

Anti asthmatic effect of *Momordica Charantia* and its comparison with montelukast an in vitro and in vivo model

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ABSTRACT

Background: *Momordica charantia* (MC) (bitter gourd) have shown the inhibition of NF- κ B and Leukotrienes expression in many inflammatory pathological conditions. Based on its anti-inflammatory action this study aimed to identify MC fruit dry powder (MCp) and MC fresh juice (MCj) action on airway inflammation in Guinea Pig model and also to find out the presence of alkaloids and flavonoides.

Methods: 18 adult Guinea pigs of both sexes (excluding mating animals) were randomly divided into three groups with six animals in each namely Montelukast, MCj and MCp groups respectively. They were given with OD oral administration of concerned drugs for 1-7 days. 6 control animals were exposed to 2% histamine aerosol for 120 seconds using histamine chamber prior to treatment with Montelukast, MCj and MCp to observe the normal Pre-Convulsive Dyspnea (PCD) in seconds. After six hours of daily OD oral dose of test drugs administration, all the groups were exposed to 2% Histamine aerosol one by one for 120 sec on day1 & 7 to observe PCD. The MCj and MCp were also tested with chemical assay, TLC to confirm the presence of alkaloids, flavonoides.

Results: ON DAY 1 and 7: MCj and MCp showed significant decrease in PCD occurrence and it is statistically significant compared to the control. PCD blocking action of MCj group was effective in DAY 1. TLC and Chemical Assay were not supportive for bronchodilator action.

Conclusions: MCj and MCp have promising preventive role in asthma. This study had expressed the positive protective role of *Momordica charantia* in asthmatic condition based on its known anti-inflammatory action.

Keywords: Guinea pig, *Momordica charantia*, Pre-convulsive dyspnea

INTRODUCTION

Asthma is a controllable chronic inflammatory disorder of the airways with high difficulty to cure completely.¹ Long term use of anti-inflammatory drugs is the treatment of chronic asthma. Most potent and consistent anti-inflammatory agent available is Corticosteroids and it's not free of adverse effects even in the inhaled form.

As a result, there is continuous search for an alternative especially from natural sources. *Momordica Charantia* (bitter gourd), a common edible vegetable, is known traditionally to possess medicinal values.^{2,3} Many studies

had confirmed the anti-inflammatory property of *Momordica Charantia* with cytokine inhibiting activity.^{4,5} It inhibited the expression of inflammatory genes like IL-1, IL-2, IL-6, IL-8, TNF- α and Matrix Metallo Proteinases (MMPs) in animal studies.⁶ Like Montelukast a Leukotrienes receptor antagonist used in asthma, *Momordica Charantia* fruit also inhibits NF- κ B expression.⁷

Very few animal studies were done on its anti-inflammatory property utilizing the prostaglandins and inflammatory cytokine inhibition by *Momordica Charantia*. So far, no study has been done to prove its anti-inflammatory effect in asthma. Hence this study has

aimed to understand the bronchodilator property of *Momordica Charantia* as an agent to reduce airway inflammation in a whole animal model using Montelukast as a comparative standard.

METHODS

Method I: Animal study-histamine induced bronchospasm in guinea pig model

Animal which used for the study were adult guinea pigs with weight 400-700gram of both sex excluding animals used for breeding.

Period of the study was 15 days to 2 months including animal selection.

Total number of animals were 18 as 3 groups with 6 animals in each.

Study parameters were PCD (Pre-convulsive Dyspnea) per 120 seconds.

Histamine chamber with nebulizer instrument was used with standard technique to apply.

Momordica fruit dry powder and fresh juice

Momordica Charantia was bought from local farm after getting botanical certification. Then it was used to prepare fresh juice and dry powder after removing seeds.

Fresh juice

Fresh juice was prepared by using fresh 500gram of *M. Charantia* unripe fruits. Then it was cut into small pieces after removal of the seeds. These fruits were crushed with a commercial blender and juice was obtained after filtration through soft muslin cloth.

