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Research Article

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Evaluation of the anti-inflammatory properties of *Boswellia serrata* (Boswegex®) on carrageenan-induced paw edema in albino Wister rats: an *in vivo* study

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Abstract

Inflammation is a primary physiological defense mechanism that helps the body protect itself from infection, toxic chemicals, or other noxious stimuli. Anti-inflammatory medicines have stronger therapeutic values and minimal toxicity. It is a defense mechanism of the body to get rid of harmful stimuli and start the tissue's healing process. *B. serrata*, commonly known as frankincense, has been widely consumed since time immemorial. The objective of the present study was to investigate whether the anti-inflammatory properties of an aqueous ethanolic extract of *B. serrata* (Boswegex®) against carrageenan-induced paw edema at doses of 22 and 44 mg/kg.bw were significant. After 4 hours, both Boswegex® and the Standard Indomethacin (10mg/kg.bw) each produced excellent outcomes of 41.66% and 50.88%, respectively. Boswegex®has shown powerful anti-inflammatory properties and established its ability to manage several inflammatory disorders, according to the findings of the current experiment.

Keywords: Boswegex®, Carrageenan induced paw edema, Anti-inflammatory

Introduction

Inflammation is a host defense mechanism against harm to the tissue, infection, and outside stimuli. Although swelling is an inevitable component of our body's immunological response, when it gets out of hand, it can harm the body. Acute inflammation is a typical component of the body's defense mechanism, but chronic inflammation is a complex process triggered by the activation of immune or inflammatory cells. Inflammation is a natural response in any tissue. However, if the specialized repair and removal of harmful stimuli are not properly regulated, inflammation persists and leads to diseases and issues. There are many different anti-inflammatory treatments available, including the most well-known steroidal and non-steroidal anti-inflammatory drugs. Research is

always being done to develop safe and effective anti-inflammatory drugs because these medications have serious side effects. Damaged by injury, infection, or noxious stimuli. It promotes tissue healing, recovery, and repair.

Ancient Indian Ayurveda makes extensive use of the plant *Boswellia serrata.* Indian frankincense is a synonym for it Siddiqui (2011). In Asian and African traditional medicine, resin extracted from it has been used for generations to treat chronic inflammatory diseases linked to arthritis Aggarwal *et al.* (2011). According to the texts of Ayurveda and Unani, it is used as an effective treatment for conditions such as syphilitic diseases, syphilitic diarrhoea, ringworm, boils, fevers, mouth sores, bad throats, bronchitis, asthma, cough, vaginal discharges, hair loss, jaundice, haemorrhoids, irregular menstruation, and liver disorders. Furthermore, it has diaphoretic, astringent, diuretic, and stimulating properties both internally and externally Sharma *et al.* (2004) and Dhiman (2006). Additionally, it has been shown in recent research to have hepatoprotective, anti-arthritic, anti-inflammatory, anti-hyperlipidemic, anti-atherosclerotic, and analgesic effects Mathe *et al.* (2004).

B. serrata gum resin extracts comprise monoterpene essential oil (3%–10%), water-soluble gum (approximately 20%), and resin acids (60%–70%) [6]. The triterpenoids in these extracts, according to an examination of their individual components, are beneficial in relieving inflammation, rheumatoid arthritis, ulcerative colitis, skin allergies, tumors, and osteoarthritis Rashan *et al.* (2019) and Hussain *et al.* (2017). 11-keto-β-boswellic acid (KBA), 3-O-acetyl-11-keto-β-boswellic acid, α-boswellic acid, β-boswellic acid, 3-O-acetyl-α-boswellic acid, and 3-O-acetyl-β-boswellic acid are among the pentacyclic triterpene acids that make up the boswellic acids (BAs) of *B. serrata*, and they play an important role in the plant's anti-inflammatory effects Hussain *et al.* (2017) and Roy *et al.* (2019). A class of medications known as non-steroidal anti-inflammatory medicines (NSAIDs) is frequently prescribed to treat inflammation. By inhibiting both COX-1 and COX-2, NSAIDs (non-steroidal anti-inflammatory medicines) suppress the synthesis of prostaglandins (PGs). The majority of these drugs are associated with well-known gastrointestinal complications and, less frequently, renal adverse reactions Singh *et al.* (2009).Therefore, it is essential to discover novel therapeutic drugs that fight inflammation.

