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Anticancer and Anti-inflammatory Potentials of Thymoquinone (an Active Ingredient from *Nigella sativa* seeds) Studied in 7, 12-Dimethylbenz(α)anthracene Induced Experimental Breast Cancer

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ABSTRACT

Plant derived compounds have been reported for their anticancer as well as antioxidant potential. Thymoquinone, the bioactive principle of Nigella sativa seeds possess a wide array of beneficial as well as pharmacological effects. The present study was aimed to evaluate the effect of thymoquinone on the levels of tumor markers and proinflammatory cytokines, NF-κB and nitric oxide in experimental groups of rats. A single dose of 7, 12-Dimethylbenz (α) anthracene (DMBA) (20 mg/kg/rat) diluted in olive oil was given orally to induce breast cancer. Thymoquinone (25 mg/kg body weight) was orally administered to DMBA Induced breast cancer rats. All animals were sacrificed after 13 weeks of experimentation. The important diagnostic biochemical indices of breast cancer (tumor markers) such as Alpha-fetoprotein (AFP), Carcinoembryonic Antigen (CEA) and Cancer Antigen 15-3 (CA 15-3), Serum Total Sialic Acid (TSA) and Lipid-bound Sialic Acid (LSA) were assayed in the serum. The anti-inflammatory potential of thymoquinone was determined by measuring the levels of proinflammatory cytokines such as Tissue Necrosis Factor (TNF-α), Interleukin-1β (IL-1β), Interleukin-6 (IL-6) in the liver and kidney tissues. There was an observed decrease in the levels of tumor markers and declined proinflammatory cytokines level in the rats treated with thymoquinone. The results of the present study indicate that thymoquinone possess anti-inflammatory potential.

Keywords: Thymoquinone, DMBA, Anticancer, Antiinflammatory

INTRODUCTION

Breast carcinoma is the leading cause among women in most developed countries [1]. It is not a single disease, which comprises of many biologically different entities with distinct pathological features and clinical implications [2-4]. Although current therapies have shown some promise against breast cancer, there is still no effective cure for the majority of patients in the advanced stages of breast cancer. Development of effective agents to slow, reduce, or reverse the incidence of breast cancer in high-risk women is necessary. Chemoprevention of breast cancer by natural products is advantageous, as these compounds have few side effects and low toxicity compared to synthetic compounds [5].

Nigella sativa L., belongs to the botanical family Ranunculaceae. *N. sativa* extract has been shown to possess immunomodulatory, antioxidant, antitumoral and antidiabetic properties. Most of the reported biological properties were mainly due to thymoquinone which is the main active ingredient of the volatile oil isolated from the black seeds [6,7]. Thymoquinone (TQ) has been reported for wide array of pharmacological activities such as anti-inflammatory, antioxidant and anti-neoplastic effects both *in vitro* and *in vivo* [8-10]. Recently we have reported the anticancer and liver protective role of thymoquinone in DMBA induced breast cancer rats [11,12]. In the present study an attempt has been made to evaluate the effect of thymoquinone on the levels of proinflammatory cytokines in control and experimental groups of rats.

MATERIALS AND METHODS

Female Wistar rats, at the age group of 45-48 days were used in the study and the rats were housed spaciously in individual cages and maintained under standard experimental conditions: temperature 25 ± 1°C, relative humidity 60 ± 5% and 12 ± 1 h (light/dark cycle) in Dr. Almpgibms, University of Madras, Taramani campus, Chennai-600113. The animals were fed with commercially available balanced pellet diet (Amrut laboratory Animal Feed, Bangalore, India) and water *ad libitum*. The animals were acclimatized for one week prior to the initiation of experiments. The experimental design was performed in accordance with the current ethical norms approved by the Ministry of Social Justice and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines (IAEC No.: 01/02/2013).

Experimental design

The rats were divided into four groups each comprising of six rats as detailed below: Group I: Normal control animals fed with standard diet and pure drinking water, Group II: Animals treated with 7, 12-Dimethylbenz (α) anthracene (DMBA) (20 mg/kg.b.w) in 1.0 ml olive oil by gastric incubation to induce breast cancer. Group III: Breast cancer bearing animals treated with thymoquinone (25 mg/kg b.wt) after the administration of DMBA from 10th week to 15th week, Group IV: Control Animals treated with thymoquinone alone (as in group III).

