin integrity is often induced by activated caspases. It is interesting to note that hence, the MCF-7 cells used in the current study are inherently deficient for functional caspase-3 because of mutation activation (22,23).

Several previous studies have shown that this cell line is resistant to more conventional anti tumour agents due to lack of caspase-3. The methanol extract significantly induced chromatin condensation in MCF-7 cells despite the absence of caspases.

It was reported that plant-derived extract containing antioxidant principles showed cytotoxicity towards tumor cells and anti-tumor activity in experimental animals. Anti-tumor activity of these antioxidants is either through induction of apoptosis or by inhibition of neovascularization. The implication of free radicals in tumors is well documented. In addition various studies reported that *Caesalpinia bonduc (L) Roxb* possess antioxidant properties. The free radical hypothesis supported the fact that the antioxidants effectively inhibit the tumor, and the observed properties may be attributed to the antioxidant and anti-tumor principles present in the extract [13]. Several antiangiogenic strategies have been developed to inhibit tumor growth by targeting different components of tumor angiogenesis. Many phytochemicals could have a tremendous potential as anti-angiogenic agents to check the cancer development and metastasis (24).

Methanol extract of *Caesalpinia bonduc (L) Roxb* at a dose of 200 mg/kg body weight reduced the tumor burden effectively. The results of present study proved that MECB extract can reduce the mammary tumor burden. Effective dose of MECB extract have increased the life span of tumor bearing animals. Usually, in cancer chemotherapy the major problems that are being encountered are of myelosuppression and anemia. The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or hemoglobin percentage, and this may either due to iron deficiency or due to hemolytic or myelopathic conditions (13). Treatment with MECB brought back the hemoglobin content, RBC and WBC count more or less to normal levels. Average life span of tumor treated groups was increased than the standard Adiramycin at a dose of 2.5 mg/kg body weight. The findings of tumor angiogenesis inhibition study suggested that MECB treated tumor showed minimal neovascularization compared to tumor control.

Conclusion

In the present study, our findings propose a underlying mechanism by which methanol extract of *Caesalpinia bonduc (L.) Roxb* (MECB) causes cytotoxicity against human MCF-7 cells by inducing chromatin condensation. Anti-tumor activity of MECB is either through induction of apoptosis or by inhibition of neovascularization.

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References

1. Merlin N J, Parthasarathy V, Santhoshkumar T R, Induction of apoptosis in human breast cancer cell line MCF-7 by phytochemicals from Gmelina asiatica. African Journal of Biotechnology, 2010, 28, 4451-4456.

2. Watanapokasin Ramida, Jarinthanan Faongchat, Nakamura Yukio, Sawasjirakij Nitisak, Jaratrungtawee Amornmart, Suksamrarn Sunit, Effects of α-mangostin on apoptosis induction of human colon cancer. World Journal of Gastroenterology, 2011, 17(16), 2086-95.

3. Kirtikar KR, Basu BD, Indian Medicinal Plants, vol-II. 2nd ed. International Book Distributors, Dehradun, India, 2005, 842-4.

4. Rastogi P Ram, Mehrotra BN. Compendium of Indian medicinal plants, Vol-1. CDRI and NISC, Cataloguing-in-publication Data, 1990, 67.

5. Mathapati S, Sidramayya, Hogade M, Patil K, Wadkar G, Hindurao, Effect of Caesalpinia bonduc (Linn) Roxb. seed extracts on human neutrophil. Journal of Pharmacological Research, 2010, 3(3), 467-9.

6. Kannur DM, Hukkeri VI, Akki KS, Adaptogenic activity of Caesalpinia bonduc seed extracts in rats. Journal of Ethnopharmacology, 2006, 108, 327-31.

7. Tasleem A, Mandal TK, Nareshkumar, Bhosale, JD, Hole A, Sharma GL, Padhi MM, Lavekar GS, Dabur R, Invitro and invivo antimicrobial activities of seeds of Caesalpinia bonduc (Lin.) Roxb. Journal of Ethnopharmacology, 2009, 123, 177-80.

