

ORIGINAL ARTICLE

Evaluation of Anti Asthmatic Effect of Extra Virgin Olive Oil (*Olea europea*) Against Milk Induced Leukocytosis and Eosinophilia

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ABSTRACT

From the last few decades, the management of chronic pain has remained a challenge for medical science research. Currently, available analgesic drugs such as NSAIDs have few adverse effects. As a result, the traditional system has gained importance in the field of medicine. To evaluate the anti asthmatic activity of extra virgin olive oil (*Olea Europea*) for the management of bronchial asthma. In the current study, commercially available virgin olive oil (1.3 ml/kg p.o) was screened for the management of bronchial asthma using milk induced leukocytosis and eosinophilia in mice. Our study results have shown that test olive oil dose (1.3 ml/kg p.o) significantly decreased milk induced leukocytosis and eosinophilia in mice when compared with control group. Conclusions: We conclude that olive oil has anti asthmatic activity against milk induced leucocytosis and eosinophilia. further, working is going on various asthmatic models to evaluate its efficacy in the management of asthma.

Keywords: Asthma, Extra Virgin Olive oil, Leucocytosis, Eosinophilia. Inflammation.

Received 21/11/2014 Accepted 21/12/2014

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How to cite this article:

Farooq Ahmed W, Shaik R, Bilal Ahmad T. Evaluation of Anti Asthmatic Effect of Extra Virgin Olive Oil (*Olea europea*) Against Milk Induced Leukocytosis and Eosinophilia. Adv. Biores., Vol 6 [1] January 2015: 15-18. DOI: 10.15515/abr.0976-4585.6.1.1518

INTRODUCTION

Bronchial asthma is characterized by airway hyper-responsiveness leading to inflammation and narrowing of airways [1]. Asthma affects about 300 million people worldwide and it has been estimated that a further 100 million will be affected by 2025 [2]. Environmental and genetic factors together play an important role in the etiology of asthma [3]. Nowadays various types of anti asthmatic drugs are available in the market for the management of asthma. However, all of these are limited to short symptomatic relief and result in complex side effects. The adverse effects of these synthetic drugs prompt a switch over to traditional complementary and alternative medicine [4]. In spite of voluminous literature on the subject, the treatment of asthma continues to be far from satisfactory. Therefore, there is a dire need to identify effective and safe remedies to treat bronchial asthma [4].

In Mediterranean countries Olive oil has been widely used in traditional medicine in the management of several diseases [5]. Olive oil has been commercialized as a food supplement which can be consumed in the form of syrup and capsules. Previous studies reported the potential activities of olive oil (*Olea europea*) such as antioxidant [6], hypotensive [7], cardiovascular [8], hepato-protective [9], hypoglycemic activity [10] anti inflammatory [11] and anti microbial activities [12]. Several studies have shown that people who consumed about 2 tablespoons of virgin olive oil daily for 1 week showed less oxidation of LDL cholesterol [13]. Olive oil is very well tolerated by the stomach. In fact, olive oil has a protective function and beneficial role in gastric ulcers and gastritis [14]. Only few studies reported that ripe olives have anti histaminic activity [15]. More studies are needed to explore the exact role of olive oil in asthma. Keeping in view, the present study was aimed to evaluate the anti-asthmatic activity of extra virgin olive oil using milk induced leukocytosis and eosinophilia in mice.

MATERIALS AND METHODS

Animals

Adult Swiss albino mice of either sex (20-25 g) were obtained from animal house, College of Medicine, Aljouf university. All animals protocols were approved by the college ethical committee, Aljouf university. All mice were fed *ad libitum* with standard laboratory pellet diet and free access to tap water. The experimental mice were maintained under a constant 12 hour light and dark cycle at room temperature. Animals were acclimatized to the new experiment environments for 3 days before the study.

Chemicals

All the chemicals and reagents used in this experiment were research analytical grade from sigma USA. Extra virgin olive oil was purchased from the local market, Sakaka, Aljouf, K.S.A.

