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Original Article

Anti-inflammatory activity of BCM-95 (bio-enhanced formulation of turmeric with increased bioavailabilty) compared to Curcumin in Wistar rats

Sayeli Vinaykumar[1], Urval Pundarik Rathnakar[2], Ullal Sheetal Dinkar[1*], Kamath Priyanka[1], Tiwary Gaurav[1], Ashok Shenoy Kudgi[1], Revappala Sekhar Nishith[3]

[1]Department of Pharmacology, Kasturba Medical College, Mangaluru, Manipal University, Manipal, INDIA.
[2]Department of Pharmacology, Kanachur Institute of Medical Sciences, Deralakatte, Mangaluru. 575018, INDIA.
[3]Scoop Med Inc, Bengaluru, INDIA.

ABSTRACT

Objective: To evaluate anti-inflammatory activity of bioenhanced turmeric formulation (BCM-95) compared to commercial Curcumin formulation (Curcuminoids 95%) in Carrageenan-induced acute inflammatory model. **Materials and Methods:** Thirty six Wistar rats were divided into six groups-Normal control (2 ml of vehicle), Standard control (Indomethacin 10 mg/kg), 2 doses of BCM 95 (10 and 20 mg/kg) and Curcuminoids 95% (10 and 20 mg/kg). Paw volume was measured using a digital plethysmometer. Vehicle or test drugs were given to rats 30 min before carrageenan administration. Baseline paw volume reading (V_0) was noted just prior to administration of 0.1 ml of 1% carrageenan to right hind paw of the rat. Test paw volume readings (V_t) were measured at 30, 60, 120, 180, 240, 300 and 360 min, after carrageenan injection. Oedema expressed as increased paw volume (v_t-v_0) was noted and percentage inhibition of oedema was calculated for all treatment groups. **Statistical analysis**: Difference between groups were analyzed with ANOVA followed by Tukey test. **Results:** All treatment groups demonstrated significant ($p<0.05$) anti-inflammatory activity (oedema suppression) compared to normal control**.** Anti-inflammatory activity of BCM 95 treated groups were comparable to standard control group except at certain time points, whereas the same activity at all-time points with Curcuminoid 95% treated groups were significantly less than standard control group. Percentage inhibition of paw oedema was maximum with standard control group followed by BCM 95 treated groups followed by Curcuminoid 95% treated groups. **Conclusion:** BCM 95 treated groups showed significant anti-inflammatory activity compared to Curcuminoid 95% treated groups.

Key words: Curcumin, Bioavailability, Inflammation, Anti-Inflammatory agents, Wistar rats.

Corresponding author: Dr. Sheetal D Ullal, Associate professor, Department of Pharmacology, Kasturba Medical College, Mangaluru. 575001, Manipal University, Manipal INDIA.

Phone No: 9448306242

Email: sheetal.ullal@manipal.edu
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INTRODUCTION

Inflammation has become one of the leading areas of global scientific research because it plays a role in virtually all human and animal diseases. Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used group of drugs to contain inflammation.[1] Yet, as a result of adverse effects with these drugs like gastric irritation, nephrotoxicity and cardiovascular concerns with newer selective cyclooxygenase-2 (COX-2) inhibitors, NSAIDs have not been satisfactory in many cases, especially in those conditions that require long term use.[2] Hence, new anti-inflammatory drugs lacking these adverse effects are being researched as alternatives to NSAIDs.

Turmeric and its extracts have been used commonly in traditional Indian medicine as effective anti-inflammatory agents.[3] However, the oral bioavailability of many of these formulations is poor for Curcumin (diferuloylmethane; active principle) and this limits therapeutic use of turmeric formulations.[4,5]

Several turmeric formulations were developed to increase bioavailability of Curcumin. These formulations include nano-formulations, PEGylated formulations and turmeric formulations combined with natural substances like Curcumin-lecithin-piperine.[4]

BCM-95 is bioenhanced turmeric formulation, with higher oral bioavailability for Curcumin.[5] It is a reconstitutedturmeric extract, where Curcumin is reconstituted with non-curcuminoid components. In a study conducted by B. Anthony *et al*, BCM 95 was found to have better bioavailability than normal Curcumin and Curcumin-lecithin-piperine combination formulations in humans.[5]

Curcuminoid 95% is the concentration of Curcuminoid present in commercially available Curcumin formulation.[6]

Hence this study was carried out to evaluate the anti-inflammatory action of BCM-95 in comparison to Curcumin (Curcuminoid 95%) by using carrageenan-induced acute inflammatory model.

