

**ANALGESIC ACTIVITY STUDIES WITH A POLYHERBAL FORMULATION
CONTAINING PLANT PARTS OF *ALLIUM CEPA*, *ALLIUM SATIVUM* AND *CURCUMA
ZEDOARIA***

Akmal Hosain Nipu, Sahara Akter and Mohammed Rahmatullah*

Department of Pharmacy, University of Development Alternative, Lalmatia, Dhaka-1207, Bangladesh.

*Corresponding Author: Prof. Dr. Mohammed Rahmatullah

Department of Pharmacy, University of Development Alternative, Lalmatia, Dhaka-1207, Bangladesh.

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ABSTRACT

A polyherbal formulation (MEAAC) was prepared by combining methanol extracts of bulbs, cloves and rhizomes, respectively of *Allium cepa*, *Allium sativum* and *Curcuma zedoaria* in a ratio of 1:1:1 (w/w). In acetic acid induced writhing tests in mice, MEAAC at doses of 50, 100, 200 and 400 mg per kg body weight reduced the number of writhings, respectively, by 34.6, 42.3, 53.8, and 65.4% versus the 50.0% reduction observed with a standard analgesic drug, aspirin, at a dose of 400 mg per kg. Individual extract of *A. cepa*, *A. sativum*, and *C. zedoaria*, at a dose of 400 mg per kg, respectively, reduced the number of writhings by 46.2, 42.3, and 50.0%. Thus the three different extract in combination (MEAAC) demonstrated synergistic analgesic activity, which activity at MEAAC dose of 400 mg per kg was greater than aspirin. MEAAC may thus be considered a potential source to obtain relief from pain.

KEYWORDS: Analgesic, *Allium cepa*, *Allium sativum*, *Curcuma zedoaria*.**INTRODUCTION**

Pain is an unpleasant sensation triggered in the nervous system and possibly is the most common problem faced by human beings. Pain can arise from a simple cause like bumping against something hard or pinching a finger in which case the pain may be acute or pain may be chronic and arise from diseases like arthritis or cancer. Over the counter drugs like aspirin or paracetamol are available for pain. However, the first drug, if taken for long time periods may cause gastric ulceration and thinning of blood, while the second when taken for long time periods or over dosage can cause hepatic damage.

The rural people of Bangladesh commonly suffer from pain because of the hard labor necessary for cultivation and harvesting. This may need analgesic or pain killing medications to be taken on a daily basis. Any analgesic drug or formulation that has less or no side effect can be a boon to people who suffer from pain on a continuous basis. We had been focusing our efforts to find out new sources of plant-based formulations for treatment of pain, which plants or formulations are readily available and affordable to the rural and urban poor people.^[1-7]

Allium cepa (onion, local name peyaj) and *Allium sativum* (garlic, local name roshun) are frequently used spices cultivated in Bangladesh. *Curcuma zedoaria*, known in English as white turmeric and locally as shoti, can be commonly seen growing in the country in the

wild. The rhizomes of the plant, besides being considered medicinal, are also dried, powdered and boiled in water to feed children as a nutritive food. The analgesic and anti-inflammatory effects of fresh onion juice in mice has been reported.^[8] The analgesic and anti-inflammatory effects of garlic powder in animal models have also been described.^[9] Methanol extract of rhizomes of *C. zedoaria* reportedly demonstrated mild analgesic activity.^[10] Under the circumstances, it was of interest to evaluate a polyherbal formulation containing extracts of bulb, clove and rhizome of the three plants (*A. cepa*, *A. sativum*, and *C. zedoaria*, respectively) for its analgesic activity, for this can lead to an easily available and affordable formulation for treatment of pain.

MATERIALS AND METHODS**Plant material collection**

Allium cepa bulbs and *Allium sativum* cloves were collected from a local market in Dhaka city in August 2016. *Curcuma zedoaria* rhizomes were collected from Gazipur district, Bangladesh in August 2016.

Preparation of methanolic extract of the various plant parts

Bulbs, cloves and rhizomes were separately sliced, dried in the shade and pulverized to a fine powder. The various powders were extracted separately with methanol (100g powder each extracted with 500 ml methanol) for 48 hours at ambient temperature with constant stirring. The

extracts were filtered and the filtrate collected and methanol dried of at 40°C. After completely drying off any residual methanol, extracts were maintained at -20°C till use (typically within 96 hours). Extracts were dissolved in Tween 20 prior to administration to mice by gavage. Methanol extracts of *A. cepa*, *A. sativum* and *C. zedoaria* are, respectively, referred to as MEAC, MEAS, and MECZ in this manuscript. The combined extract (MEAAC) of the three extracts contained individual extracts (MEAC, MEAS, and MECZ) in an equal weight by weight ratio. This means that when MEAAC was used at a dose of 50 mg per kg body weight, the actual weight was 150 mg and contained 50 mg each of MEAC, MEAS, and MECZ. Combination of the three extracts was done following dissolving in Tween 20 and administration to mice.

Chemicals and Drugs

Aspirin was obtained from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals were of analytical grade.

Animals

Swiss albino mice, which weighed between 11-15g were used in the present study. The animals were obtained from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). The animals were acclimatized for three days prior to actual experiments. The study was conducted following approval by the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh in accordance with "Principles of Laboratory Animal Care".