The experimental animals were fasted overnight and sacrificed by sodium pentothal anesthesia followed by cervical decapitation. Blood was collected with and without anticoagulant and the serum was centrifuged at 5000 rpm for 15 min to obtain a clear supernatant and stored at -70°C until its use for further biochemical analysis. Breast tissues from control and experimental groups of rats were immediately excised, washed in ice-cold Phosphate-buffered Saline (PBS) to remove the blood stains, blotted, weighed and homogenized in Tris-HCl buffer (0.1 M, pH 7.4) using a Teflon homogenizer to prepare 10% (w/v) tissue homogenate. This homogenate was centrifuged at 12,000 g for 30 min at 4°C to obtain a clear supernatant. This supernatant was pooled and used for further analysis.

Evaluation of markers of tumorigenicity

Tumor markers Alpha-fetoprotein (AFP), Carcinoembryonic Antigen (CEA) and Cancer Antigen 15-3 (CA 15-3) were quantified based on solid phase enzyme linked immunosorbent assay method using UBI MAGIWELL (USA) enzyme immunoassay kit according to the manufacturer's instructions. The serum levels of Serum Total Sialic Acid (TSA) and Lipid-bound Sialic Acid (LSA) were determined by the method of Plucinsky et al. [13].

Assay of Tissue Necrosis Factor (TNF-α), Interleukin-1β (IL-1β), Interleukin-6 (IL-6) and NF-κB/p65 subunit

The levels of proinflammatory cytokines such as TNF-α, IL-1β and IL-6 in breast, liver and kidney tissue homogenates of control and experimental groups of rats were determined by Enzyme-linked Immunosorbent Assay (ELISA) kits from Biosource, Camarillo, CA.

