

Original Research Article

Anti-inflammatory and analgesic activity of leaf and callus extracts of *Coleus forskohlii*

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Abstract

Though all sixteen combinations of NAA + BAP, and NAA + KN induced callus from leaf explants of *Coleus forskohlii* with a variable response, 0.5 mg/l NAA + 1.5 mg/l BAP was the most efficient (100%) followed by 1.0 mg/l NAA + 2.5 mg/l KN (86%). Investigations were made for anti-inflammatory and analgesic activity of hydroalcoholic extracts of leaf and callus on Swiss albino mice. Anti-inflammatory studies were performed by Carrageenan induced paw edema (acute) and Cotton pellet induced granuloma (chronic) models, while the analgesic activity was tested by: glacial acetic acid induced writhing and tail immersion models. Maximum anti-inflammatory activity was exhibited by 200 mg/kg callus extract (43% in acute and 22.3% in chronic) followed by 200 mg/kg leaf extract (37% in acute and 21.8% in chronic) compared to standard drug, the aspirin (27.4% in acute and 29.9% in chronic). In writhing model, 200 mg/kg callus extract exhibited highly significant ($p < 0.01$) reduction in writhing count compared to 200 mg/kg ($p < 0.01$) leaf extract. In tail immersion model also the reaction time was significantly reduced ($p < 0.01$) by both callus and leaf extracts throughout observation period of 3 hrs. The results obtained supported the use of this plant in traditional medicine.

Keywords: *Coleus forskohlii*, callus, inflammation, analgesic, edema.

Introduction

Inflammation is a patho-physiological response of living tissue to injury that leads to local accumulation of plasmatic fluid and blood cells. The inflammatory process is invariably characterized by the production of prostaglandins, leukotrienes, histamines, bradykinins, platelet-activating factors (PAF) and the release of chemicals from tissues and migrating cells. The defense mechanism helps body to protect itself against infections, burns, toxic chemicals, allergens or other noxious stimuli [1,2]. Carrageenan-induced local inflammation is commonly used to evaluate non-steroidal anti-inflammatory drugs (NSAID). It appears that onset of the Carrageenan induced local inflammation has been linked to neutrophil infiltration and the production of neutrophil-derived free radicals, such as hydrogen peroxides, superoxides and hydroxyl radicals, besides the release of other neutrophil-derived mediators. Pain has been defined, by the International Association for the Study of Pain (IASP), as an unpleasant sensory and emotional experience associated with actual or potential tissue damage [3]. Drugs that are currently used for the management of pain, including acute postoperative pains, are opioids and non-opioids which are highly toxic in nature [4]. A study revealed that risk of gastrointestinal bleeding is significantly associated with acute use of non-steroidal anti-inflammatory drugs (NSAIDs) like aspirin, diclofenac, ketorolac, naproxen or nimesulide while the drug piroxicam increased the risk of bleeding in both acute and chronic therapy [5].

Hence, there is a need to develop safe and new anti-inflammatory agents without side effects. In this scenario, use of plant derived products to treat inflammation and related conditions becomes a viable and valid approach.

Coleus forskohlii is an ancient medicinal plant, belongs to mint family, the lamiaceae, and variously called as Pashan bhedi, Pather chur, Makandi, Mayini, False boldo, Coleonol etc. in different languages, It has been cultivated in India (Tamil Nadu, Karnataka and Gujarat), Thailand and parts of South-East Asia as a spice. It is mostly used in ayurvedic medicine in the treatment of heart ailments, asthma, bronchitis, insomnia, epilepsy, anemia and inflammation [6].

Phyto-chemical investigations, into the roots of this plant, revealed the presence of a diterpene compound, the forskolin [7]. Qualitative analysis carried out on this plant showed the presence of terpenoids, tannins, flavonoids, phlobatannins, saponins and cardiac glycosides [8]. One of the pharmacologically active compounds found in leaves of *Ocimum* is rosmarinic acid, a phenylpropanoid [9]. an ester of caffeic acid, the 3, 4-dihydroxyphenyllactic acid, is a natural compound present and has been reported to have a multitude of biological activities, including anti-oxidative, anti-microbial and anti-inflammatory effects [10]. The antioxidant property of rosmarinic acid is protection against oxidants and free radical injuries. Its activity, especially against

rheumatic and inflammatory conditions makes it a sought-after substance for use in phytotherapy [11].