Acute toxicity

The method described by Lorke [16] with slight modification was used to determine the safety of the extra virgin olive oil dose for mice. Briefly, normal healthy mice were divided into groups of five mice in each cage. Olive oil doses of 0.5, 1, 1.3 ml/kg b.w. were orally administered to animals 10 in number. Free Access to food and water was provided. Toxic symptoms and the general behavior of mice were observed continuously for 1 h after the treatment, intermittently for 4 h and thereafter over a period of 24 h. The mice were further observed for up to 14 days following treatment for any signs of toxicity and mortality.

Milk-induced leucocytosis in mice.

Animals were divided into five groups of six animals in each group. Blood samples were collected from retro-orbital plexus. Control group (Group 1) received 1% Tween-80 solution, Test groups (groups II-IV) received extra virgin oil dose of 1.3 ml/kg b.w., group V received standard drug dexamethasone (50 mg/kg i.p). Total leukocyte count was carried out on each group before drug administration and 24 hr after milk injection (boiled and cooled milk 4 ml/kg, s.c.). Difference in total leukocytes count before and after 24 h drug administration was calculated [17].

Milk-induced eosinophilia in mice

Animals were divided into four groups of six animals in each group. Blood samples were collected from retro-orbital plexus. Normal control group (Group 1) received 1% Tween-80 solution. Group II animals received only boiled and cooled milk referred as milk intoxicant group. Test group animals (Group III) received extra virgin oil dose of 1.3 ml/kg b.w. Group IV animals received standard drug dexamethasone (50 mg/kg i.p). Total leukocyte count was carried out on each group before drug administration and 24 hr after milk injection (boiled and cooled milk 4 ml/kg, s.c.). Difference in total leukocytes count before and after 24 h drug administration was calculated [17].

Statistical analysis

The results were reported as mean \pm SEM and using one way ANOVA analyzed for statistical significance followed by Newman-Keuls test $P < 0.05$ was considered significant.

RESULTS

Acute toxicity

The signs of toxicity and mortality were not found in any group of animals after 14 days observation. Hence, dose of highest dose of 1.3 ml/kg p.o, was selected for the experimental study.

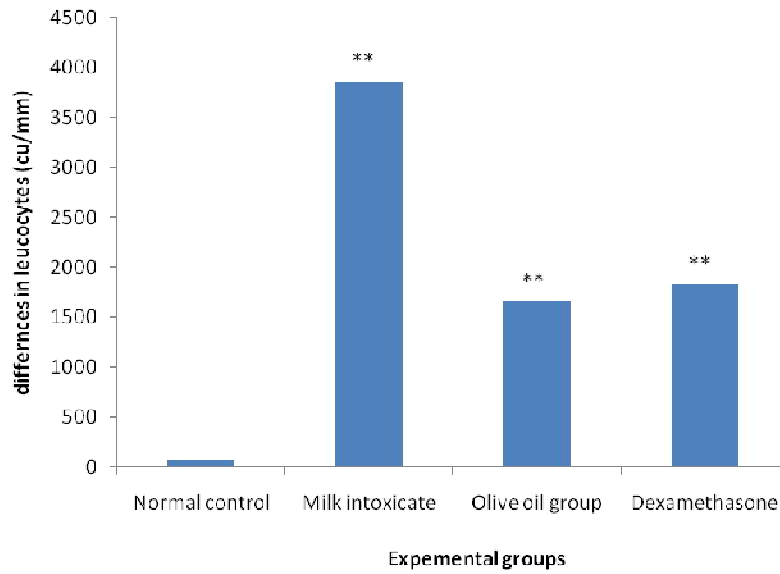
Effect of Olive oil on milk induced leucocytosis

Subcutaneous administration of boiled and cooled milk in milk intoxicant group (4ml/kg s.c) animals showed significant increase ($P < 0.05$) in leukocyte count after 24 hr as compared to leukocyte count before milk administration. Olive oil group (1.3ml/kg oral) showed significant inhibition of leucocytosis after 24 hr of treatment as compared to milk intoxicant group ($P < 0.05$) shown in Figure 1.

