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Antinociceptive and Anti-Inflammatory Activity of Beta-Sitosterol

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Abstract

Background: Phytosterols are commonly present in various plants, among which sitosterol is the abundant one. Various extracts of leaves of the plant were screened but a purified phytosterol like β-sitosterol (BS) is a compound of interest. **Objective:** The aim of the study is to evaluate for analgesic and anti-nociceptive property of activity BS. Materials and Methods: Hot plate test and Acetic acid-induced writhing's test was used for evaluating the central and peripheral Antinociceptive activity and Anti-inflammatory activity was evaluated by carrageenan-induced hind paw edema method. **Results:** BS (10 and 20 mg/kg, i.p.) was responsible for the significant and dose-dependent activity comparable with the standard. **Conclusion:** The dose dependent activity of β-sitosterol might be responsible for analgesic and anti-inflammatory activity, this may also be a vital observation for further studies.

Keywords: Analgesia, Ibuprofen, Morphine, Nociceptive, Paracetamol, β-sitosterol

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Introduction

Analgesia is known as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage,"[1] it is basically a sign to elicit a protective reflex to avoid the basic cause of the pain and its responses.[2] In spite of extensive research on the mechanisms of nociception and pathophysiology of pain, drugs acting on the anti-inflammatory mechanisms have been the only least successful over the last decades, with very few novel selective mechanisms shown to be effective in clinical practice;[3] natural products have played an important role in the development of new sources to treat inflammatory diseases.[4] Historically, the screening of sources for natural products led to the discovery of clinical drugs currently used in the pharmacological therapy and natural products may provide compounds with a unique structural activity in identifying the potential biological therapeutic in new plant extracts.[5]

Phytosterol phytocompounds are structurally similar to cholesterol; β-sitosterol (BS), one of the common and a major phytosterol, has a wide spectrum of biological effects including a protective effect against various chronic ailments.[6] BS is a phytosterol found in plants such as rice, wheat, corn, nut, peanut, and particularly in cat's claw (*Uncaria tomentosa*), where it has been suggested to be involved in the curative properties attributed to the plant on inflammation, viral damage, ulcer, cancer development, as well as in the enhancement of the immune system.[7] BS is a chemical structurally related to cholesterol, but more slowly absorbed into the intestinal tract, interfering with the cholesterol absorption and preventing its rise in serum.[8] Besides, BS is suggested to modulate the immune function, inflammation, and pain levels by controlling the production of inflammatory cytokines.[9] Sterols among which BS are the most prevalent phytosterol reported to possess antioxidant, anticancer, antiatherosclerotic, antinociceptive, anti-inflammatory, antipyretic, immunomodulatory, and insulin releasing effects.[10] In view of these facts, the aim of the study is to investigate the effects of BS on its antinociceptive and anti-inflammatory activity.

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Materials and Methods

Chemicals

BS, morphine, ibuprofen, naloxone, and dexamethasone were commercially purchased from MERCK-Life Science, Bengaluru, India; Paracetamol and essentially pure λ carrageenan (C3889) were purchased from Sigma-Aldrich Chemicals Private Limited.

Animals

Young Swiss albino mice aged about 4–5 weeks with average weight of 25–35 g and adult Wistar albino rats of average weight of 150–200 g were used for the experiment housed in standard cages

under standard environmental conditions of room temperature at 24 ± 1°C and 55–65% relative humidity with 12 h dark-light cycle and provided with standard food and water *ad libitum*. The study was approved by the Institutional Animal Ethics Committee (IAEC No. SU/CLAR/RD/021/2019).

Method for the Evaluation of Analgesic Effect

Eddy's hot plate test

The central nociceptive activity was evaluated using Eddy's hot plate method.[11] The mice will be placed individually on the hot plate maintained at 55°C ± 0.2°C and latency of nociceptive response such as licking, flicking of the hind limb, or jumping will be noted. The reading will be taken at 0, 15, 30, 60, and 120 min after treatment. The experiment will be terminated 20 s after their placement on the hot plate to avoid damage to the paws. The treatment groups were divided into seven groups of control – saline and morphine (10 mg/kg i.p), two different doses of BS (10 mg/kg i.p) (20 mg/kg i.p) and morphine (10 mg/kg i.p); after 15 min naloxone (1 mg/kg s.c), BS (10 mg/kg i.p); after 15 min naloxone (1 mg/kg s.c), BS (20 mg/kg i.p); and after 15 min naloxone (1 mg/kg s.c).

