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Chemomodulatory Influence of Melatonin Rich *Morus Alba* L. on N-Methyl-N-Nitrosourea (NMU) Induced Mammary Carcinoma in Sprague-Dawley Rats

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ABSTRACT

We show that Melatonin rich *Morus alba* Linn is a potent inhibitor of mammary carcinogenesis induced by N-methyl-N-nitrosourea (NMU) in Sprague Dawley rats. Rats were first treated with a single dose of NMU (50 mg/kg body weight) and then treated with Methanolic extract of *Morus alba Linn* (MMA) with different concentrations 300mg/kg and 500mg/kg body weight. MMA was highly effective in reducing tumor incidence, tumor volume, and average tumor burden, as well as extending tumor latency. The MMA was particularly effective; when compared to the NMU only group tumor-free at autopsy and significantly diminished tumor number and tumor burden. MMA at the dose of 300mg/kg is effective when compared to 500mg/kg body weight. Compared with the Melatonin (MEL) and standard Tamoxifen (TAM) MMA also shown equivalent activity in tumor latency, tumor burden and tumor volume and compared with NMU only group tumor latency, tumor burden and tumor volume decreased.

Keywords: Melatonin; MMA; Tamoxifen; *Morus alba Linn*; Mammary tumor; Tumor latency; Tumor volume; Tumor incidence; and Tumor burden.

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INTRODUCTION

The occurrence of breast cancer is the most common cancer disease in women is still rising. This fact indicates an urgent need for improving not only the treatment but also the prevention of the disease. A wide range of substances acting on various stages of tumor progression is already known [1]. Cancer originating in the mammary gland is the most common type of cancer in women. The lifetime risk of breast cancer for a woman in developed countries has been calculated at around 1 in 7 to 1 in 10. When it comes to Catalonia the latest analyses report an accumulated life risk of breast cancer of 1 in 11 with a 1 in 33 probability of death due to this disease. This means around 10% of the female population will be diagnosed with the breast cancer at some point of their life. Out of these patients, around 30-40% will eventually die of this disease, mainly due to the development of metastases, an incurable cancer in most type of cancer [2]. The current controversy over the safety and efficacy of the use of tamoxifen to prevent breast cancer in women at high risk emphasizes the need to develop new agents for suppression of carcinogenesis [3]. Since melatonin is useful agent for Chemoprevention of cancer, and treatment with natural agents containing melatonin is one of the best approaches in cancer prevention. The recent findings that melatonin is a high-affinity ligand for both the mel_1 and mel_2 families of melatonin receptors and focus attention on the critical role in mammary gland physiology [4]. To date, there have been no reports on the use of MMA to prevent breast cancer, either in experimental animals or women. We now show that MMA is highly effective for preventing mammary cancer in the standard rat model which uses a single dose of NMU as carcinogen. Furthermore, we show that MMA is therapeutically useful for this purpose and that MMA have the chemopreventive activity in standard animals. Finally, we show that MMA shows low tumor latency, tumor volume and tumor burden compared to the NMU only group and having slightly decreased the activity when compared to standard and MEL treated animals. Since loss of functional integrity has been implicated in breast cancer progression [5-11], these results suggest that MMA may be useful, not only in the prevention of breast cancer, but also in its treatment.

MATERIALS AND METHODS

Materials

Melatonin obtained from Himedia (Mumbai), Tamoxifen was obtained as a gift sample from Torrent pharmaceuticals (Mumbai), Methanol obtained from Loba chemicals (Mumbai) and *Morus alba* Linn leaves were collected locally from Erode district of Tamilnadu. Other reagents and solvents used were of reagent grade.

Extraction

Extraction of *Morus alba* was done by methanol using continuous soxhlet extraction up to the disappearance of colour of the leaves and then redistilled with the same apparatus and evaporated the excess solvent in extract and the resulted extract given to animals.

Animals

Female Sprague dawley rats at the age of 21 days were obtained from Thrisur veterinary college, Thrisur, Kerala.

Methodology

Mammary Carcinogenesis Studies**.** A standard protocol for induction of breast cancer in Sprague-Dawley rats was used [12, 13] with a single i.v. dose of NMU, 50 mg/kg body weight. All palpated tumors were confirmed at autopsy. MMA at the dose of 300mg/kg, 500mg/kg, MEL 1mg/kg and TAM 10mg/kg were administered orally daily in the evening at 4:00pm and fed ad libitum, beginning after formation of tumors in animals after injection with NMU. MEL (Himedia Company, Mumbai), NMU (Sigma, Mumbai) and TAM (Torrent pharmaceuticals, Mumbai) were dissolved in distilled water and given orally [13].