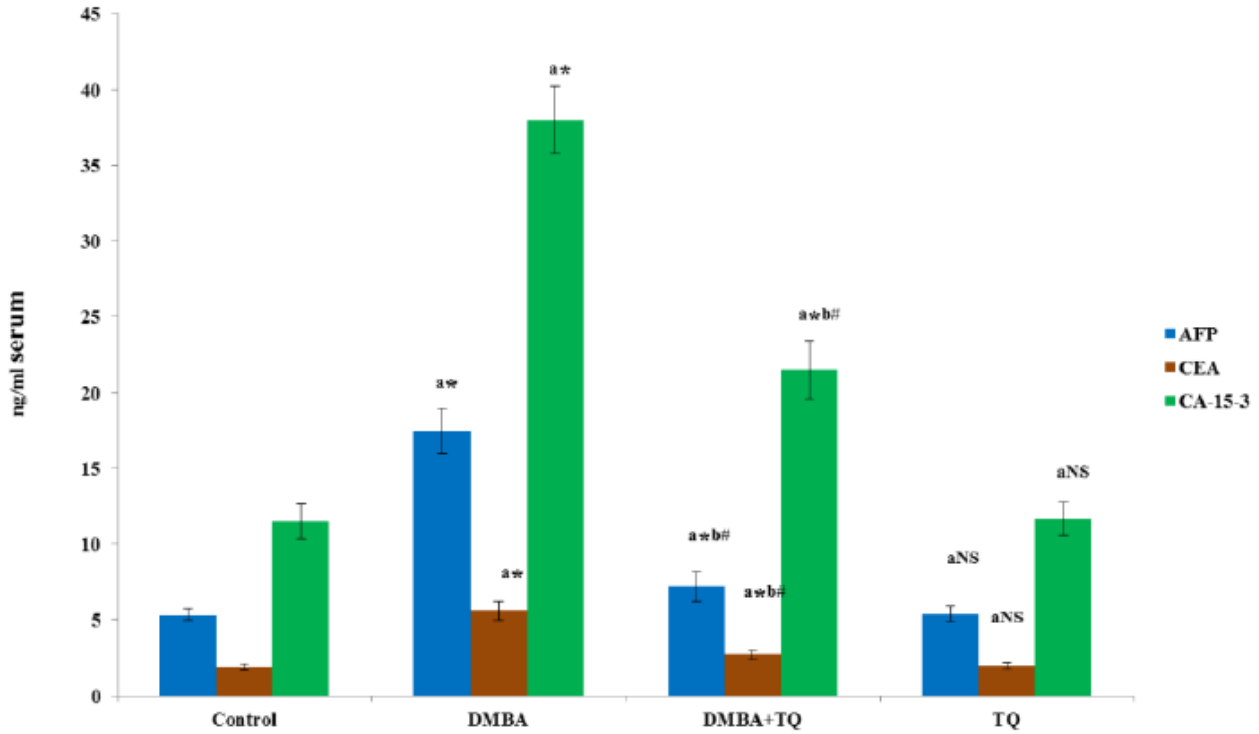
Statistical analysis

Statistical analysis was performed using SPSS 16.0 (SPSS, Inc., Chicago) statistical package. The results were expressed as mean ± S.D One-way ANOVA followed by post hoc test LSD was used to correlate the difference between the variables. Values were considered statistically significant if *P<0.001; @ P <0.05; #P<0.01.

RESULTS AND DISCUSSION

Effect of thymoquinone on the tumor markers

Figures 1 and 2 shows the effect of thymoquinone on the levels of serum tumor markers in the control and experimental groups of animals. There was a significant increase in the AFP, CEA, CA 15-3, TSA and LSA levels in the DMBA induced rats. Thymoquinone treatment to DMBA induced animals significantly decreased the levels of all the tumor markers suggesting the therapeutic value of thymoquinone in cancer treatment.



Values were given as mean ± S.D for groups of six rats in each. Statistical was compared within the group as follows: a-compared with control rats; b-compared with DMBA induced rats

Figure 1: The effect of thymoquinone on the levels of serum tumor makers ASP, CEA, CA 15-3 of control and experimental groups of rats