Dry powder

500gram of *M. Charantia* unripe fruits were cut into small pieces after removal of the seeds. These pieces were allowed to dry in hot air oven at 60 degrees for 24 hrs.⁸ Once the fruit pieces were dried enough, they were made into fine powder using blender. Powder thus prepared was passed through a sieve and fine particles was obtained and stored in air tight containers.

IAEC

The animals were maintained at ambient temperature $25\pm 2^{\circ}\text{C}$ and 55% relative humidity. The Institutional Animal Ethics Committee of PSG and IMSR approved the study and the study was conducted strictly with guidelines of ICMR and CPCSEA. 6 animals out of 18, randomly selected to observe the control values for PCD per 120 seconds. Control was exposed to 2% Histamine

aerosol for 120 seconds.⁷ These values were compared with Montelukast, MCp (*Momordica Charantia* dry powder) and MCj (*Momordica Charantia* fresh juice) exposure to understand the protective effect on histamine induced Bronchospasm.

If any animals showed severe bronchospasm in any of these groups were immediately exposed to normal air and Inhalational corticosteroid + salbutamol aerosol using nebulizer to revert the histamine induced bronchospasm. After completion of the study, all the animals were observed for a period of 1 week in their own environment with the normal water, food for any reactions.

Method II

A. Chemical analysis for alkaloid material⁹

Detection of alkaloids

Dry powder and Fresh Juice were dissolved individually in dilute Hydrochloric acid was heated for 30 min, allowed to cool and filtered.

- Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide).
- Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide).
- Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide).
- Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution).
- Marquis test was performed with both dry powder and fresh juice.¹⁰

1 drop reagent made up of 8-10 drops (approx. 0.25ml) of 37% formaldehyde solution to 10 ml of glacial acetic acid. Then added 3 drops of concentrated sulfuric acid

Results are present in Table 4.

- Chen-Kao Test: Test for ephedrine and pseudoephedrine.¹⁰
 - Reagent 1: Added 1 ml of glacial acetic acid to 100 ml of water (=1% (v/v) aqueous acetic acid solution).
 - Reagent 2: Dissolved 1 g of copper (II) sulphate in 100 ml of water (=1% (w/v) aqueous CuSO₄ solution).
 - Reagent 3: Dissolved 8 g of sodium hydroxide in 100 ml of water (=2N aqueous sodium hydroxide solution).

Procedure

Placed a small amount (1-2 mg of powder, or 1-2 drops of a liquid) of the suspected material in a depression on a spot plate.

- Added 2 drops of Reagent 1.
- Added 2 drops of Reagent 2 and then added 2 drops of Reagent 3 and stirred. Results were shown in Table 5.

B. Thin layer chromatography¹¹

MC Dry powder and fresh juice were subjected to thin layer chromatography (TLC) with silica gel F254 plates using Chloroform-Methanol solvent was used and the extract showed spots on Merck silica gel (MSG) pre-coated TLC plate of thickness of about 0.2mm.

The plates were saturated in chromatography tank and spots were detected following drying of the solvent. Band of separated fractions was detected under UV light. The retention fraction (Rf) of each fraction or band was calculated and recorded.

Statistical analysis

Analysis was done by comparing the before and after values, and also between groups student t- test was done for comparing before and after values, whereas ANOVA was used in comparing between all three groups. Analysis was done using SPSS version 19.

RESULTS

On day 1

MCj and MCp were showed significant decrease in PCD occurrence and it was statistically significant compared to the control. MCj group PCD blocking action was significant, when all the three groups were compared (Table 1, 2 and 3).

Table 1: Mean±SD of PCD in all three groups in percentage.

Montelukast	PCD (120 sec) in % Mean±SD
Control	61.10±9.74
Day 1	79.16±16.21
Day 7	87.35±21.16
Momordica dry powder (MCp)	
Control	61.10±9.74
Day 1	93.47±12.36
Day 7	93.47±10.52
Momordica fresh juice (MCj)	
Control	61.10±9.74
Day 1	73.46±16.48
Day 7	100.00±0.00

On day 7

MCj and MCp showed significant decrease in PCD occurrence statistically, compared to the control. TLC

and chemical assay were not supportive for bronchodilator action (Table 1, 2, 3).