Analgesic activity evaluation through abdominal writhing test

Analgesic activity of methanolic extract of boiled bulbs, cloves rhizomes, and combination (MEAC, MEAS, MECZ, MEAAC)) was examined as previously described.^[11] Mice were divided into nine groups of five mice each. Group 1 served as control and was administered vehicle only. Group 2 was orally administered the standard analgesic drug aspirin at a dose of 400 mg per kg body weight, respectively. Groups 3-6 were administered MEAAC at doses of 50, 100, 200 and 400 mg per kg body weight, respectively. Groups 7, 8 and 9 were administered, respectively, MEAC, MEAS, and MECZ at a dose of 400 mg per kg body weight. Following a period of 60 minutes after oral administration of standard drug or MEAC, MEAS, MECZ, MEAAC, all mice were intraperitoneally injected with 1% acetic acid at a dose of 10 ml per kg body weight. A period of 15 minutes was given to each animal to ensure bioavailability and onset of chemically

induced irritation of acetic acid after a slight modification of procedure described earlier,^[5] following which period, the number of abdominal constrictions (writhings) was counted for 10 min. The percent inhibitions of abdominal constrictions were calculated according to the formula given below.

$$\text{Percent inhibition} = (1 - W_e/W_c) \times 100$$

where W_e and W_c represents the number of writhings in aspirin or MEAC, MEAS, MECZ, MEAAC administered mice (Groups 2-9), and control mice (Group 1), respectively.

Phytochemical analysis

Preliminary phytochemical analysis of MEAC, MEAS, and MECZ for presence of saponins, tannins, alkaloids, and flavonoids were conducted as described before.^[12]

Statistical analysis

Experimental values are expressed as mean \pm SEM. Independent Sample t-test was carried out for statistical comparison. Statistical significance was considered to be indicated by a p value < 0.05 in all cases.^[6]

RESULTS AND DISCUSSION

Phytochemical analysis of MEAC, MEAS, and MECZ

Preliminary phytochemical analysis of crude extract of *A. cepa* (MEAC) revealed the presence of tannins and flavonoids; analysis of crude extract of *A. sativum* (MEAS) showed that flavonoids are present, while analysis of crude extract of *C. zedoaria* showed the presence of alkaloids and flavonoids. Thus flavonoids seemed to be a common group of phytochemicals present in all three extracts.

Analgesic activity results

In acetic acid induced writhing tests in mice, MEAAC at doses of 50, 100, 200 and 400 mg per kg body weight reduced the number of writhings, respectively, by 34.6, 42.3, 53.8, and 65.4% versus the 50.0% reduction observed with a standard analgesic drug, aspirin, at a dose of 400 mg per kg. Individual extract of *A. cepa* (MEAC), *A. sativum* (MEAS), and *C. zedoaria* (MECZ), at a dose of 400 mg per kg, respectively, reduced the number of writhings by 46.2, 42.3, and 50.0%. The results are shown in Table 1. A synergistic analgesic action was noted with MEAAC (combination of MEAC, MEAS, and MECZ), which was much higher than aspirin or individual extracts, suggesting that the extracts in combination can be a potent means for obtaining relief from pain.

Table. 1: Analgesic effect of MEAC, MEAS, MECZ and MEAAC in acetic acid-induced pain model mice.

Treatment	Dose (mg/kg body weight)	Mean number of abdominal constrictions	% inhibition
Control	10 ml	5.2 ± 0.20	-
Aspirin	400 mg	2.6 ± 0.24	50.0*
(MEAAC)	50 mg	3.4 ± 0.51	34.6*
(MEAAC)	100 mg	3.0 ± 0.32	42.3*
(MEAAC)	200 mg	2.4 ± 0.51	53.8*
(MEAAC)	400 mg	1.8 ± 0.37	65.4*
(MEAC)	400 mg	2.8 ± 0.20	46.2*
(MEAS)	400 mg	3.0 ± 0.32	42.3*
(MECZ)	400 mg	2.6 ± 0.51	50.0*

All administrations (aspirin and extract) were made orally. Values represented as mean ± SEM, (n=5); **P* < 0.05; significant compared to control.

Preliminary phytochemical analysis showed the presence of alkaloids, flavonoids, and tannins in the extracts. While the exact identification of the responsible bioactive component(s) awaits further studies, the three groups of phytochemicals, both individually and in various combinations have been reported to produce analgesic effects. The analgesic action of Ephedra herb has been attributed to its alkaloids.^[13] Flavonoids like quercetin and derivatives have proved useful in pain management.^[14] Tannins have been implicated among other phytochemicals behind the analgesic effects of *Dalbergia saxatilis*.^[15] A combination of alkaloids, flavonoids and tannins has been hypothesized to be responsible for the analgesic effects shown by methanol extract of root of *Schoenoplectus grossus*.^[16]

CONCLUSION

Since the polyherbal formulation (MEAAC) contains extracts from plant parts of three very common and affordable plants of Bangladesh, the results suggest that the combination of the three extracts, MEAC, MEAS and MECZ can be useful for alleviating pain. This can prove beneficial to the rural and low income group population of Bangladesh and elsewhere.

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The authors declare that they have no conflicts of interest.

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