Acetic acid-induced writhing test

The peripheral nociceptive activity will be evaluated using acetic acid-induced writhing test.[11] Thirty minutes before intraperitoneally 0.6% solution of acetic acid (10 ml/kg) were administered, after acetic acid injection, the mice will be observed for the number of writhing responses for a period of 30 min for each animal. The treatment groups were divided into four groups of control – saline and paracetamol (50 mg/kg i.p) and two different doses of beta-sitosterol (10mg/kg i.p) (20 mg/kg i.p)

Carrageenan-induced paw edema

Anti-inflammatory activity was evaluated using the carrageenan-induced paw edema method.[12] After 30 min of drug treatment, 0.05 ml of 1% w/v carrageenan in saline will be injected in the subplantar tissue of the left hind paw of the animal. The degree of paw edema of all the treatment groups will be measured using a digital Vernier caliper at 0, 15, 30, 60, and 120 min after administration of carrageenan to each group**.** 0th min reading will be the initial paw volume of the animal. The treatment groups were divided into five groups of control – saline and ibuprofen (50 mg/kg), two different doses of beta-sitosterol (10 mg/kg) (20 mg/kg) and dexamethasone (1 mg/kg).

Statistical Analysis

All data were expressed as mean ± SEM. The statistical analysis of all the observations was carried out using one-way analysis of variance followed by multiple comparison test of Tukey–Kramer, where necessary. $P = 0.05$ was considered as significant compared with the control group.

Results

Eddy's Hot Plate Test

In the hot plate test, BS (10 and 20 mg/kg, i.p.) showed promising dose-dependent activity comparable to control [Table 1]. To study the involvement of opioid receptors, isolated BS was given after opioid antagonist, naloxone (1 mg/kg, s.c.). Results showed that naloxone reversed its antinociceptive activity [Table 1].

Acetic Acid Writhing Test

BS (10 and 20 mg/kg, i.p.) showed significant inhibition of writhing reaction induced by acetic acid as compared to the control group and comparable activity with standard drug paracetamol, 50 mg/kg, i.p. and the activity was dose dependent [Table 2].

Carrageenan-Induced Paw Edema

In the acute inflammation model, that is, carrageenan-induced rat paw edema method, BS (10 and 20 mg/kg, i.p.,**)** produced significant ($P < 0.05$) inhibition of paw edema as compared to the control. BS (5, 10, and 20 mg/kg, i.p.,) showed comparable activity with standard drug ibuprofen (50 mg/kg, i.p.) and the activity was dose dependent [Table 3].

Discussion

The hot plate test is the specific central antinociceptive test. BS showed significant results in this test, so there may be involvement of opioid receptors. The opioid agents exert their analgesic action through the supraspinal (μ1, k3, δ1, and σ2) and spinal (μ2, k1, and δ2) receptors. To evaluate the effect of on thermic stimulus-induced pain involvement in the opioid mechanism of β-sitosterol 10 and 20mg and an opioid antagonist, naloxone, was used, the interaction between naloxone and β-sitosterol exerted the possible mechanism of central analgesic activity on the pain threshold of mice. Results showed that the naloxone reversed its antinociceptive activity. Therefore, it is possible that the BS exerts its effect through the central opioid receptor or promoted release

Table 1: Effect of β-sitosterol and effect of naloxone on thermal stimulus-induced pain (hot plate test)