Earlier attempts have been made to study the identification of poly-phenolic compounds in the root cultures of *Coleus forskohlii* [12]. However, reports on the properties of these compounds, including antioxidant, anti-inflammatory, analgesic and anti-depressant activities, on animals have not been documented till now in the leaf and callus extracts of *Coleus forskohlii*. Therefore, for the first time, the present study is initiated for induction of callus and to evaluate the leaf and callus extracts of *Coleus forskohlii* for their anti-inflammatory and analgesic activity on an animal model, the Swiss albino mice.

Materials and Methods

Authentication of plant material

Coleus forskohlii plants were raised, at the Plant Genetics Experimental Farm, Dept. of Genetics, Osmania University, from the cuttings obtained from ANGRAU Herbal Gardens, Rajendra Nagar, Hyderabad, A.P, India. A herbarium of this plant was authenticated by a taxonomist, bearing Voucher specimen No.0786, *Coleus forskohlii* (wild) briq, Dept. of Botany, Osmania University, has been preserved.

Induction of callus

MS [13] medium was prepared by supplementing sixteen different combinations of an auxin: 0.5 and 1.0 mg/l NAA, with two cytokinins: 1.0, 1.5, 2.0 and 2.5 mg/l of BAP; and 1.0, 1.5, 2.0 and 2.5 mg/l of KN were added. Later, agar was added and autoclaved at 15 lbs at 121° C for 20 min and poured into the borosil culture tubes. The tubes were inoculated with leaf explants (1.0 -1.5cm) of *Coleus forskohlii* and incubated at 25 ± 1° C under 16/8 h photoperiod with cool white fluorescent lights for a period of 25-30 days for callusing. The induced callus was sub - cultured on to the fresh MS medium after 30 days. The yield of callus was recorded after 30 days of subculture.

Extraction and Qualitative evaluation

Fresh leaves from the field grown plants and the callus obtained from the leaves were shade dried, powdered and extracted with hydroalcohol [methanol: water (1:1)] by using rotary vacuum evaporator. The leaf and callus extracts were subjected for identification quantification and antioxidant activity of secondary metabolites using standard methods [14].

Swiss albino mice

The leaf and callus extracts were evaluated for their acute toxicity, anti-inflammatory and analgesic activity against the experimental test animal, the Swiss albino mice. The mice of both sexes, weighing 20-25 g, procured from the mice breeding unit (NCLAS) of NIN, Hyderabad, were used in the present study. The study was

performed by adapting the protocols and procedures reviewed and approved by the Institutional Animal Ethics Committee and the experiments were conducted in accordance to the guidelines laid down by the CPCSEA (383/01/a /CPCSEA) for the use and care of experimental animals.

Acute toxicity

Acute toxic effects, of leaf and callus extracts suspended in hydroalcohol, were determined by oral administration in Swiss albino mice. The experimental animals were observed for 14 days for product-related symptoms. However, the test substances did not produce any signs of toxic effects even 14 days after administering the extracts and all the animals survived. Since the extracts did not exhibit any toxic effect even up to the dose of 2000 mg/kg body weight, the LD₅₀ value of hydroalcoholic extracts of *Coleus forskohlii* in mice by oral route may be considered to be greater than 2000 mg/kg body weight.

Anti-inflammatory activity

Anti-inflammatory activity of leaf and callus extracts was studied by two methods - Carrageenan-induced hind paw edema in mice and Cotton pellet granuloma models.

Carrageenan-induced hind paw edema in mice

A total of 48 Swiss albino mice were divided into eight groups (one control, one standard and six test groups), containing six mice in each group. Carrageenan, an inflammation producing agent, prepared as 1% w/v solution in 0.9 % w/v NaCl and injected 0.1 ml of solution to the mice hind paw underneath the sub plantar region [15,16]. Acute inflammation was induced in mice as mentioned below.