Table 2: P value of PCD in all the three groups.

	Between groups	't' value	P value
PCD (Pre convulsive dyspnoea)			
Montelukast	Control and day 1	2.225	0.077
	Control and day 7	2.521	0.053
	Day 1 and day 7	0.955	0.383
MC powder	Control and day 1	4.187	0.009
	Control and day 7	5.194	0.003
	Day 1 and day 7	0.000	1.000
MC juice	Control and day 1	2.733	0.041
	Control and day 7	7.950	0.001
	Day 1 and day 7	3.176	0.034

Table 3: Anova difference between all 3 groups.

PCD		
Time period	Between group	Significance
Day 1	MCj is significant over MCp	P =0.037
Day 7	No Significance between all 3 groups	P >0.05

Table 4: Chemical analysis.

Test for Alkaloids	Dry powder	Fresh juice
1. Mayer's test	Positive	Positive
2. Wagner's test	Positive	Positive
3. Dragendroff's test	Negative	Negative
4. Hager's test	Positive	Positive

Table 5: Test for Ephedrine- Marquis test.

Compound	Positive color reaction	Dry powder	Fresh juice
Ephedrine	Pale yellow	-	-
Pseudoephedrine	Pale yellow (greenish)	-	-
Norephedrine	Pale yellow (brownish)	-	-
Norpseudoephedrine	Yellow (brownish)	-	-
Norpseudoephedrine	Yellow (brownish)	-	-
N-methylephedrine	Brownish red	-	+
Chlorpseudoephedrine	no change	-	-

- Chemical tests: Test for alkaloid and ephedrine were revealed in tabulation (Table 4, 5).
- Chen-Kao test: Test for ephedrine and pseudoephedrine¹¹ - Both dry powder and fresh juice didn't show violet color positive reaction.

Thin layer chromatography¹¹

- Solvent System: Chloroform-Methanol (9:1)
Detection at UV: 366nm.
- Color of bands: Reddish spots

Results were shown in the Table 6 and Table 7.

Table 6: Thin layer chromatography.

Formulation	No. of spots	Rf value
Dry powder (MCp)	1	0.84
Fresh juice (MCj)	2	0.51
		0.64

Table 7: TLC of MCj and MCp with atropine and ephedrine.

Formulation	No. of spots	Rf value
Atropine	3	0.1
		0.54
		0.6
Ephedrine	1	0.56
Dry powder (MCp)	1	0.56
Fresh juice (MCj)	5	0.1
		0.54
		0.6
		0.68
		0.76



Figure 1: Thin layer chromatography of MCp and MCj.

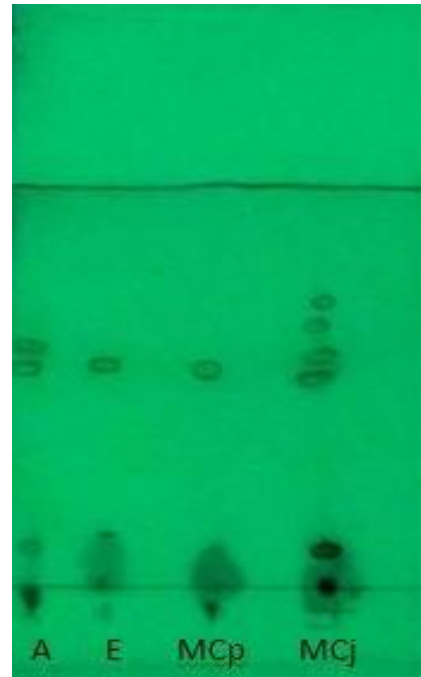


Figure 2: Solvent system: Toluene: Chloroform: Ethanol (28.5:57:14.5) Detection at UV: 254nm expressed the presence of A-atropine, E- ephedrine, MCp, MCj.