Treatment groups	*Reaction time (sec)*				
	0 min	*15 min*	*30 min*	*60 min*	*120 min*
Control – Tween-20	2 ± 0.6	3 ± 0.6	3 ± 1.0	3 ± 0.0	2 ± 0.0
STD morphine 10 mg/kg/mL i.p	3 ± 0.6	7 ± 1.0	10 ± 0.6	11 ± 0.6	11 ± 1.0
BS 10 mg/kg B.Wt. i.p	2.3 ± 0.6	6.3 ± 0.6	9.3 ± 1.5	9.7 ± 0.6	9.7 ± 0.6
BS 20 mg/kg B.Wt. i.p	3 ± 0.6	7 ± 0.6	9 ± 1.0	10 ± 0.6	10 ± 0.0
Morp 10 mg after 15 min naloxone 1 mg/kg B.Wt. s.c	2 ± 0.6	7 ± 0.6	7 ± 1.5	5 ± 0.6	4 ± 1.0
BS 10 mg after 15 min naloxone 1 mg/kg B.Wt. s.c	2 ± 0.6	4 ± 0.6	7 ± 0.6	8 ± 0.6	8 ± 0.0
BS 20 mg after 15 min naloxone 1 mg/kg B.Wt. s.c	3 ± 0.6	4 ± 0.6	8 ± 0.6	8 ± 0.6	8 ± 0.0

Each value represents mean±SEM of six observations. Values in the table indicate the reaction time (paw licking and jumping), *P<0.05 when compared to vehicle treatment (one-way analysis of variance, Tukey's test)

Table 2: Effect of β-sitosterol on acetic acid writhing on mice

Treatment groups	*No. of writhing and percentage*
Control – Tween-20	39.2±1.9 (60.8)
Paracetamol 50 mg/Kg B.Wt i.p	30.8±1.3 (69.2)
BS 10 mg/Kg B.Wt i.p	21.2±2.2 (78.8)
BS 20 mg/Kg B.Wt i.p	19.4±1.1 (80.6)

Each value represents mean±SEM of six observations. Values in the table indicate the number of writhing and percentage inhibition of writhing, *P<0.05 when compared to vehicle treatment (one-way analysis of variance, Tukey's test)

Table 3: Effect of β-sitosterol on carrageenan-induced rat paw edema

Treatment groups	*Paw thickness (cm)*				
	0 h	*1 h*	*3 h*	*5 h*	*24 h*
Control – Tween-20	0.25±0.0	0.25±0.0	0.25±0.0	0.25±0.0	0.25±0.0
Ibuprofen 50 mg/kg B.Wt i.p	1.5±0.0	1.4±0.1	0.7±0.1	0.5±0.1	0.3±0.1
BS 10 mg/kg B.Wt i.p	1.4±0.1	1.2±0.1	0.8±0.1	0.7±0.1	0.4±0.1
BS 20 mg/kg B.Wt i.p	1.4±0.1	1.0±0.1	0.7±0.1	0.5±0.0	0.3±0.1
Dexamethasone 1 mg/kg B.Wt i.p	1.5±0.1	1.1±0.1	0.3±0.0	0.2±0.0	0.2±0.0

Each value represents mean±SEM of six observations. Values in table indicate the percentage inhibition of paw edema, *P<0.05 when compared to vehicle treatment (analysis of variance, Tukey's test)

of endogenous opioid peptides. Intraperitoneal injection of acetic acid produced pains through activation of chemosensitive nociceptors nor through the irritation of visceral surface, which led to liberation of histamine, bradykinins, prostaglandins and serotonins. Thus, the antinociceptive activity of opioid partial agonist and nonsteroidal anti-inflammatory agents can be determined by the writhing test. The mechanism of the analgesic effect of BS could probably be due to the blockage of effect or release of endogenous substances that excite pain nerve endings. Overall, BS showed potent antinociceptive activity in both the tests. Prostaglandins and bradykinin were suggested to play an important role in analgesia. Carrageenan-induced edema is a biphasic response. In the first phase, mediator is through the release of serotonin, histamine, and kinins, whereas the second phase is related to the release of prostaglandin and slow-reacting substances which peak at 3 h. In case of analgesia, prostaglandins and bradykinin were suggested to play an important role in the pain process. Some sterols and triterpenes are responsible for anti-inflammatory and analgesic activity. From this, we can conclude that BS has analgesic and anti-inflammatory action; it works through central mechanism through opioid receptors.

Conclusion

The present study demonstrated the anti-nociceptive potential of betasitosterol and its anti-inflammatory activity, there was a prominent anti-inflammatory action in the carrageenan paw edema assay in which the effect which was clearly demonstrated similar to the selected reference anti-inflammatory compounds.

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