Methods for statistical analysis

One way ANOVA, followed by Tukey's multiple comparison of all pairs of columns test was performed. NMU only (Group 2) is compared with Normal control (Group 1), MMA 300 mg/kg (Group 3), MMA 500 mg/kg (Group 4), MEL- 1 mg/kg (Group 5) TA- 10 mg/kg (Group 6) and MEL only 1mg/kg (Group 7).

RESULTS

Inhibition of Mammary Carcinogenesis. In our laboratory, greater than 66.6% of Sprague-Dawley rats treated with NMU alone (50mg/kg body weight) develops invasive mammary adenocarcinomas within 128 days of a single i.v. injection. We report here results obtained on a total of 30 rats treated with NMU, using MMA at 300mg/kg and 500mg/kg, MEL and TAM, to inhibit the development of mammary carcinoma. We chose TAM because of the current ongoing clinical trials being conducted in thousands of women worldwide, evaluating the efficacy of this agent for prevention of breast cancer. At optimal doses, TAM is highly effective in the NMU rat model [14]. To evaluate therapeutic efficacy of MMA, MEL and TAM, we deliberately used fixed doses with reference to previous experiments. The results are shown in Table 1 and Fig. 1. In all cases, treatment with chemopreventive agents began before 15 days of i.v NMU induction. The data indicate that MMA 300mg/kg is highly effective in this animal model, and in particular, that it is markedly superior to MMA 500mg/kg. Daily injection of MMA caused significant suppression of carcinogenesis, as measured by four end points, i.e., tumor incidence, average number of tumors per rat, average tumor burden (the total weight of all of an animal's tumors), and tumor latency. The effect of MMA on the number of rats that were tumor free at autopsy is particularly striking; in the two experiments (Table 1), only 2 of 6 (33.3%) of the control rats treated with NMU alone was tumor free, while 5of 6 (83.3%; $P < 0.001$) of the rats treated with NMU plus MMA 300mg/kg was tumor free, 4 of 6 (66.6%; $P< 0.001$) of the rats treated with NMU plus MMA 500mg/kg was tumor free, 5 of 6 (83.3%; $P < 0.001$) of the rats treated with NMU plus melatonin was tumor free, 5 of 6 (83.3%; $P < 0.001$) of the rats treated with NMU

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plus tamoxifen was tumor free. In addition, statistical analysis indicates that the effects of MMA, MEL and TAM on inhibition of tumor number and tumor burden are also highly significant ($P < 0.001$). Fig. I also shows that all treated groups greatly increases tumor latency compared to NMU only group. Pooling the results of Experiments indicates that the higher dose of MMA 500mg/kg, doubles the number of rats that are tumor free at autopsy ($P < 0.001$), decreases the average number of tumors/rat from 0.66 to 0.33 ($P < 0.001$), and lower dose of MMA 300mg/kg, four times of the number of rats that are tumor free at autopsy ($P < 0.001$), decreases the average number of tumors/rat from 0.66 to 0.165 ($P < 0.001$). Finally, when all 6 animals treated with MMA 300 mg/kg, MMA 500mg/kg, MEL 1mg/kg and TAM 10mg/kg are compared with NMU only group, the P values for the efficacy all treated groups are all < 0.001, whether one measures effects on inhibition of tumor incidence, inhibition of average number of tumors/rat, or on average tumor burden. None of the chemopreventive agents caused any grossly evident toxicity. Although there was some slightly diminished weight gain in NMU only animals. In treated animals with MMA 300mg/kg shows little weight gain compared to remaining treated groups.

Table: 1 shows the effect of *Morus alba* on mammary tumor genesis.

S.No.	Groups	Latency	Tumor Incidence	Tumor volume
1.	NMU only	48.33±2.028	4/6 (66.66 %)	3.8±0.4
2.	NMU+ MA LExt 300mg/kg	75.67±2.028^{A}	1/6 (16.66%)^{A}	1.5±0.6^{B}
3.	NMU+ MA LExt 500mg/kg	70.00±2.082^{A}	2/6 (33.33%)^{A}	1.9±0.4^{A}
4.	NMU+MEL 1mg/kg	81.33±2.728^{A}	1/6 (16.66%)^{A}	1.3±0.7^{A}
5.	NMU+TA 10mg/kg	84.00±2.646^{A}	1/6 (16.66%)^{A}	0.9±0.8^{A}