DISCUSSION

Pre convulsive dyspnea

The mean of the control group was found to be 61.10 ± 9.74 and comparison done for each group with before and after values using student t- test (Table 1).

Montelukast Group compared with the baseline Day 1 control values, the mean was found to be 79.16 ± 16.21 which was more than the control mean of 61.1%. But it was not statistically significant with P value of 0.077. Day 7 values of Montelukast, the mean was found to be 87.35 ± 21.16 and was not statistically significant with P value of 0.053 even though the average PCD was increased from baseline control value on both day 1 and day 7.

This study also compared the difference between PCD on Day 1 and Day 7. The mean PCD was found to be 79.16 ± 16.21 on Day 1 against 87.03 ± 21.16 on day 7. This was also not statistically significant even though there is increase in average value.

MCp group compared for the baseline control value with Day 1 values, mean was found to be 93.47 ± 12.36 which was more than the control mean of 61.1%. Also this difference in PCD was statistically significant with P value of 0.009 which showed the dry powder was effective in delaying PCD. MCp group were compared for Day 7 values, the mean was found to be 93.47 ± 10.52 . This too was statistically significant with P value of 0.003

which proved that the dry powder of *Momordica Charantia* was effective in delaying PCD in Histamine exposed guinea pigs significantly.

MCp group's were compared for the difference between PCD on Day 1 and Day 7. The mean PCD was found to be 93.47 ± 12.36 on Day 1 against 93.47 ± 10.52 on day 7. This was not statistically significant with P value of 1.000 as there was not much difference in the average value of PCD.

MCj group were compared the baseline Day 1 control value the mean was found to be 73.46 ± 16.42 which was more than the control mean of 61.1%. Also this difference in PCD was statistically significant with P value of 0.041 which shows Fresh juice was effective in delaying PCD. MCj group compared for the Day 7 values, the mean was found to be 100.00 ± 0.00 . This too was statistically significant with P value of 0.001. This proved that the fresh juice of *Momordica Charantia* was significantly effective in delaying PCD in Histamine exposed guinea pigs.

MCj group compared for the difference between PCD on Day 1 and Day 7, the mean PCD was found to be 73.46 ± 16.42 on Day 1 against 100.00 ± 0.00 on day 7. This was statistically significant with P value of 0.034 which justifies *Momordica* was effective in both short term and long-term management.

Further analysis of both study groups of *Momordica* dry powder and fresh juice with student t-test. On day 1 in dry powder group the mean was found to be 93.47 ± 12.36 and in fresh juice group it was found to be 73.46 ± 16.42 . This was statistically significant with P value of 0.038; which showed the dry powder was better on Day 1 of treatment with quick response. On day 7 in dry powder group the mean was found to be 93.47 ± 10.52 whereas in fresh juice group it was 100.00 ± 0.00 . This was not statistically significant as both groups had similar protective effect on day 7. The average was more in fresh juice group compared to the dry powder group on day 7.

When ANOVA was used to analyze the difference between the groups, (Table 2) on Day 1, MCp was significantly better than MCj with P value of 0.037 whereas it was not statistically better than Montelukast. On day 7, there was no statistical significance between all 3 groups since all were effective in abolishing PCD occurrence.

These results showed that, there was a significant improvement in both groups of *Momordica* after starting treatment, whereas there was no much difference between groups. It's a pilot study and each group strength was six may be the limitation of this study.

Momordica charantia had been extensively studied for various metabolic, antihypertensive and its role in Diabetes with its anti-inflammatory background and not

much significant reference for the direct effect of MC on smooth muscle activity.¹²⁻¹⁵

CONCLUSION

This study had expressed the positive protective role of *Momordica charantia* in asthmatic condition based on its known anti-inflammatory action. Further, MC both dry powder and fresh juice protecting role on histamine aerosol induced bronchospasm as an anti asthmatic action may be explored and proved in clinical situation using suitable models to ensure the usefulness and effectiveness in chronic asthma treatment compared to standard care of management in future.

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