The most important enzyme for leukotriene production in inflammatory illnesses, 5 lipoxygenase, has been reported to be inhibited by boswellic acids from *B. serrata.* The other molecular targets of boswellic acids are human leukocyte elastase, topoisomerase I and II, as well as IκB (inhibitor of nuclear factor kappa B) kinases Iram *et al.* (2017) and Kunnumakkara *et al.* (2018). *B. serrata* inhibited the edema during the acute phase of inflammation probably by inhibiting the chemical mediators of inflammation. The anti-inflammatory mechanism of *B. serrata* is through the inhibition of the leukotrienes synthesis Safayhi *et al.* (1992).

This study was established to assess the efficacy of standardized boswellia extract on carrageenan-induced rat paw edema in rats based on the aforementioned subjects. Due to their characteristics, boswellic acids show great promise as a therapeutic agent; nevertheless, two key drawbacks are their low solubility and hydrophobicity. Consequently, it is essential to develop that is highly biocompatible.

Based on the hydrophobicity of boswellic acids, our Boswegex® is standardized with 65 to 85% of boswellic acids. This offers a product that is more bioavailable than other boswellia products due to the fact that it dissolves quickly and maintains solubility over time. In order to assess Boswegex®s anti-inflammatory effects, an *in vivo* investigation was used in this study.

Materials and Methods

Preparation of the plant extract Boswegex®

Boswegex® is a mixture of fractions derived from an aqueous ethanolic extract of the gum resin from *B. serrata*. Boswegex® is standardized with

65 to 85% total boswellic acids (BAs) each in order to maintain quality and batch-to-batch consistency.

Standardization of Boswegex®

Processes for extraction and purification of Boswegex® were standardized. Accurately weighed 1.5 g of the sample and then dissolved it in 100 ml of alcohol. 8–10 drops of phenolphthalein were added as an indicator. This was titrated against 0.1 N NaOH. The 100 ml of alcohol were given as blank. The End points were observed as pinkish-brown to colourless. The following formula was used to calculate the values:

$$\frac{\text{Titre value} \times 45.67 \times \text{Normality of 0.1 N NaOH} \times 100}{\text{Wt. of sample in mg} \times 0.1}$$

Chemicals and reagents

Carrageenan was bought from Hi Media in Mumbai, India. In Bangalore, India, Recon supplied the common anti-inflammatory drug Indomethacin. Sodium carboxymethylcellulose (GIOZ/1710/3006/13) was bought from SDFCL in India. HI Media India delivered picric acid (0000175497), while Otsuka in India provided normal saline (G/280/LVP/5).

In vivo Anti-Inflammatory Effect of Boswegex®

Experimental Design

30 male Wistar albino rats (180–200g) were used in this experiment. They were collected from the animal house of Radiant Research Services Pvt. Ltd., Karnataka, India. Animal experiments were conducted in accordance with the guidelines of the committee for the purpose of controlling and supervising experiments on animals (CPCSEA Registration Number: 1803/PO/RcBi/S/2015/ CPCSEA). Each animal was marked with picric acid, and numbering was given individually to each animal. Each cage was numbered separately to identify the group. In each cage, a single animal was housed in a standard stainless steel cage with facilities for pelleted food and drinking water in bottles. Aquaguard on line water was provided *ad libitum*. Animals had continuous access to fresh, potable, uncontaminated drinking water. They were kept in these cages under conventional laboratory settings, which included a temperature of 22± 3^{o}C, relative humidity of 30–70%, and a 12-hour light and 12 hour dark cycle. Every procedure involving animals was carried out in an ethical way, under the supervision of competent professionals. The research protocol was reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of Radiant Research Services Pvt. Ltd. before the study could begin.