8. Yadav PP, Arora A, Bid KH, Konwar RR, Kanojiya S. New cassane butenolide hemiketalditerpenes from the marine creeper Caesalpinia bonduc and their antiproliferative activity. Tetrahedron Letter, 2007, 48, 7194-98.

9. Aswar PB, Khadabadi SS, Deokate UA, Jain SP, Biyani KR, Jawarkar RD, Evaluation of Antidiabetic activity of Caesalpinia bonduc(L.) Roxb Leaves (Caesalpianceae) in Alloxan induced diabetic mice. Indian Journal of Pharmaceutical Education and Research, 2009, 43(3), 280-3.

10. Gaur RL, Sahoo MK, Dixit S, Fatma N, Rastogi S, Kulshreshtha DK, Chatterjee RK, Murthy PK, Antifilarial activity of Caesalpinia bonducella against experimental filarial infections. Indian Journal of Medical Research, 2008, 128, 65-70.

11. Datte JY, Traore A, Offoumou AM, Ziegler A, Effect of leaf extract of Caesalpinia bonduc (Caesalpiniaceae) on the contractile activity of uterine smooth muscle of pregnant rats. Journal of Ethnopharmacology, 1998, 60,149-155.

12. Md Rajib Ahsan, Islam KM Monirul, Bulbul Israt Jahan, Musaddik, Md Ashik, Haque Ekramul, Hepatoprotective activity of methanol extract of some medicinal plants against carbon tetrachloride induced hepatotoxicity in rats. European Journal of Scientific Research, 2009, 37(2), 302-310.

13. Gupta M,Upal K, Mazumder, Ramanathan S, Thangavel S, Antitumor activity and antioxidant status of Caesalpinia bonducella against Ehrlich ascites Carcinoma in swiss albino mice. Journal of Pharmacological Sciences, 2004, 94,177-184.

14. Wagner H, Bladt S. Plant drug analysis A thin layer chromatography atlas 2nd edition, Springer, 1996,1-14.

15. Ana, Antibacterial Effects of Plectranthus amboinicus (Lour.) Spring (Lamiaceae) in Methicillin Resistant Staphylococcus aureus (MRSA). Latin American Journal of Pharmacy, 2009, 28, 460-4.

16. Kale, Decreased B16F10 Melanoma Growth and Impaired Tumour Vascularization in BDF1 Mice with Quercetin–Cyclodextrin Binary System. Journal of Pharmacy and Pharmacology, 2006, 58, 1351-1358.

17. Vanicha V, Kanyawim K, Suforhodamine B colorimetric assay for cytotoxicity screening. Nature Protocols, 2006, 1(3), 1112-1116.

18. Yu-Ping Y, Hsien-Shou K, Hsin-Da T, Yi-Chen P, Yuh-Ling L, The p53-dependent apoptotic pathway of breast cancer cells (BC-M1) induced by the bis-type bioreductive compound aziridinylnaphthoquinone. Breast Cancer Research, 2004, 7(1), R19-R26.

19. Jung-Sun K, Tae- Young J, Chong Kwan C, Yeon- Weol L, Hwa Seung Y, Antitumor effect of Venenum Bufonis in a NCI-H460 Tumor regression model. Journal of Accupuncture Meridian Studies, 2010, 3(3), 181-187.

20. Lowe SW, Lin AW, Apoptosis in cancer. Carcinogenesis, 2000, 21, 485-495.

21. Holmes HN, Sprague IS, Kowalak JP, Hughes AS, Johnson PH, Mills EJ, Malignant neoplasms: Breast Cancer; in The Professional Guide to Diseases, 7th ed., Springhouse publishing Co., Spring House, USA, 2001, 47-150.

22. Taraphdar Amit K, Roy M, Bhattacharya R K, Natural products as inducers of apoptosis:Implication for cancer therapy and prevention. Current Sciences, 2001, 80(11), 1387-1396.

23. David HJ, Vegetables, fruits and phytoestrogens in the prevention of diseases. Journal of Postgraduate Medicine, 2004, 50(2), 145-149.

24. Kerr, Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. British Journal of Cancer, 1972, 26, 239-257.

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