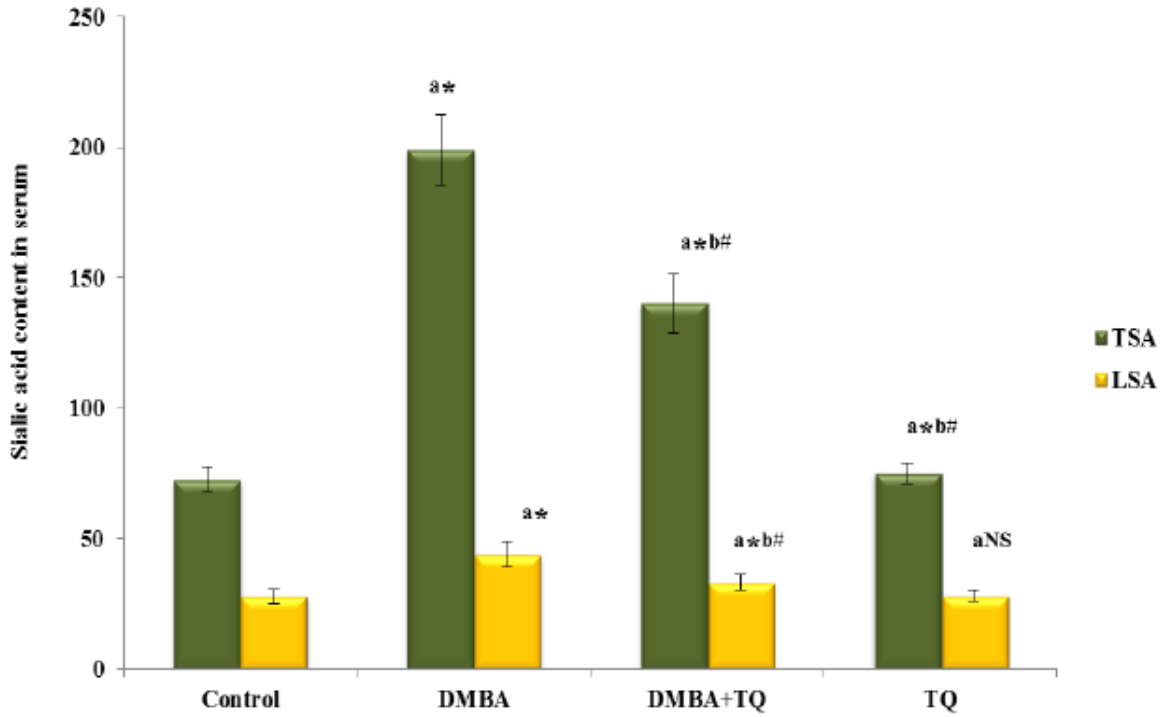


Figure 2: The effect of thymoquinone on the levels of total sialic acid (TSA) and Lipid bound sialic acid (LSA) in serum of control and experimental animals

Biochemical marker enzymes are routinely assayed to screen cancerous state for diagnosis, prognosis and monitoring the progress in response to therapy. Alpha-fetoprotein (AFP) is a major plasma protein produced by the yolk sac and liver during fetal development. It is thought to be the fetal form of serum albumin [14]. AFP is often used as a biomarker to detect a subset of tumors.

CEA are Glycosylphosphatidyl Inositol (GPI) cell surface anchored glycoproteins whose specialized glycoforms serve as functional carcinoma L-selectin and E-selectin ligands, which are involved in cancer cell adhesion and metastasis respectively. CEA is produced in gastrointestinal tissue during fetal development, but the production ceases before birth. However, the serum levels of CEA are raised in some types of cancer, which indicates its prominence as a tumor marker in clinical tests [15].

Carcinoma Antigen 15-3 (CA 15-3), derived from Mucin-1 (MUC-1) gene is used as a tumor marker for breast cancer. It is used to monitor the response to breast cancer treatment and disease recurrence [16]. Sialic acid is found as the end moiety of the carbohydrate chain. Sialic acid is essential for the functions of glycoconjugates, found to be present mostly in glycoproteins and gangliosides and their levels reported to be altered in cancer patients. These glycoconjugates are released into the circulation through increased turnover, secretion, or shedding from malignant cells [17]. Sialic acid is found either as TSA or LSA in glycoproteins and glycolipids. Its level rapidly increases in breast cancer [18].

The effect of oral treatment of thymoquinone on the levels of TNF-α, IL-1β, and IL-6 in serum, liver and renal tissues of control and experimental groups of rats is demonstrated in Figures 3-5 respectively. The levels of these proinflammatory cytokines in drug control rats did not reveal any statistical difference when compared with that of control rats. Conversely, these proinflammatory cytokine levels were escalated significantly in DMBA induced rats in comparison with control rats. Moreover, thymoquinone treated rats showed significantly altered levels when compared with DMBA induced rats.

Cytokines are intercellular short-acting soluble mediators that are involved in the pathogenesis of cancer [19]. IL-1β is a pro-inflammatory regulatory cytokine, which plays a key role in breast cancer progression by regulating the function of tissue and immune cells within the tumor micro-environment via expression of IL-1β receptors. DMBA induced rats showed increased levels of TNFα, IL-1β and IL-6 which has been found to play an important role in proliferation and differentiation of tumor cells and apoptosis [20], invasion and growth of malignancies [21] and cell migration and attachment leading to metastasis [22].