Assessment of Boswegex® Anti-Inflammatory Properties

Carrageenan-induced paw edema

The acute anti-inflammatory effect was evaluated using a model of rat paw edema caused by carrageenan. By injecting 0.1 ml of 1% carrageenan (0.9% NaCl solution) under the outer layer of the rats' right hind paw, acute inflammation was generated. There were five groups of six male Wistar albino rats used in this study, each weighing between 180 and 200 g. The drug dosages administered to the various groups are given as follows: Normal saline was used as the control in Group I (10 mL/kg), Group II Carrageenan was the negative control, Group III Boswegex® (22 mg/kg.bw.), Group IV Boswegex® (44 mg/kg.bw.), and Group V Indomethacin (10 mg/kg.bw) of the experiments, respectively. Indomethacin is the standard anti-inflammatory drug. All groups except the control group were pretreated for 1 hour prior to inducing paw edema. The duration of the linear paw was determined at intervals of 0, 30, 60, 120, 180, 240, and 360 minutes. The Vernier calipers were used to measure the paw's circumference. The formula used to determine the ability to reduce inflammation was as follows:

% inhibition of paw edema = (T − T0) / T) × 100

T: Thickness of paw edema in the positive control group
T0: Thickness of paw edema in the test sample groups

Statistical analysis

The results have been presented as Mean±SEM. One-way anova and the Dunnet test were used to determine the significance of the *in vivo* data. Statistical significance was defined as P <0.05.

Results

Carrageenan-Induced Paw Edema By injecting 0.1 mL of 1% Carrageenan in normal saline into the subplantar tissue of the right hind paw, inflammation was elicited in rats. Rats received Boswegex® one hour before the induction of inflammation. Normal saline, 10 mL/kg body weight, was given orally to the control group. Edema was measured as the difference in paw volume between the control and 0, 30, 60, 120, 180, 240, and 360 minutes (Table 2 and Figure 2) after the inflammatory agent, inhibition, was administered. The study's usage of Boswegex®at doses of 22 and 44 mg/kg b.w. resulted in a significant decrease in paw edema following Carrageenan injection, suggesting that Boswegex® has a similar anti-edematous action as indomethacin. According to data from these preclinical models, Boswegex® can cause an inhibition, which leads to a reduction in prostaglandins. As a result, our findings confirmed the hypothesis that Boswegex®'s anti-inflammatory effects result from a decrease in prostaglandins via cyclooxygenase inhibition and suppression of leukotriene production. Table 3 and Figure 3 demonstrate these results.

Table 1Effect of Body weight on experimental animals

Groups	Treatment	Body weight (gm)
Group I	Normal Control	188.3±1.40
Group II	Negative Control	188.5±1.38
Group III	Boswegex®(22 mg/kg.bw)	189.5±0.76
Group IV	Boswegex®(44 mg/kg.bw)	190.8 ±1.62
Group V	Indomethacin (10 mg/kg.bw)	190.8±1.75