Control group: Carrageenan + Normal Saline H₂O (0.1ml)
 Standard group: Carrageenan + Aspirin (91 mg/kg)
 Group1: Carrageenan + Hydro alcoholic leaf extract (50 mg/kg)
 Group2: Carrageenan + Hydro alcoholic leaf extract (100 mg/kg)
 Group3: Carrageenan + Hydro alcoholic leaf extract (200 mg/kg)
 Group 4: Carrageenan + Hydro alcoholic callus extract (50 mg/kg)
 Group 5: Carrageenan + Hydro alcoholic callus extract (100 mg/kg)
 Group 6: Carrageenan + Hydro alcoholic callus extract (200 mg/kg)

The hind paw edema volume was measured using Vernier calipers at 0 hours and from 2 to 6 hours, with an interval of 1 hour, and finally once after 24 hrs of Carrageenan injection.

The Percentage of anti-inflammatory activity of leaf and callus extracts was calculated by the following equation:



Percentage inhibition of paw edema (% EI) = $(1 - V_t/V_c) \times 100$

Where EI is the edema inhibition, V_t is the average inflammation caused by the drug treatment (extracts or test drug aspirin) at the same time, V_c represents average inflammation in the control mice at a given time.

Cotton pellet induced granuloma model

Anti-inflammatory effects of leaf and callus extracts were evaluated against six groups (Control, Standard and 4 test groups) of Swiss albino mice, each of six animals, by the Cotton pellet granuloma model. Animals were anaesthetized by oral administration of ketamine at 10 mg/kg. About 30 min after anaesthetization, the fur was removed and incisions were made on the axilla to insert the sterile cotton pellets of 50 mg. Later, the mice were administered daily with doses of 100 and 200 mg/kg of leaf and callus extracts separately for a period of seven days. The cotton pellets were removed surgically on 8th day along with the layer of connective tissue formed, if any, and made free from extraneous tissues. The pellets, along with the connective tissue, were incubated at 37°C for 24 h and dried at 60°C. Increase in dry weight of the pellets was taken as a measure of granuloma formation [17]. Three phases of inflammatory response to subcutaneously implanted cotton pellets in mice: (A) a transudative phase, that occurs during the first 3h, (B) an exudative phase, occurring between 1 and 72h, after implanting the pellets and (C) a proliferative phase, measured as the increase in dry weight of the granuloma that occurs between 3 and 6 days after implantation of pellets have been described [18].

Analgesic activity

Analgesic activity of leaf and callus extracts of *Coleus forskohlii* were evaluated by two methods – the Acetic acid induced writhing test and the Tail immersion method in mice.

Acetic acid induced writhing test in mice

In this method, peripheral analgesic activity was investigated by administering separately the doses of 100 and 200 mg/kg of leaf and callus extracts orally to all the six groups of mice. One hour after administering the extracts, acetic acid (1% v/v) was injected intraperitoneally to all the six groups at a dose of 1 ml/kg body weight. These animals were placed in a large glass cylinder and the intensity of nociceptive behavior was quantified by counting the total number of writhes occurring at 0, 10, 20 and 30 min after the stimulus injection. The writhing response consists of a contraction of the abdominal muscles together with simultaneous stretching of hind limbs [19,20].

The percentage of Inhibition in writhing = $[1 - \text{Mean No. of writhes in treated group} / \text{Mean No. of writhes in control group}] \times 100$

Tail immersion method

In this method, the central analgesic activity of leaf and callus extracts of *Coleus forskohlii* was assessed against the Swiss albino mice by immersing only the 5 cm lower portion of the tail in a cup containing hot water maintained at 40 ± 2 C. Later, the mice were placed into individual restraining cages, leaving the tails hanging out freely for 30 min to adapt to the cages before testing. Within a few seconds of immersing tails in hot water cups, the mice reacted by withdrawing their tails. After every reaction, the time was recorded in 0.5 s units by a stopwatch and later the tail was dried. The reaction time was determined before and after 0.5s units of oral administration of extracts. The cut off time of tail immersion was only 15s. The withdrawal time of untreated animals was recorded between 1.0 and 5.5 s. The withdrawal time of more than 6 s in treated mice was regarded as a positive response [21,22].

Statistical Analysis

Results were expressed as Mean \pm Standard error of mean (S.E.M.). Statistical significance was determined by one-way analysis of variance (ANOVA) coupled with Dunnet's post test. p -values < 0.05 were considered as the significant difference between control vs test groups.