Effect of Olive oil on milk induced eosinophilia

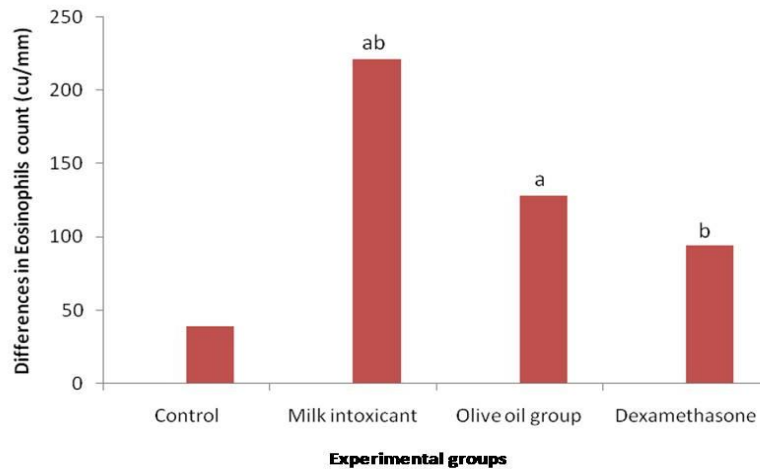
The milk intoxicant groups showed significant eosinophilia ($P < 0.05$) after 24 hr treatment with subcutaneous injection of boiled and cooled milk (4ml/kg). However animals receiving olive oil (1.3ml/kg oral) showed inhibition of milk-induced eosinophilia as compared to milk intoxicant group with significant p value ($P < 0.05$) shown in Figure 2.. The standard drug treatment (50mg/kg i.p) has shown significant inhibition of eosinophilia.

Figure 1: Effect of olive oil on milk induced leucocytosis in mice.



** Significantly difference at $p < 0.05$

Figure 2: Effect of olive oil on milk induced eosinophilia in mice



a, b, ab Significantly difference at $p < 0.05$

DISCUSSION

Globally around about 300 million people are asthmatic and it has been estimated to increase 100 million more by 2025 [18]. Asthma is pathologically characterized by narrowing of airways and its associated inflammatory changes. During asthmatic inflammation, mainly the leukocytes are responsible to release the several inflammatory mediators like histamine, cytokines etc. which enhance inflammatory process further [19]. Infiltration of leucocytes in surrounding tissues in asthmatic inflammation causes increased oxidative stress which is characterized as the main pathogenic feature of asthma [20]. In this study we observed that the inhibition of leucocytosis was significant in animals treated with olive oil as compared to normal control group (Figure1). These results suggest that olive oil suppresses the milk induced leucocytosis by stabilizing the oxidative stress in the surrounding tissue [21].

Bronchial biopsies in asthmatic patients have shown increased number of eosinophils in the submucosal and epithelial layers. Eosinophilia is defined as an abnormal increase in peripheral eosinophil count to more than 4% of total leukocyte [22]. Eosinophils plays an important role in the late phase of asthmatic inflammation. Eosinophils secrete several mediators such as tumor necrosis factor, eosinophil-derived neurotoxin and eosinophil cationic protein which are responsible for the shedding of epithelial layer,

broncho-constriction and respiratory inflammation that leads to allergic condition [23]. Our study results revealed that, the groups treated with olive oil orally inhibit the milk induced eosinophilia significantly, whereas, the milk intoxicant group showed significant increase in the eosinophil count. This probably indicates the olive oil may help to reduce type I hypersensitivity in asthma. Previous studies on *Olea europaea* reported only ripe olives have anti-histaminic activity [15] but our study reveals that extra virgin olive oil also possessed anti-asthmatic activity which may be useful in the management of bronchial asthma as an alternative drug therapy. The anti-asthmatic activity of olive oil may be due to its antioxidant properties and photochemicals present in it. Our study results conclude that olive oil has anti-asthmatic activity against milk-induced leucocytosis and eosinophilia. Further, work is going on various asthmatic models to evaluate its efficacy in the management of asthma.

ACKNOWLEDGMENT

The authors are thankful to Department of Pathology, College of Medicine, Aljouf University, K.S.A for providing necessary facilities. The authors are also grateful to laboratory persons, College of medicine, Aljouf University for their cooperation during this study.

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