*Values were expressed as Mean± SEM

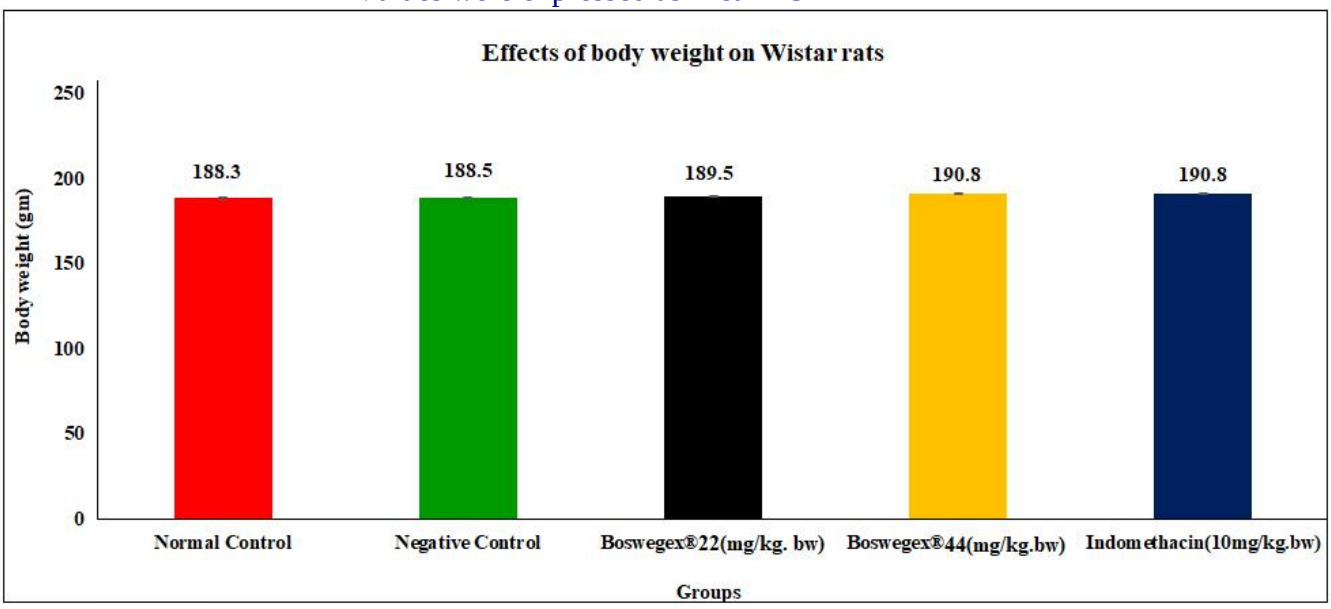

Figure 1 Effect of Body weight on male Wistar rats

Table 2 Modulatory effect of Boswegex® on paw edema thickness in Wistar rats at various time intervals (Paw edema thickness in mm)

Group	Treatment	0 Min (mm)	30 Min (mm)	60 Min (mm)	120 Min (mm)	180Min (mm)	240 Min (mm)	360 Min (mm)
Group I	Normal Control	3.06±0.02	3.06±0.02	3.06±0.02	3.06±0.02	3.06±0.02	3.06±0.02	3.06±0.02
Group II	Negative Control	3.11±0.01	6.41±0.07	6.68±0.08	6.80±0.08	6.95±0.05	7.04±0.04	7.11±0.0
Group III	Boswegex® (22 mg/kg.bw.)	3.08±0.02	6.21±0.02 *	6.36±0.04*	6.02±0.08***	5.75±0.08 ***	5.58±0.07 ***	5.47±0.07***
Group IV	Boswegex® (44 mg/kg.bw.)	3.12±0.005	6.05±0.03 ***	6.21±0.10***	5.81±0.06***	5.11±0.08 ***	4.18±0.04***	4.15±0.18 ***
Group V	Indomethacin (10mg/kg.bw)	3.10±0.02	6.01±0.05 ***	5.89±0.08 ***	5.60±0.11***	4.91±0.05***	4.04±0.6 ***	3.14±0.03 ***