MATERIALS AND METHODS

Animals

A total of thirty six Wistar rats (150-200 g) of both sexes were used for the study. These rats were inbred at the Institutional Central Animal House. They had free access to standard commercial rat chow (VRK nutritional solutions) and water *ad libitum*. Proper care was given to animals as per CPCSEA guidelines. All experiments were done between 09.00-17:00 hrs. This study was approved by our Institutional Animal Ethics Committee.

Test and Standard drugs

BCM-95 and Curcuminoid 95% (or) Curcumin (Arjuna Naturals Pvt Ltd.) were the test drugs used in this study. Indomethacin (Tablet. Microcid, Microlabs ltd., Batch no MIAD0032) 10 mg/kg was used as a standard drug. These drugs (test/ standard drugs) were given per orally to Wistar rats using 2% tween 80 (Hi media, Lot no: 0000172935) as vehicle to their respective groups; while normal control group received only 2% Tween 80 as placebo.

Other chemicals

0.1 ml of 1% Carrageenan (Sigma Aldrich, Lot no: 118F0368), which was dissolved in freshly prepared normal saline was injected to the plantar

surface of right hind limb paw of Wistar rats to induce inflammation or oedema.

Instruments used

Digital plethysmometer (Ugo-Basile plethysmometer, Model no: 7140) was used to measure paw volume. Plethysmometer measures paw volume readings by estimating amount of fluid displaced after dipping paw of the animal. Right hind limb paw volume readings of Wistar rats were measured in this study. While taking these readings, right hind limb paw of the rat was dipped only up to the ankle joint.

Study design and treatment

Study design used in this study was prospective experimental design. A total of thirty six Wistar rats were divided into six groups containing six animals each (Normal control, Standard control, 2 doses of BCM 95 and Curcuminoids 95% respectively). All groups received treatment only for one day based on their treatment schedule given in Table 1 and inflammation induced in various groups was evaluated.

Experimental procedure

Drugs according to treatment schedule (Placebo, test or standard drugs), were given to overnight fasted rats 30 min before carrageenan administration, according to treatment schedule of various groups. Baseline paw volume (V_0), was noted just prior to administration of Carrageenan. Rats were injected with 0.1 ml of 1% Carrageenan in 0.9% NaCl intradermally to the plantar surface of right hind paw. Test paw volume readings after Carrageenan administration (V_t) were measured at 30, 60, 120, 180, 240, 300 and 360 min. Each reading (V_0/ V_t) is a mean of three readings.[7,8]

Oedema, expressed as increased paw volume (V_t-V_0) was noted at various time points and percentage inhibition of paw volume were calculated for all treatment groups using this formula.[8]

Statistical analysis

Paw oedema values(V_t-V_0) was expressed as mean ± SD. To compare this parameter between various groups, one-way ANOVA with drug treatment as the independent factor was applied. Post-hoc analysis was performed by applying Tukey test. $p<0.05$ was considered as statistically significant. Data has been analysed using SPSS Version 16.

RESULTS

Paw oedema (V_t-V_0) values of various groups when arranged in descending order, Normal control group was the group with very high paw oedema values followed by curcuminoid 95% treated groups (10 mg/kg, 20 mg/kg) and this was followed by BCM 95 treated groups (10 mg/kg, 20 mg/kg), the values were lowest in Standard control (Indomethacin 10 mg/ kg) at all the time points after treatment with carrageenan (Table 2 and Figure 1).

Percentage inhibition of paw oedema compared to normal control at all-time points were high with standard control group (>80%) followed by BCM 95 groups (>60%) and Curcuminoid 95% groups (< 60%) (Table 2, Figure 2).

DISCUSSION

The injection of carrageenan to the hind paw of rats is a common model to study inflammation. Carrageenan causes an increase in paw volume