Results and Discussion

The objective of this study was to induce callus from the leaves obtained from the field grown plants and to evaluate the anti-inflammatory and analgesic activity of extracts of leaves of *C. forskohlii*. Among the sixteen different combinations, 0.5 mg/l NAA + 1.5 mg/l BAP was found to be the most effective and efficient (100%) followed by 1.0 mg/l NAA + 2.5 mg/l KN (86%) in inducing callus. This suggests that the combination of NAA with BAP at lower concentration itself is more effective while the combination of NAA with KN even at higher concentrations is not as effective as NAA with BAP. In vitro raised callus cultures are being popularly used for the production of metabolites, and the presence of sucrose concentration mainly influences the production of metabolites in phenylpropanoid pathway [23].

Induction of callus

Though, all the sixteen combinations of NAA with BAP, and NAA with KN produced callus with a variable response, the 0.5 mg/l NAA + 1.5 mg/l BAP found to be the most effective and efficient (100%) followed by 1.0 mg/l NAA + 2.5 mg/l KN (86%) in inducing callus (Table I). The extracts of callus and leaves were evaluated for their anti-inflammatory and analgesic activity.



Table 1. Induction of callus in *Coleus forskohlii*

Hormonal combinations (mg/L)	Percentage of Callusing	
NAA+BAP	0.5 + 1.0	85
	0.5 + 1.5	100
	0.5 + 2.0	82
	0.5 + 2.5	80
	1.0 + 1.0	42
	1.0 + 1.5	64
	1.0 + 2.0	48
NAA + KN	1.0 + 2.5	52
	0.5 + 1.0	22
	0.5 + 1.5	34
	0.5 + 2.0	36
	0.5 + 2.5	48
	1.0 + 1.0	52
	1.0 + 1.5	74
1.0 + 2.0	44	
1.0 + 2.5	86	

Anti-inflammatory activity of leaf and callus extracts on Swiss albino mice Carrageenan induced paw edema (Acute model)

This model is generally used in detecting and assessing the anti-inflammatory activity of orally administered natural products. In the present investigation, though the leaf and callus extracts at doses of 50, 100 and 200 mg/kg body weight exhibited significant anti-inflammatory effects on paw edema formation, at all recorded times, the callus extract at a dose of 200 mg/kg exhibited highest (43.1%) anti-inflammatory activity followed by 200 mg/kg dose of leaf extract (36.4%) where as the Aspirin, a cyclo-oxygenase (COX) inhibitor, exhibited anti-inflammatory activity of 27.4% at a dose of 91 mg/kg, after 3 hrs of 1% carrageenan injection (Table-II). Carrageenan-induced hind paw edema in mice, the standard experimental model of acute inflammation (acute method), a biphasic process, [24], frequently used to assess the anti-inflammatory effects of natural products [25]. The first phase of inflammation occurs within an hour of carrageenan injection, partly attributed to trauma of injection to histamine and serotonin components. Significant inhibition of paw edema in the early hours of study by *Coleus forskohlii* could be attributed to the inhibition of histamine and or serotonin. The second phase is associated with the production of bradykinin

Table 2. Anti-inflammatory activity of hydroalcoholic extracts of *C. forskohlii* on Swiss albino mice by acute method

Group	Paw edema volume after 1% carrageenan injection							
	2 h		3 h		4 h		5h	
	EV	% EI	EV	%EI	EV	% EI	EV	% EI
Control	0.77 ± 0.07	-	0.85 ± 0.06	-	0.97 ± 0.08	-	1.07 ± 0.09	-
Aspirin, 91 mg/kg	0.65 ± 0.07	21.7	0.62 ± 0.05**	27.4	0.73 ± 0.05**	24.1	0.80 ± 0.04**	25.0
Leaf extract								
50 mg/kg	0.68 ± 0.12	10.2	0.83 ± 0.14	6.0	0.95 ± 0.04	12.2	1.02 ± 0.07	12.1
100 mg/kg	0.68 ± 0.12	11.0	0.60 ± 0.03**	29.4	0.75 ± 0.03**	22.4	0.77 ± 0.03**	28.1
200 mg/kg	0.58 ± 0.06**	29.5	0.53 ± 0.04**	36.4	0.68 ± 0.04**	27.5	0.73 ± 0.04**	28.7
Callus extract								
50 mg/kg	0.63 ± 0.12*	19.7	0.77 ± 0.13	10.3	0.88 ± 0.04**	13.7	0.97 ± 0.05*	12.5
100 mg/kg	0.58 ± 0.06**	26.5	0.77 ± 0.13	11.1	0.68 ± 0.03**	29.3	0.73 ± 0.02**	31.2
200 mg/kg	0.55 ± 0.04**	28.2	0.48 ± 0.04**	43.1	0.65 ± 0.05**	32.7	0.68 ± 0.06**	35.9