Values were expressed as Mean± SEM

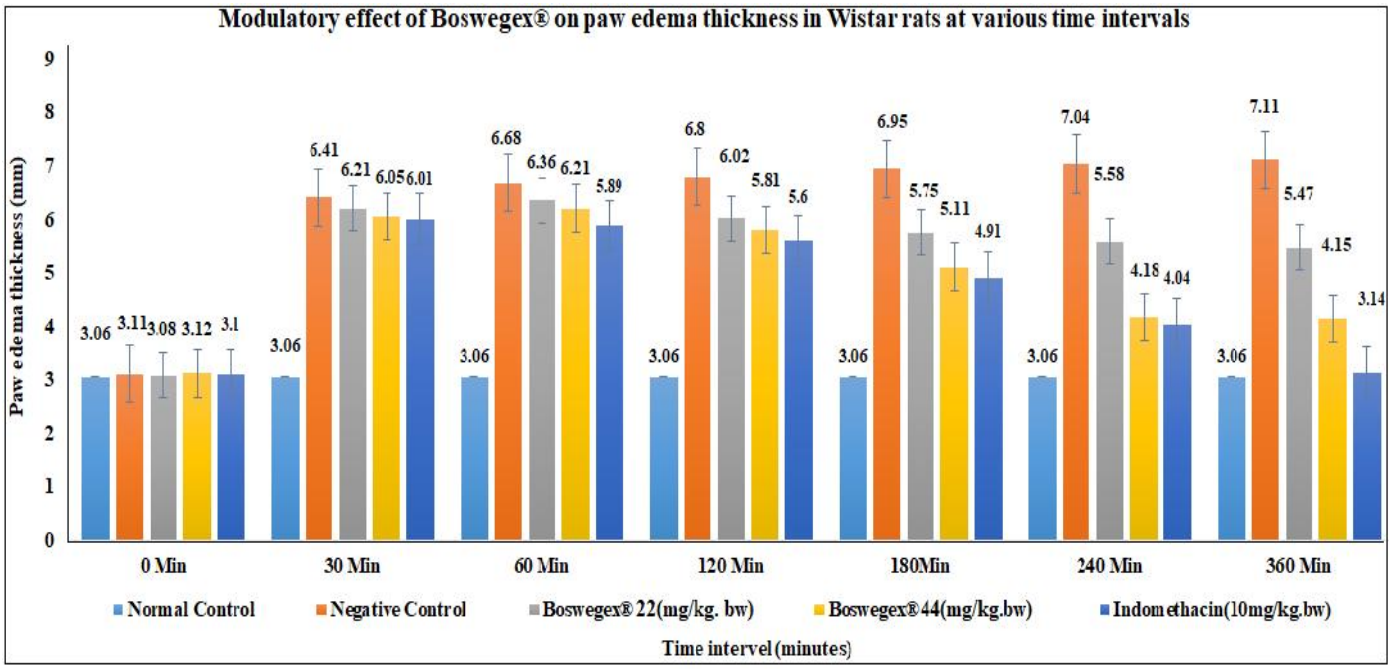

Figure 2 Modulatory effect of Boswegex®and Indomethacin on paw edema thickness in Wistar rats at various time intervals (Paw edema thickness in mm).Values were expressed as Mean± SEM

Table 3: *In vivo* anti-inflammatory effect of Percentage inhibition on Boswegex® against carrageenan-induced paw edema in albino Wistar rats

Group	Treatment	Percentage inhibition of paw edema
Group I	Normal Control	--
Group II	Positive Control	--
Group III	Boswegex® 22 mg/kg.bw.	23.13 %
Group IV	Boswegex® 44 mg/kg.bw.	41.66 %
Group V	Indomethacin (10mg/kg.bw)	50.88 %