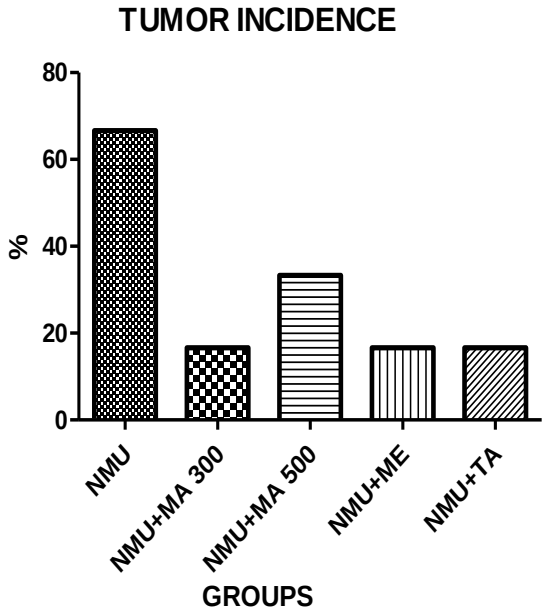

Fig : 1 Tumor Incidences

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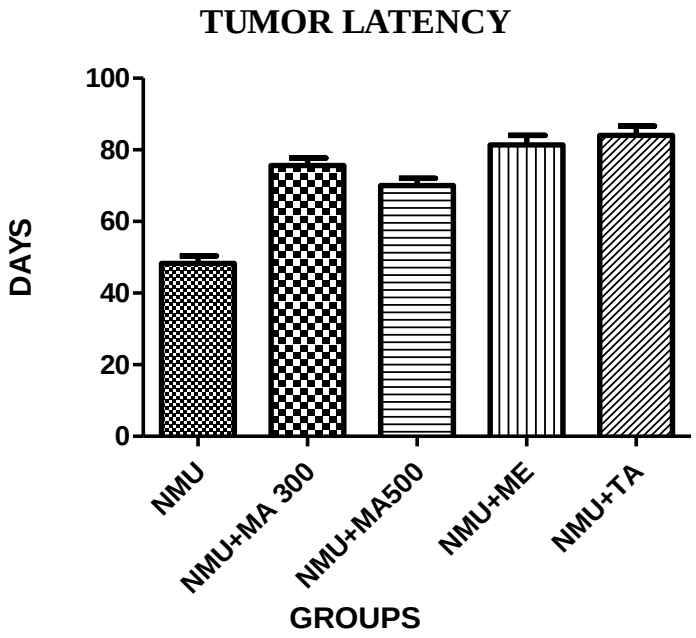

Fig : 2 Tumor Latency

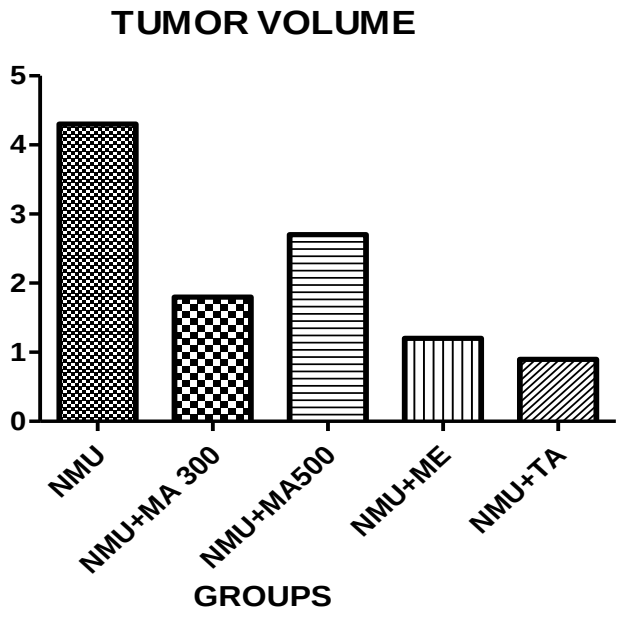

Fig: 3 Tumor Volume

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Body Weight Analysis

Table 2 shows the effect of MA on body weight on 0th day and at the end of 14th week

S.NO	Groups	Body weight	
		0th Day	14th week
1.	NMU only	101.3±5.34	154.6±5.875
2.	NMU+ MMA300mg/kg	98.5±4.344	167.7±4.709
3.	NMU+ MMA500mg/kg	97.3±2.477	166.2±4.07
4.	NMU+MEL 1mg/kg	98.5±4.726	179.8±4.922
5.	NMU+TA 10mg/kg	102.8±7.288	185.5±3.948

DISCUSSION

We report here, for the first time, the use of MMA for the prevention of breast cancer in a widely used animal model. In this MEL has high affinity for MEL_1 as well as MEL_2, broader receptor activation may contribute to the greater activity of MMA. In the dose of 300mg/kg shows greater activity when compared to 500mg/kg. There is concern at present regarding the safety of chronic administration of the standard 10 mg/kg daily dose of TAM, which is widely used currently under study in long-term human chemoprevention trials. The present data suggest that it may be possible to be a chemotherapeutic agent in the prevention of mammary tumor. The present data emphasize the importance of obtaining new information about the safety of chronic administration of MMA to human populations at high risk for development of cancer so that it may be clinically evaluated for suppression of carcinogenesis. Although we have emphasized the use of MMA for chemoprevention in the present study, Melatonin also inhibits the growth of many overtly malignant breast cancers.

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