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Antidiarrheal and anti inflammatory activities of Lupeol, Quercetin, β - Sitosterol, Adene-5-en-3-ol and Caffeic acid isolated from *Rhizophora Mucronata* Bark

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ABSTRACT

The anti inflammatory and antidiarrheal effect of Rhizophora mucronata bark extracts led to the isolation of active phytoconstituent/s(1-6) such as lupeol , a new terpenoid adeneneol , quercetin , and caffeic acid , all of which were subjected to carrageen induced paw edema and castor oil induced diarrheal method to assess the activity, it was observed that decrease in percentage of paw volume as caffeic acid < adeneol < lupeol < β - sitosterol, quercetin at 10 mg/kg with respect to control and quercetin and caffeic acid reduced the number of wet feces during the 4 h study ; it would be hypothesized that quercetin , sitsterol and caffeic acid exerted anti inflammatory and anti diarrheal effect by inhibiting the prostaglandins synthesis and due their antioxidant action, while failure of anti diarrheal action and positive response of anti inflammatory action of lupeol and adene-5-en-3-ol,would be through non prostaglandins biosynthesis

Key words lupeol, adene-5-en-3-ol,, quercetin , caffeic acid, Rhizophora mucronata

INTRODUCTION

Diarrhea is a common cause of death in developing countries and the second most common cause of infant deaths worldwide. The loss of fluids through diarrhea can cause dehydration and electrolyte imbalances; diarrhea can be classified as secretory, osmotic, exudative and inflammatory. Inflammatory diarrhea occurs when there is damage to the mucosal lining or brush border, which leads to a passive loss of protein-rich fluids, and a decreased ability to absorb these lost fluids. It can be caused by bacterial infections, viral infections, parasitic infections, or

autoimmune problems such as inflammatory bowel diseases. Drugs possessing anti-inflammatory activity have been shown to delay castor oil-induced diarrhea, suggesting the involvement of prostaglandins in this mechanism[1] *Rhizophora mucronata* (Rhizophoraceae) occurs on the coasts of the Indian Ocean and the West-Pacific and commonly called as Asiatic mangrove, is a folk remedy for angina, diabetes, diarrhea, dysentery, hematuria, and hemorrhage old leaves and/or roots are for childbirth [2] In continuation of our work on anti diarrhea and anti inflammatory activity of *R mucronata* bark extracts (RMB) [3, 4], the present study was emphasized on phytoconstituents isolated from the bark extract to evaluate their activity. This is first report to establish the activity of new terpenoid adene-5-en-3-ol, along with other known compounds isolated from RMB extract.

MATERIALS AND METHOD

Rhizophora mucronata bark was collected from Kundapur of Mangalore district, India. The bark was authentication was done from Bangalore University. Animals' experiments were done following ethical committee guide lines. The dried bark powder was extracted from methanol by soxhlet extraction. From the extract lupeol, adeneneol, β -sitosterol, quercetin, and caffeic acid were isolated by column chromatography, the structures were characterized from spectral analysis [5]. These isolated compounds were used for the present study.

Carrageenin- induced paw odema model

Forty-eight female wistar albino rats weighing 150-200 g were used. The rats were housed in colony cages in an animal house, at an ambient temperature of $25\pm 2^\circ\text{C}$, with 12 h light/ dark cycle. The rats were allowed standard laboratory feed and water *ad libium*

Animals were divided into seven groups (n = 6); group I received indomethacin (10 mg/kg) and group II served as control were given 1 % aq tween 80 suspension; group III- VII were orally administered 10 mg /kg of isolated phytoconstituents from RMB (lupeol, quercetin, β -sitosterol, caffeic acid and adene-5-ene -3-ol, suspended in 1 % aq tween 80) one hour before administration of an intradermal injection of carrageenan (0.1 ml of 1% in 0.9% saline), into the plantar surface of the right hind paw. The doses of test samples were based from their published reports [6-9]. The paw volume up to a fixed mark at the level of lateral malleolus, was measured by recording the volume displacement by plethysmometer, just before, and three hours after the injection of carrageenan [9]. The average percent increase in paw volume of each group was calculated, and compared with that of the control and positive control groups.

The data were analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's test. $P < 0.01$ was considered as statistically significant. The data are expressed as mean \pm SEM. The results are shown in Table 1.

Percent anti-inflammatory activity = $(1-T/C) \times 100$

Where, T= Mean volume of oedema in drug treated group
C= Mean volume of oedema in control group

Antidiarrheal activity of isolated compounds*Castor oil induced diarrhea*

Wistar rats were divided into seven groups (n = 6) and, fasted for 18 h and water was provided *ad libitum* prior to the experiment. A dose of 10 mg/kg was selected for all the compounds tested. Group I served as control, to which saline 3 ml/kg was administered orally; group II received atropine 3 mg/kg p. o, which was taken as positive control, animals of groups III, IV, V, VI and VII received 10 mg/kg of lupeol, β -sitosterol, quercetin, caffeic acid and adene-5-ene -3-ol respectively. After one hour, all the animals were orally administered castor oil 1 ml/kg and were observed for onset of defecation, number of wet stools for period of 4 h [10]. The frequency of defecation was noted in transparent plastic sheet placed beneath the individual rat cages up to 4 h.