Table 1: Treatment Schedule

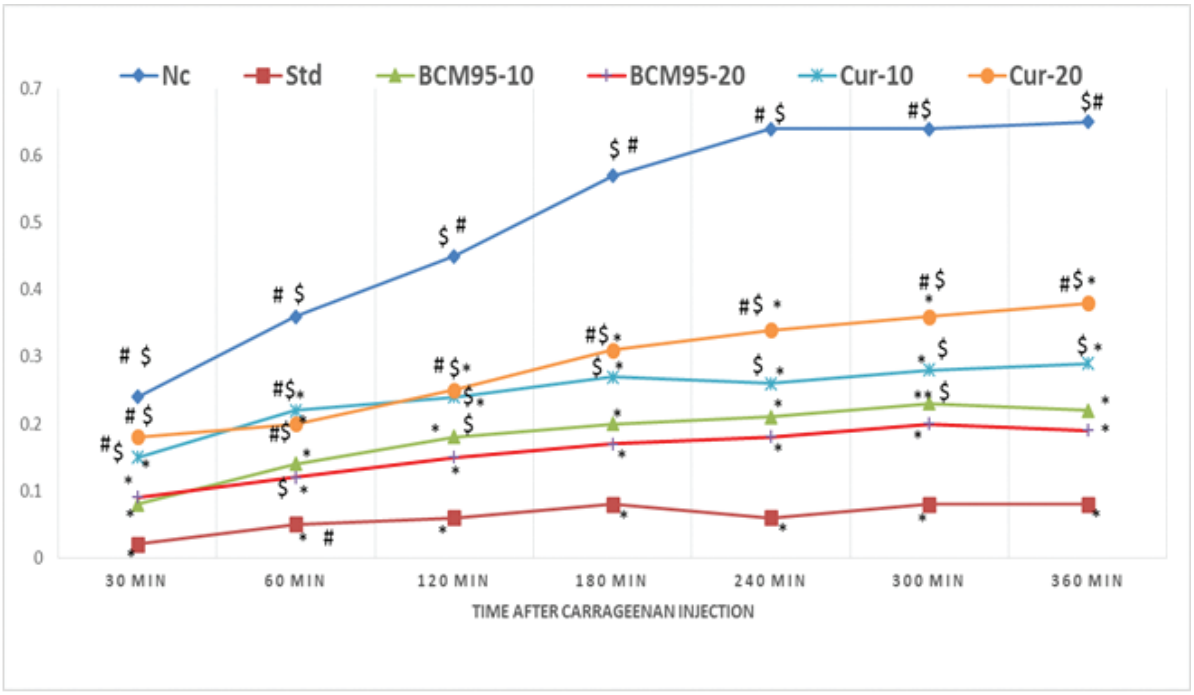
Group number	Group	Treatment	Dose
1	**Normal control (Nc)**	Normal control (2% tween 80-vehicle)	2 ml, per oral
2	**Standard control (Std)**	Indomethacin (Standard control) in 2% tween 80	10 mg/kg, per oral
3	**BCM 95-10 mg/kg (BCM 95- 10)**	BCM 95 in 2% tween 80	10 mg/kg, per oral
4	**BCM 95-20 mg/kg (BCM 95-20)**	BCM 95 in 2% tween 80	20 mg/kg, per oral
5	**Curcuminoid 95%-10 mg/kg (Cur-10)**	Curcuminoid 95% in 2% tween 80	10 mg/kg, per oral
6	**Curcuminoid 95%-20 mg/kg (Cur-20)**	Curcuminoid 95% in 2% tween 80	20 mg/kg, per oral

All drugs were administered using oral gavage tube.

Table 2: Paw Oedema and Percentage inhibition readings

Groups	Oedema or Difference in paw volume in ml [V_t-V_0] (percentage inhibition of oedema compared to normal control)						
	30 min	60 min	120 min	180 min	240 min	300 min	360 min
Nc	0.24 ± 0.06	0.36 ± 0.05	0.45 ± 0.09	0.57 ± 0.12	0.64 ± 0.15	0.64 ± 0.12	0.65 ± 0.11
Std	0.02 ± 0.01 (91.67)	0.05 ± 0.01 (86.11)	0.06 ± 0.02 (86.67)	0.08 ± 0.03 (85.96)	0.06 ± 0.03 (90.63)	0.08 ± 0.04 (87.50)	0.08 ± 0.05 (87.69)
BCM 95-10	0.08 ± 0.04 (66.67)	0.14 ± 0.03 (61.11)	0.18 ± 0.03 (60.00)	0.20 ± 0.04 (65.56)	0.21 ± 0.05 (67.86)	0.23 ± 0.06 (64.06)	0.22 ± 0.06 (66.82)
BCM 95-20	0.09 ± 0.01 (62.50)	0.12 ± 0.04 (66.67)	0.15 ± 0.05 (67.33)	0.17 ± 0.05 (71.58)	0.18 ± 0.06 (71.88)	0.20 ± 0.06 (69.44)	0.19 ± 0.06 (72.18)
Cur-10	0.15 ± 0.08 (37.50)	0.22 ± 0.05 (38.89)	0.24 ± 0.06 (47.60)	0.27 ± 0.08 (54.21)	0.26 ± 0.09 (59.38)	0.28 ± 0.11 (57.38)	0.29 ± 0.12 (57.05)
Cur-20	0.18 ± 0.05 (25.50)	0.20 ± 0.04 (44.44)	0.25 ± 0.07 (45.78)	0.31 ± 0.10 (47.44)	0.34 ± 0.10 (46.88)	0.36 ± 0.07 (45.06)	0.38 ± 0.08 (43.20)

Oedema values were summarized as mean ± SD (Percentage inhibition).



