



The anti-inflammatory and antipyretic effects of clove oil in healthy dogs after surgery



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ABSTRACT

The current study was designed to evaluate the anti-inflammatory and antipyretic properties of clove oil in dogs. So thirty adult male dogs were used. After a surgical incision on the abdominal area, animals were divided into four group. The first group received 25 mg/kg of clove oil while the second group was considered as a control. The third and fourth groups received betamethasone (20 mg/kg) and phenylbutazone (15 mg/kg) as anti-inflammatory and anti-pyretic agents, respectively. All injections were performed for five consecutive days. All tests (measurement of edema, complete blood count, histopathology, and rectal temperature) were performed on all groups. Our results showed that in the clove oil-treated animals, the amount of edema was significantly decreased as compared to control ($P \leq 0.05$). The number of white blood cells, neutrophils and band neutrophils was decreased in clove-oil treated dogs as compared to control ($P \leq 0.05$). There was no significant difference in the number of red blood cells and hematocrit between clove-oil treated and vehicle-treated groups ($P > 0.05$). Rectal temperature significantly decreased in the clove oil-treated group as compared to control ($P \leq 0.05$). Histopathology revealed that the clove oil-treatment significantly reduced the inflammation. We showed that clove oil administration has anti-inflammatory and antipyretic properties in dogs.

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1. Introduction

A number of natural products have been tested on several animal models for the development of new anti-inflammatory compounds. Inflammation can be defined as a comprehensive, nonspecific but beneficial reaction of tissues to injury. Inflammation consists of both local and systemic adaptive responses to tissue damage. The local reactions of inflammation include

recruitment of phagocytic cells for elimination of endogenous or exogenous harmful agents. The systemic responses can change the 'milieu interior' to permit these processes to happen more efficiently [1]. There are several anti-inflammatory drugs currently available which are associated with various side effects. Most people tolerate Nonsteroidal antiinflammatory drugs (NSAIDs) and acetaminophen without any trouble. However, side effects can happen in cardiovascular system, gastrointestinal system, liver toxicity and kidney toxicity by these drugs [2]. However many anti-inflammatory agents are available with low level of side effects at effective doses, such as acetaminophen and NSAIDS but it is essential to find new more effective and safe compounds with fewer side effects and good acceptability in patients. Even now,

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plant products retain a significant role in primary health care as therapeutic medications in numerous developing countries. There is a constant need to find new compounds with a variety of chemical structures and unique mechanisms of action for novel and reemerging diseases. Therefore, investigators are progressively turning their attention to traditional medicine, looking for new information to be used in the development of better agents for numerous health problems as alternative medicine [3]. Essential oils and their components are extensively used in medicine as ingredients in a diverse range of medical products, and in food manufacturing as flavoring additives. In addition to their antimicrobial properties, in food systems they can be considered as an extra intrinsic factor to increase the safety and shelf life of foods. They are also used in cosmetics as perfumes [4]. *Eugenia caryophyllus* (clove), belonging to the family *Myrtaceae*, has several medicinal effects and its systemic and local use is widespread in traditional medicine. Cloves are reported to have anti-oxidant [5], anti-bacterial [6], anti-candida [7], local anesthetic [8], anti-halitosis [9], and aphrodisiac [10] properties. The aim of the present study was to investigate the possible anti-inflammatory and antipyretic effects of clove oil in an experimental animal model using male dogs.

2. Materials and methods

2.1. Animals

The study protocol was approved by the Prefectural Animal Care and Use Committee. Thirty mixed breed male dogs aged from 1 to 5 years were used. In order to ascertain the good health of the dogs, a complete physical examination, complete blood count (CBC), serum biochemical analyses, and urinalysis were done on each dog before the experiment. Two weeks before commencement of the study, the animals received parasite prophylaxis in the form of levamisol 8 mg/kg and praziquantel 5 mg/kg orally. The animals were acclimatized to their environment for at least one week prior to the experiment. They were housed indoors in individual rooms and had outdoor access twice daily. Commercial dry food was offered to them twice daily and water *ad libitum*.