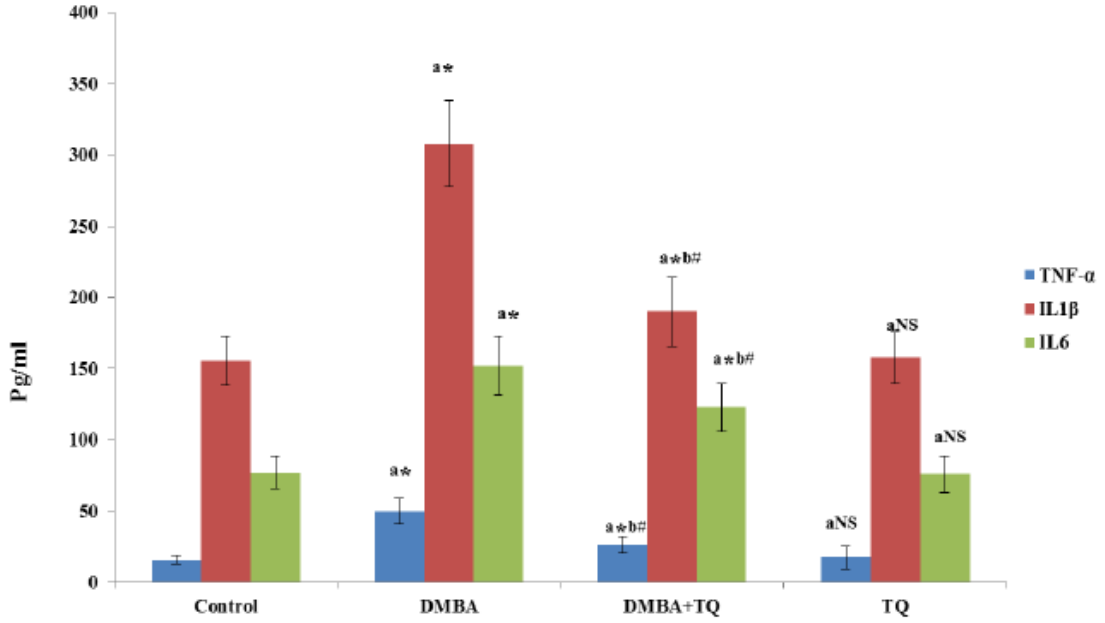


Figure 3: The effect of thymoquinone on the levels of TNF-α, IL-β, AND IL-6 in serum of control and experimental animals

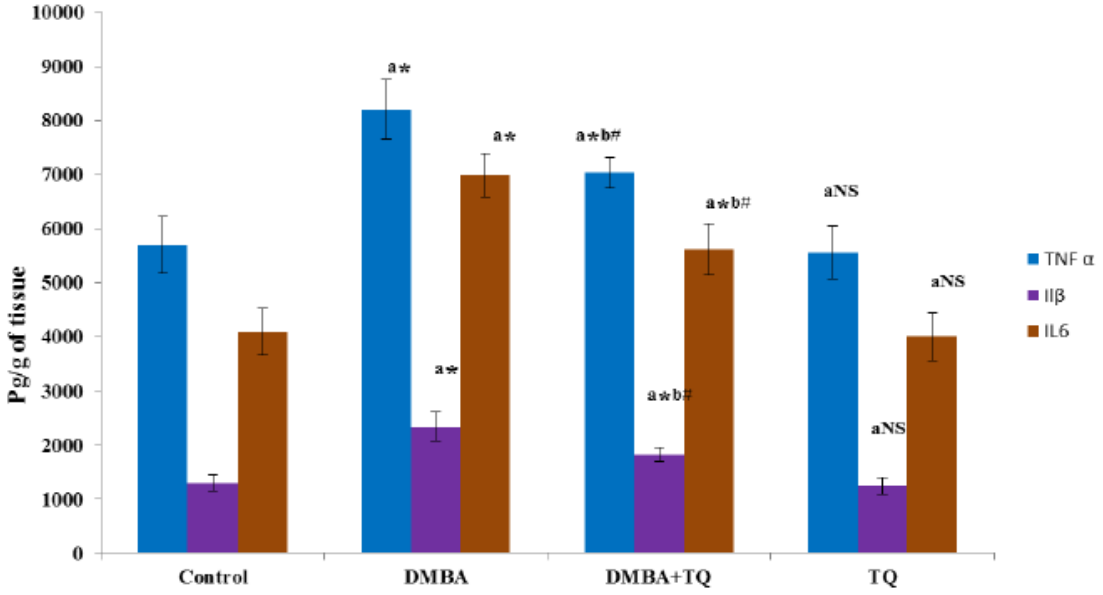


Figure 4: The effect of thymoquinone on the levels of TNF-α, IL-β, AND IL-6 in hepatic tissue of control and experimental animals

IL-6 has been proved to be implicated in the etiology of these major complications in breast cancer patients. TNF-α is one of the most important pro-inflammatory cytokines which induces apoptosis via the TNF-α receptor 1 and an intracellular signaling cascade involving both activation of caspase-8 and caspase-3 [23]. Increased levels of TNF-α l in breast cancer individuals correlates with aggressive tumor biology. Hence, TNF-α blockade is a novel approach to breast cancer therapy. In the present study, the DMBA induced rats showed elevated cytokine levels of TNF-α, IL- 1β, IL-6. Oral administration of thymoquinone reduced the levels of these cytokine levels, thereby contributing to a reduction in tumor burden indicating the antiinflammatory potential of thymoquinone.