* p< 0.05 compared to Normal control respectively
$ p< 0.05 compared to Standard control
# p< 0.05 compared to BCM 95 20 mg/kg

Figure 1: Paw oedema values ($V_t - V_0$).

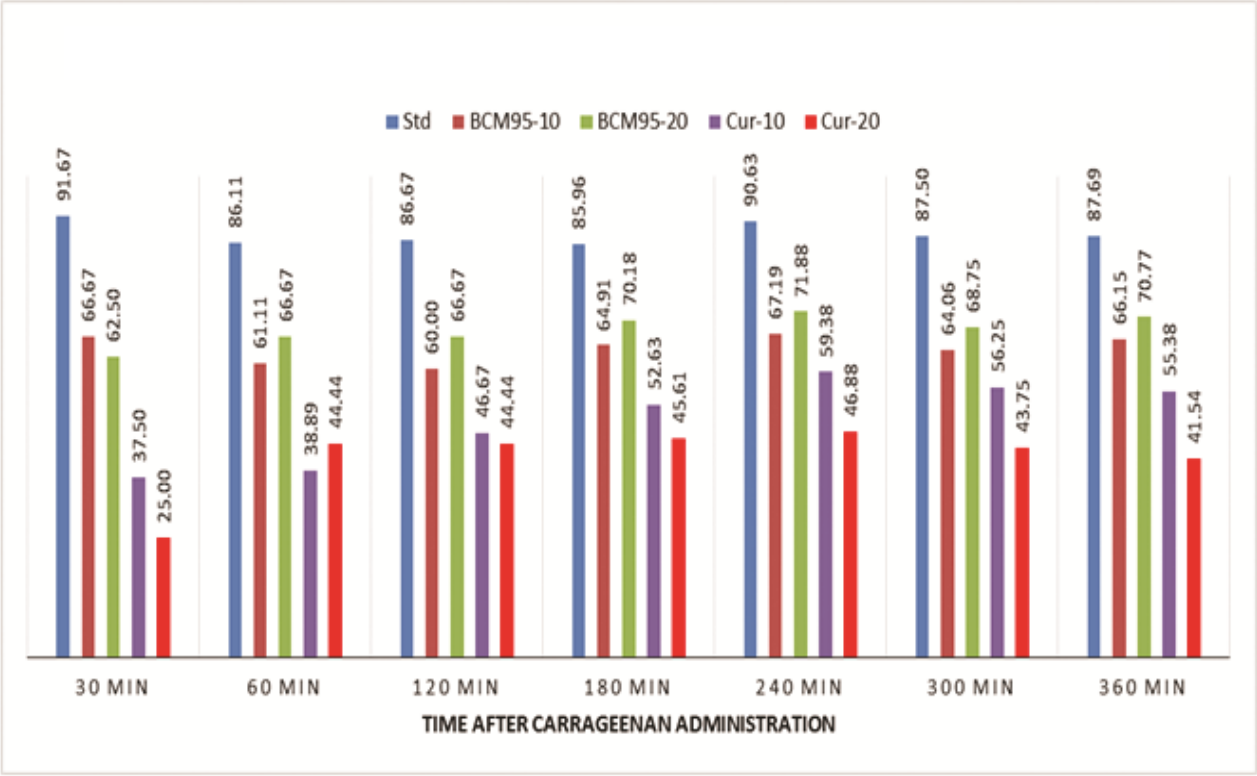


Figure 2: Percentage inhibition of paw oedema compared to Normal control.

by causing oedema. In this model, there is an increase in COX-2 levels, along with an increase in prostaglandin production.[9] Increase in mean paw oedema after carrageenan administration (v_t-v_0) can be correlated to inflammatory activity induced by carrageenan.[7]

All groups treated with active drugs (test/ standard drug) had significantly reduced paw oedema values compared to Normal control group from 60th min after carrageenan administration. All treated groups had significantly lower paw oedemavalues (v_t-v_0) compared to Normal controls from 30th min reading onwards till 360th min reading. However there was no significant difference compared to Normal control group at 30th min reading with Cur-20 group.