*Values were expressed as Mean± SEM

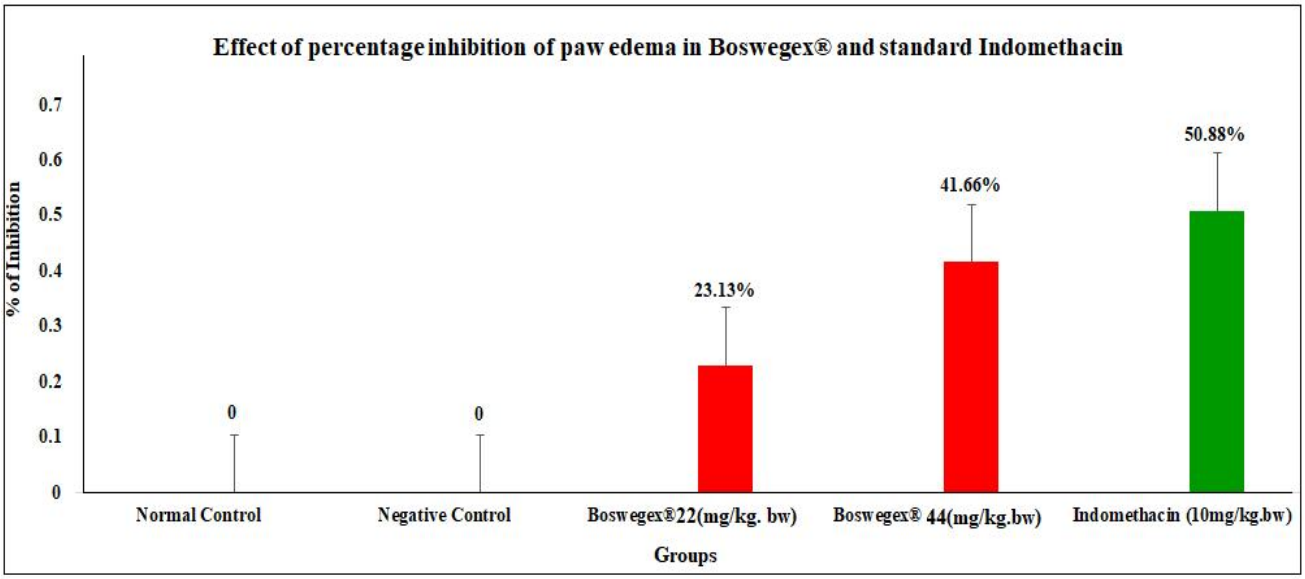

Figure 3 Effect of percentage inhibition of paw edema in Boswegex® and Indomethacin

Discussion

The challenging biological reaction of vascular tissues to damaging stimuli, such as pathogens, damaged cells, or irritants, comprises inflammation. Redness, swollen joints, pain in the joints, stiffness, and loss of joint function are its primary symptoms. These manifestations of inflammation include edema, leukocyte infiltration, and granuloma development. However, it serves as a defense mechanism. Numerous reactions can be caused by or made worse by the intricate processes and mediators involved in the inflammatory response. Acute inflammation caused by carrageenan is one of the best test methods for anti-inflammatory therapy assessment. A Vernier caliper is used to depict the progression of swelling in carrageenan-induced edema over time. After receiving a carrageenan injection, the first stage of inflammation sets in, which is partly brought on by the trauma of the injection and partially by the serotonin and histamine components. Numerous systemic disorders are largely influenced by inflammation. The development of new therapeutic agents derived from natural products or traditional herbal medicines has received more attention recently as a consequence of their larger safety spectrum. Various natural items have been thoroughly investigated as potential sources of therapeutic substances Ye *et al.* (2012).

In certain situations scientists have used topical formulations to provide more potent anti-inflammatory activity with the least amount of systemic side effects. Despite this, systemic administration of NSAIDs and various herbal medicines is generally advised to treat inflammatory diseases.

Frankincense, a popular herbal remedy, has been shown to have anti-inflammatory and anti-cancer effects Banno*et al.* (2006) and Frank *et al.* (2009). Our studies, however, only concentrate on the effect of the crude extract of frankincense compounds, such as boswellic acids (BAs), AKBA, and KBA, on anti-inflammatory activity. In the present study, the carrageenan-induced paw edema method was used to examine the anti-inflammatory effect of *B. serrata*. The typical models for inflammatory experiments are rat paw edema caused by carrageenan and xylene, respectively Khan *et al.* (2011) and Cai *et al.* (2014).

Because of its sensitivity in detecting orally active anti-inflammatory drugs, particularly in the acute phase of inflammation Dirosa *et al.* (1971) and Dirosa (1972), the carrageenan test was used. Rats' paw edema results from a carrageenan intraplantar infection. Its initial stage (0–2.5 hours after carrageenan injection) is caused by the simultaneous release of mediators such as histamine, serotonin, and kinins on the vascular permeability. Leukotrienes are associated with the second phase. The second phase of inflammation is suppressed by *B. serrata* when taken orally. The highest inhibitory responses have been observed at 44 mg/kg b.w. of oleo-gum-resin extract. By inhibiting the molecular mediators of inflammation, these *B. serrata* reduced edema during the acute phase of inflammation. Leukotriene production is reduced by *B. serrata* as part of its anti-inflammatory properties Safayhi *et al.* (1992). Our findings indicate that frankincense-derived boswellic acids exhibit significant anti-inflammatory effects.

Overall, the results showed that Boswegex® has exhibited potential anti-inflammatory properties at low and high doses of 22 mg and 44 mg/kg.bw respectively. Boswegex®has shown promising anti-inflammatory properties gives hopes for natural alternative to synthetic non-steroidal anti-inflammatory drugs. Additionally, it has been suggested that Boswegex®'s mode of action involves a decrease in prostaglandins via cyclooxygenase inhibition and suppression of leukotriene production. These findings demonstrate that Boswegex® has powerful anti-inflammatory activity and support its use as a natural anti-inflammatory drug.

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Conflict of interest

The authors have no conflict of interest regarding the publication of this paper.

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