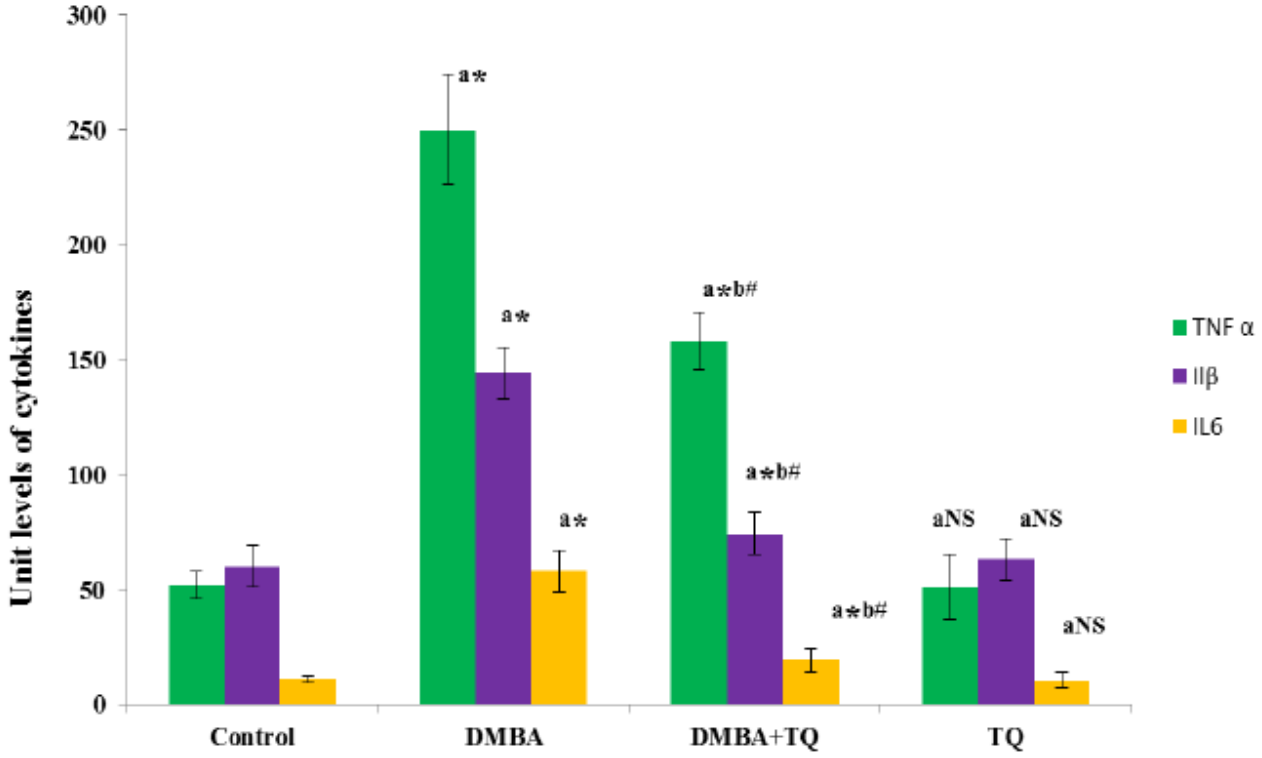


Figure 5: The effect of thymoquinone on the levels of TNF-α, IL-β, AND IL-6 in renal tissue of control and experimental animals

It can be concluded that thymoquinone, possess anti-inflammatory potential in addition to its anticancer and antioxidant potential in DMBA induced breast cancer bearing rats.

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