RESULTS

Anti inflammatory activity -The isolated lupeol, quercetin, β - sitosterol , caffeic acid and adene-5-ene -3-ol from the RMB extract exhibited significant inhibition of paw edema in rats treated with carrageenan (Table 1) . At a dose of 10 mg/kg orally, the new lupane terpenoid adene-5-ene -3-ol and caffeic acid demonstrated 55 % inhibition of paw edema while lupeol produced 54.4 % inhibition, and β -sitosterol produced 51.4 % inhibition (P<0.01), moderate activity was seen from quercetin 45 % .

Table 1 -Inhibition of paw edema in rats exhibited from isolated compounds of R mucronata

Treatment	Carragenen induced paw edema	
	Edema volume	% inhibition
Control Suspension of acica	60.4 \pm 4.6	
Indomethacin	28.6 \pm 0.6***	52.7
Lupeol (10 mg/kG)	27.6 \pm 0 .21**	54.4
Quercetin (10 mg/kg)	33.2 \pm 0.45*	45.1
β -Sitosterol (10 mg/kg)	29.4 \pm 1.00**	51.4
Caffeic acid (10 mg/kg)	26.9 \pm 1.2**	55.5
adene-5-ene -3- ol (10 mg/kg)	27.03 \pm 0.3 **	55.3

Values are mean \pm SEM; n= 6 animals in each group, ** p< 0.01as compared to control

Anti diarrhea activity

Oral administration of castor oil produced characteristic semi solid stools in treated animals. It was observed that quercetin and two phenolic compounds at 10 mg/kg showed significant reduction in the number of defecation over four hours ($p < 0.001$) when compared to the untreated group along with significant delay in the onset of defecation ; the activity was similar to the atropine control (3 mg/kg) as shown in the table 2.

Table 2 –Antidiarrheal effect of the isolated compounds from RMB in castor oil induced animal model

Treatment	Mean onset of time (min)	Mean number of wet feaces in 4 h
Control	42.36 ± 1.00	4.7 ± 0.32
atropine 3m/kg	149.0 ± 1.66 *	0.00 **
Lupeol 10 mg/kg	69.61 ± 0.22	3.80 ± 1.90
Sitosterol 10 mg/kg	54.32 ± 1.40	2.96 ± 0.98
Quercetin 10 mg/kg	142 ± 0.74*	0.92 ± 0.06**
Caffeic acid 10 mg/kg	138 ± 0.62*	1.32 ± 1.02**
adene-5-ene -3-ol	46 ± 1.90	5.90 ± 0.74

Values are mean ± SEM; n= 6 animals in each group, ** $p < 0.01$ as compared to control

DISCUSSION

The inflammatory reaction is orchestrated by a large range of mediators able to promote vascular events, recruit cells to the site of inflammation and subsequently resolve the process. The literature provides evidence showing that a vast array of inflammatory mediators including prostaglandins (PGs), kinins, platelet-activating factor, leukotrienes (LTs), amines, purines, cytokines, adhesion molecules and chemokines act on specific sites (microvasculature), leading to changes in vascular tonus, blood flow and local activation of leukocytes and endothelial cells[11]. Tumor necrosis factor α (TNF- α) is a major cytokine involved in the initiation of the inflammatory response. Its actions include the induction of other cytokines such as interleukin 1 (IL-1) and interleukin 6 (IL-6), priming of polymorphonuclear leukocytes (PMN), up-regulation of adhesion molecules and activation of arachidonic acid (AA) metabolism[12,13]. AA metabolites include PGs and thromboxanes (*via* cyclooxygenases, COX) and LTs (*via* lipoxygenase). Prostaglandin E2 (PGE2), derived from COX metabolic pathway, is able to promote changes in vascular tonus and blood flow. The inflammatory agent carrageenin stimulates macrophages/monocytes, fibroblasts and epithelial cells with TNF- α which in turn induces IL-1 α and IL-6, which ultimately lead to the release of COX products (such as PGE2). Therefore, the impairment of TNF- α synthesis/release, and of other pro-inflammatory cytokines, represents an interesting alternative for the inhibition of PGE2 and consequently of the edema[12]. Toriyabe *et al* [14] investigated the effect of peripherally released NO on COX expression/activation and production of PGs in carrageenin-induced inflammation and found that