All groups treated with test drugs, showed an increased percentage inhibition of paw oedema by at least 25% compared to Normal control group. This finding indicates that all test groups (BCM 95, Curcuminoid 95% treated groups) had significant anti-inflammatory activity. These findings also support previous studies, which stated that turmeric formulations had significant anti-inflammatory activity.[3]According to previous studies, Curcumin has significant anti-inflammatory activity to prevent arthritis in Wistar rats from a dose of 5 mg/kg to 40 mg/kg. Dose of Curcumin used in this study falls in this dose range and had significant anti-inflammatory activity compared to Normal control group.[3]

Curcumin produces its anti-inflammatory activity by down regulating COX-2 enzyme, lipoxygenase enzyme and Inducible nitric oxide synthase (I-NOS) and also by inhibiting production of other inflammatory mediators like tumor necrosis factor alpha (TNFα), Interleukins (IL-1,2, 6, 8 and 12). It also has capacity to decrease chemotaxis.[3]

The anti-inflammatory activity of BCM 95-20 group was comparable to Indomethacin-10 mg/kg (Standard control group) in 6 out of 7 readings after carrageenan administration (significantly different at 60th min reading), whereas anti-inflammatory activity of BCM 95-10 group was comparable to Standard control group at 5 out of 7 readings after carrageenan administration (significantly different at 120 and 300th min reading). But in Curcumin (or Curcuminoid 95%) treated groups anti-inflammatory activity was significantly lower than standard control group in all 7 readings after carrageenan administration (Figure 1).

Percentage inhibition of paw oedema compared to Normal control group, at all-time points of standard control group was greater than 80%. The same percentage inhibition with BCM 95 treated groups were greater than 60%, whereas percentage inhibition in Curcumin treated groups at all-time points were less than 60% (Figure 2).

The above observation suggests that BCM 95 treated groups had more anti-inflammatory activity compared to Curcumin treated groups1.

CONCLUSION

Curcuminoids 95% (Curcumin) and BCM 95 had significant anti-inflammatory activity. The anti-inflammatory activity of BCM 95-20 is comparable to standard control group and it was higher than curcuminoid 95% treated groups.

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CONFLICTS OF INTEREST

The author declare no conflict of interest.

ABBREVIATION USED

BCM-95: Bioenhanced turmeric formulation with increased bioavailability; **Cur:** Curcumin formulation with 95% curcuminoids; **Nc:** Normal control; **Std:** Standard control; **Cox:** Cyclooxygenase enzyme.

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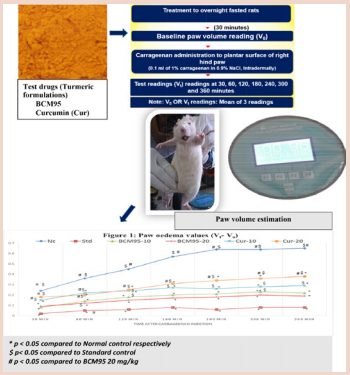
PICTORIAL ABSTRACT



SUMMARY

- Study aimed at evaluating anti-inflammatory activity of bioenhanced turmeric formulation with increased bioavailability (BCM-95) compared to commercial Curcumin formulation (Curcuminoids 95%) in Carrageenan-induced acute inflammatory model.
- The test drugs used were BCM-95 (10 and 20 mg/kg) and Curcuminoids 95% (10 and 20 mg/kg), standard control group received Indomethacin 10 mg/kg.
- BCM-95 treated groups showed significant anti-inflammatory activity compared to Curcuminoid 95% treated groups; Anti-inflammatory activity of BCM95 treated groups were comparable to standard control group except at certain time points, whereas the activity at all-time points with Curcuminoid 95% treated groups were significantly less than standard control group.
- All test groups significantly reduced oedema compared to normal control groups

ABOUT AUTHORS

Dr Vinaykumar Sayeli: Is a final year post-graduate student and tutor in Department of Pharmacology, Kasturba Medical College, Mangalore. His research work is mainly focused in fields of inflammation, diabetes, cancer and pharmacovigilance.

Dr Sheetal D Ullal: Completed her MD in Pharmacology from Manipal University in 2002. Presently, she is an Associate professor in the Department of Pharmacology, Kasturba Medical College, Mangalore, Manipal University. Herareas of interest are preclinical research in inflammation and diabetes, medical education and bioethics.