Values as mean ± S.E.M; n = 6 in each group; * $P < 0.05$; ** $P < 0.01$; EV = edema volume, EI = edema inhibition



protease, prostaglandin (PG) and lysosome [26]. Prostaglandins play a major role in the production of inflammatory reaction which is measured at 3rd hr. The doses each of 200 mg/kg leaf and callus extracts produced a significant inhibition of carrageenan induced paw edema at 3, 4, and 5 hr. The anti-inflammatory activity of *C. forskohlii* could be due to its inhibitory effect on the activity of enzyme cyclooxygenase and subsequent inhibition of prostaglandin synthesis [27]. The strong anti-inflammatory activity of the extract may also be due to the presence of a polyphenol, the rosmarinic acid, in the extracts. Polyphenols are reported to be involved in anti-inflammatory activity of plants [28]. The anti-inflammatory activity is due to the inactivation of C₃ convertase by the polyphenolic compounds present in the extracts [29]. The maximum inhibition of paw oedema exhibited by extracts of *in vitro* generated callus cultures may be attributed to the presence of higher amounts of secondary metabolites compared to extracts of leaves. The results strongly suggest that the hydroalcoholic extracts of leaf and callus possess potent anti-inflammatory activity that could inhibit the acute inflammation of paw edema in mice induced by both the carrageenan and cotton pellet granuloma models.

Cotton pellet induced granuloma (chronic model)

Cotton pellet-induced granulation, a chronic model, commonly used for, *in vivo*, assessment of anti-inflammatory activity of compounds. The fresh weight of cotton pellets correlates with transudative phase while the dry weight correlates with the amount of granulomatous tissues. In this experiment, a significantly high percentage of inhibition was observed in the dry weights of cotton pellets removed from the mice treated with 200 mg/kg of leaf (21.9%) and callus (22.3%) extracts, however, less effective compared to the percentage of inhibition caused by Aspirin (29.9%), thus avoiding the formation of granuloma compared to control. A significant reduction in the dry weights of granuloma were caused by callus extract (33.9 ± 1.71 mg) and Aspirin (30.3 ± 0.72 mg) treated group of mice compared to control group (44.1 ± 3.33 mg) (Figure 1)

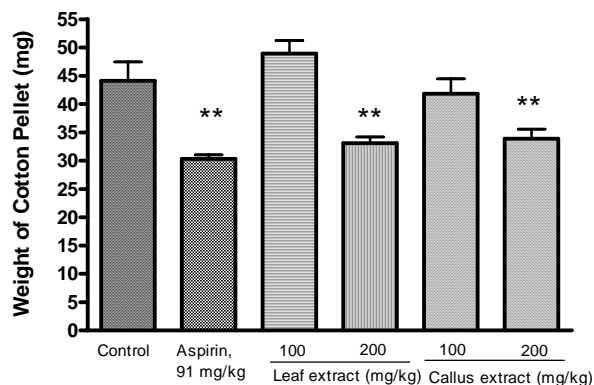


Figure 1. Anti-inflammatory activity of hydroalcoholic extracts of *C. forskohlii* on Swiss albino mice by chronic method Values as mean ± S.E.M; n = 6 in each group; Significant at ** $p < 0.01$

and may be attributed to the suppression of proliferative phase of chronic inflammation [28].

Analgesic activity of leaf and callus extracts on Swiss albino mice

Analgesic activity was evaluated by two methods – the acetic acid induced writhing method and the tail immersion method

Acetic acid induced writhing method

Acetic acid, the peripheral analgesic agent, causes inflammatory pain by inducing abdominal constrictions and stretching of hind limbs [21]. This response is believed to be mediated by the prostaglandin pathways [29]. There was a highly significant reduction in the number of writhes treated with 100 mg/kg (61.9%) and 200 mg/kg (72.6%) callus extracts while 200 mg/kg leaf extract (54.3%) in the acetic acid induced mice. However, the effectiveness of these extracts at 100 mg/kg was not as significant as that of standard drug, the diclofenac (74.1 %) (Figure 2). The anti-nociceptive activity of leaf and callus extracts of *C. forskohlii* in mice indicates the presence of analgesic compounds that might influence the prostaglandin pathways.