NO activates COX-1 and up-regulates COX-2, resulting in production of PGE₂ and PGI₂ at the site of carrageenin inflammation. Nonsteroidal anti-inflammatory agents such as indomethacin used as positive control act as nonselective inhibitors of the enzyme cyclooxygenase (COX), inhibiting both the COX-1 and COX-2 isoenzymes. Steroidal anti-inflammatory agent's effects on the leukocyte migration may also be multifactor since it could be related to the inhibition of phospholipase A₂ [15], as well as other mechanisms, for example, inhibition of TNF α and IL-1 β productions which are potent triggers of many of the actions involved in leukocyte migration. A large number of reports indicated that the lupeol isolated from various plants has been shown to possess anti-inflammatory activity [16-18]. The β -sitosterol, lupeol and adene-5-ene-3-ol inhibited carrageenin induced edema, which indicates that the compounds could inhibit different aspects of chemical mediators of inflammation (histamine, serotonin, bradykinin and prostaglandins). Fernandez et al hypothesized that anti-inflammatory effect of pentacyclic triterpene may be due to an immunosuppressive action and the inhibition of cell migration to the inflammation site as well as to a reduction in the release of pro-inflammatory chemotactic factors [19]. Lupeol failed to show any analgesic or antipyretic effect, suggests that lupeol acts via a different mechanism of action than acetylsalicylic acid, and so it does not inhibit cyclooxygenase activity and the synthesis of prostaglandins [20]. Sterol containing fraction having high concentration of beta-sitosterol failed to inhibit the histamine secretion in histamine-release from rat peritoneal mast cells and, leukocytes pre-incubated with the sterol fraction decreased the level beta-glucuronidase involved for inflammation and tissue destruction [21]. This effect could be explained by a stabilization effect on membranes, as it has been reported that sterols decrease the membrane permeability to the effects of a wide array of agents [22, 23]. In our previous studies conducted for analgesic and anti-inflammatory effect for *RMB* solvent extracts (chloroform, ethyl acetate, methanol and water) [4] it was observed the extracts failed to produce analgesic effect in the animal demonstrated but produced positive result for anti-inflammatory effect thus suggesting lupeol, adene-5-en-3-ol, and β -sitosterol isolated from the extracts of *RMB* may elicit anti-inflammatory response by inhibition of cytokine production (TNF α and IL-1 β) which are potent triggers of many of the actions involved in leukocyte migration. The anti-inflammatory activity of quercetin would be possibly due to an influence on the production of eicosanoids, including leukotrienes, prostaglandins, and also cytokines [24,25]; Since the potent antioxidant activity of PCA, QE, and CA are well-established phenomenon we cannot rule out the possibility that phenol acid and flavanoid to exerts anti-edematogenic activity through the inhibition of NO synthesis.

Ricinoleic acid released from castor oil stimulates the formation of prostaglandins PG which leads to inflammatory reaction and increases the motility and secretion of hydro electrolytes leading to diarrhea. The Geiger's criteria for the acceptance of a drug as an antidiarrheal include: (1) inhibition of the production of wet or unformed feces in animals; (2) inhibition of the production of watery stool or fluid evacuation in animal and (3) inhibition of gastrointestinal propulsive action [26]. Flavonoids are known to inhibit the intestinal motility and hydro electrolytic secretion which are known to be altered in diarrheal condition [27], From literature study its noted that Quercetin and other phenol compounds inhibited the formation of proinflammatory cytokines responsible for the formation of large amount of nitric oxide by inducible nitric oxide synthase [28]. In vitro study of Caffeic acid on anti-inflammatory effect has revealed it to inhibit significantly melittin-induced arachidonic acid release and PGE₂ production in Raw 264.7 cells, and histamine release in RBL 2H3 cells stimulated by melittin or arachidonic acid [29]; the anti

diarrhea effect of caffeic acid would be probably to the inhibition of PGE formation and free radical scavenging activity. These facts partially helps to understand the anti diarrheal effect of quercetin and caffeic acid isolated from *RMB* which would be attributed to its anti oxidant effect & inhibiting biosynthesis of PGS. These compounds, therefore, meets the Geiger's anti diarrhea criteria. β -Sitosterol and lupeol failed to produce effect in castor oil induce diarrhea this would explain & support the anti inflammatory effect of these two compounds to act through non prostaglanins biosynthesis , may be involving inhibition of IL -A, IL -6 factors involved in inflammation .

CONCLUSION

From the overview of the above discussion it would be suggested that quercetin , sitsterol and caffeic acid exerted anti inflammatory and diarrheal effect by inhibiting the prostaglandins synthesis and can be partly attributed to their anti oxidant effect , while terpenoids i.e 's lupeol and adene-5ene-3-ol failed to show antidiarrheal effect but inhibited inflammation in the carrageen induced paw edema indicated these two compound could act by inhibiting cytokine formation .further elaborate pharmacological study would reveal the mechanism involved for the isolated compounds .

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