2.2. Preparation of the extract

Hydroalcoholic extract was obtained by the soaking method. 200 g of cloves were soaked in 70% methanol in water (3 × 1) for 72 h. The acquired extract was concentrated using a rotary evaporator at the maximum temperature of 45 °C [11,12].

2.3. Experimental plan

Thirty mixed breed male dogs were randomly divided into four groups (n = 7–8). The dogs were premedicated for surgery with a combination of acepromazine (0.04 mg/kg), atropine (0.04 mg/kg) and morphine (0.4 mg/kg), injected subcutaneously. Anesthesia was induced with thiopentone, given intravenously to effect approximately 90 min after the administration of the sedative agents. After induction of anesthesia and surgery, the first group received 25 mg/kg of clove oil dissolved in dimethyl sulfoxide (DMSO) 1%, while the second group was injected with DMSO 1% dissolved in normal saline as negative control. The third group received betamethasone as an anti-inflammatory positive control at a dose of 20 mg/kg, and the fourth group was given phenylbutazone as an antipyretic positive control at a dose of 15 mg/kg. All injections were performed intravenously once daily for five consecutive days. The first injection was performed immediately after surgery. All tests (measurement of edema, CBC, histopathological study, and rectal temperature) were performed in groups

receiving clove oil or the vehicle. In the betamethasone-treated group, edema (inflammation) was studied, whereas in phenylbutazone-treated animals, rectal temperature (fever) was measured.

2.4. Surgical operation

Dogs were fasted for 24 h. Three hours after blood was taken, the dogs were anesthetized with xylazine 2 mg/kg bodyweight (BW) and ketamine 11 mg/kg BW intravenously (IV). When anaesthetized, a 5 cm long incision was made in the abdominal wall skin, the incision was then sutured closed in two layers. All of the incisions were covered with a sterile non-adherent, semi-occlusive pad (Melolin; Smith and Nephew, London, England). A secondary bandage layer consisted of strips of wide adhesive tape (Zonas; Johnson and Johnson, Berkshire, England). Bandages were changed daily. Elizabethan collars (Buster; Kruuse, Denmark) were used to keep the dogs from damaging the bandages. Analgesia was provided for the initial 24 h post-surgery by administration of butorphanol (0.2 mg/kg BW) IV at 4 h intervals.

2.5. Measurement of edema

The extent of edema was determined by measuring the thickness of the skin at the site of the surgery using a digital micrometer every hour of the first day, then every 6 h for the remaining days.

2.6. Determination of blood cells

Blood samples were taken at –3, 0, 1, 3, 6, 12, 24, 48, 72, 96, 120, 168, and 216 h after surgery and a CBC (neutrophils, band cells, lymphocytes, monocytes, platelets red blood cells and percentage of hematocrit) was performed using an optical microscope.

2.7. Histopathological study

In the tenth day after surgery, biopsy samples were taken by cutting a very small area of the skin with a scalpel under local anesthesia, and after fixation in 10% buffer formalin, histopathological sections of skin tissue in the vehicle and clove oil-treated groups were prepared and studied using an optical microscope after hematoxylin and eosin staining.

2.8. Rectal temperature

After 24 h following surgery, rectal temperature was measured using a digital thermometer, every 5 min for 45 min.

2.9. Statistical analysis

Statistical analyses were carried out using GraphPad Prism 5 software (San Diego, CA, USA).

Data were checked for normality by both Shapiro-Wilk and Kolmogorov-Smirnov normality tests by using of graph pad software and after determination of data normal distribution we used parametrical analysis. The results are presented as mean ± S.E.M. Comparisons between groups was done by two-way repeated measure analysis of variance (ANOVA) followed by Bonferroni post-hoc test. $P \leq 0.05$ were considered statistically significant.

3. Results

3.1. Measurement of edema

The skin edema was significantly decreased in the clove oil-treated group compared to the vehicle-treated group between 2

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