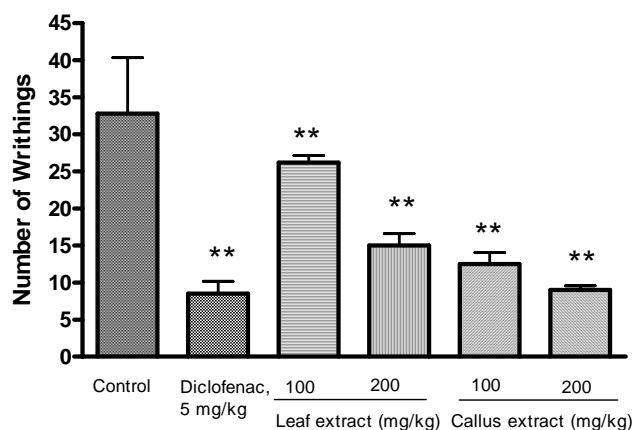


Figure 2. Analgesic activity of hydroalcoholic extracts of *C. forskohlii* on acetic acid induced writhing in Swiss albino mice Values as mean ± S.E.M; n = 6 in each group; Significant at ** $p < 0.01$

Tail immersion method

Thermally induced nociception indicates the involvement of narcotics in centrally acting analgesics which generally elevate the pain towards threshold. The increase in mean reaction time to reduce pain due to the effect of leaf and callus extracts suggests their action through centrally mediated analgesic mechanism. In this method, the mice exhibited an initial reaction only after 30 min of oral administration of 100 and 200 mg/kg doses of leaf and

callus extracts and persisted until the following 3 hours, while pentazocine, the standard compound, significantly delayed the initial reaction time to the nociceptive response at a dose of 17.5 mg/kg (Table- III).

The increase in mean reaction time to reduce pain due to the effect of leaf and callus extracts suggests their action through centrally mediated analgesic mechanism.

Anti-inflammatory and analgesic activity exhibited by these extracts, in the present study, might be due to the presence of antioxidants, which could protect against oxidant and free radical

injuries was proved by DPPH assay [14].

The present investigation amply demonstrated that the extracts of *Coleus forskohlii* have significant anti-inflammatory and analgesic properties, justifying its traditional use in the treatment of various types of inflammations and pains.

Table 3. Reaction time and percent inhibition in reaction time (RI) for Analgesic activity of hydroalcoholic extracts of *Coleus forskohlii* by tail immersion model in mice

Group	Reaction time (sec) after administration of test compound									
	0 h		0.5 h		1 h		2 h		3 h	
	Reaction time (sec)	% RI	Reaction time (sec)	% RI	Reaction time (sec)	% RI	Reaction time (sec)	% RI	Reaction time (sec)	% RI
Control	2.34 ± 0.34	-	2.54 ± 0.19	-	2.01 ± 0.41	-	2.45 ± 0.26	-	2.18 ± 0.31	-
Pentazocine , 17.5 mg/kg	2.55 ± 0.21	8.24	6.84 ± 0.91**	62.9	6.67 ± 1.16**	69.9	6.21 ± 0.99**	60.5	5.81 ± 0.78**	62.5
Leaf extract										
100 mg/kg	2.61 ± 0.32	10.3	4.86 ± 0.69**	47.7	3.48 ± 0.89*	42.2	3.14 ± 1.31	22.0	2.34 ± 1.21	6.84
200 mg/kg	2.84 ± 0.53	17.6	5.35 ± 0.93**	52.5	4.82 ± 1.01**	58.3	4.28 ± 1.71*	42.8	3.55 ± 1.91	38.6
Callus extract										
100 mg/kg	2.48 ± 0.41	5.65	5.11 ± 0.58**	50.3	4.43 ± 0.37**	54.6	4.19 ± 0.41*	41.5	3.83 ± 0.31*	43.1
200 mg/kg	2.63 ± 0.45	11.0	5.61 ± 0.37**	54.7	5.10 ± 0.33**	60.6	4.85 ± 0.41*	49.5	4.32 ± 0.39**	49.5

Values as mean ± S.E.M; n = 6 in each group; Significant at * $p < 0.05$; ** $p < 0.01$

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