

# Lack of alpha amylase inhibitory activity of monoammonium glycyrrhizinate

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## Abstract

**Introduction:** Monoammonium glycyrrhizinate (MAG) is used as a sweetener and also as a flavoring agent to enhance the flavor of dairy products, confectioneries, baked foods and beverages. It is used in cough drops, cough syrups and cosmetics. It is reported to have antioxidant, antitussive and antiviral activities. **Materials and Methods:** The *in-vitro*  $\alpha$  - amylase inhibitory activity was performed using 3,5-dinitrosalicylic acid assay. Different concentrations (1  $\mu\text{g/ml}$ , 3  $\mu\text{g/ml}$ , 5  $\mu\text{g/ml}$ , 10  $\mu\text{g/ml}$ , 30  $\mu\text{g/ml}$  and 50  $\mu\text{g/ml}$ ) of MAG were analyzed for the  $\alpha$  - amylase inhibitory potential. **Results:** The results of this study revealed that MAG lacks  $\alpha$  - amylase inhibitory potential. **Conclusion:** Documentation of pharmacological activities of phytochemicals, which have either positive or negative outcome, is required to create a database for phytopharmacological research. The result of this study can serve as one of the components of phytochemical research database.

**Key words:** add-on therapy, enzyme inhibitors, *Glycyrrhiza glabra*, monoammonium glycyrrhizinate

## INTRODUCTION

Monoammonium glycyrrhizinate (MAG) is an aglycone of glycyrrhizin obtained from liquorice (*Glycyrrhiza glabra*, *Leguminosae*). In Chinese traditional medicine, *Glycyrrhiza* root is used as a source of glycyrrhizin. Glycyrrhizin is also called glycyrrhizic acid, which is 50-60 times sweeter than sucrose.<sup>[1]</sup> It is used as a flavoring agent in dairy products, confectioneries, baked foods and beverages.<sup>[2]</sup> It is also used in the cough drops, cough syrups and cosmetics.<sup>[3]</sup> It is chemically glycyrrhizinic acid monoammonium salt which after oral administration is hydrolyzed by the glucuronidase of the intestinal bacteria to an active principle aglycone, 18 $\beta$  - glycyrrhetic acid and then absorbed into the blood.<sup>[4]</sup> It is reported to have hepatoprotective, antiulcer, antioxidant, antitussive, antiviral and cytotoxic activities.<sup>[5-14]</sup>

In addition, it is also used in toothpastes for treatment of dental plaques.<sup>[15]</sup> If the natural sweetening agents have additional pharmacological activities, they can be beneficial to diabetic patients because they can be used as add-on therapy. Currently, many of the phytochemicals have been proven to have inhibitory activity on several enzyme systems, which are serving as tools for the therapeutic management of diabetes.<sup>[16]</sup> In view of this, the present study was carried out to evaluate  $\alpha$  - amylase inhibitory activity of MAG.

## MATERIALS AND METHODS

### Materials

Starch, 3,5-dinitrosalicylic acid, sodium potassium tartrate, sodium hydroxide, sodium dihydrogen phosphate, sodium chloride and  $\alpha$  - amylase were purchased from HiMedia (Mumbai). Acarbose was purchased from Sigma Aldrich, Bangalore.

### Methodology

Different concentrations (1  $\mu\text{g/ml}$ , 3  $\mu\text{g/ml}$ , 5  $\mu\text{g/ml}$ , 10  $\mu\text{g/ml}$ , 30  $\mu\text{g/ml}$  and 50  $\mu\text{g/ml}$ ) of MAG were prepared with phosphate buffer. All samples were prepared in

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triplicate. To 0.2 ml of the each dilution, 0.4 ml of enzyme solution containing 10 mg of  $\alpha$  - amylase in 100 ml of phosphate buffer pH 6.9 (20 mM sodium dihydrogen phosphate containing 6.7 mM of sodium chloride) was added. To the above solution, 0.2 ml of buffer was added and the solution was incubated for 20 min. Later on, starch solution (1% w/w in water) was prepared by heating on water bath for 15 min and then 0.2 ml of this starch solution was added to the above solution. Then, 1ml of DNS reagent [3,5-dinitrosalicylic acid (1.5%), sodium potassium tartrate (12%) and sodium hydroxide 0.4 M in 100 ml distilled water] was added and the solution was boiled for 5 min and cooled under running tap water. The absorbance of the samples was measured at 540 nm using a spectrophotometer (Schimadzu UV-1800).

Control values were recorded replacing MAG with vehicle. The results were expressed as % inhibition calculated using the formula.

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

The  $IC_{50}$  values were determined from plots of percentage inhibition versus concentration. The total experiment was performed in triplicate.<sup>[17]</sup>

## RESULTS

The percentage inhibition of  $\alpha$  - amylase by acarbose and MAG is shown in Table 1. The  $IC_{50}$  value of acarbose was found to be 59.63  $\mu\text{g/ml}$  and that of MAG was found to be 80.51  $\mu\text{g/ml}$ . The  $IC_{50}$  value of MAG indicates that it doesn't have  $\alpha$  - amylase inhibitory activity.

**Table 1:  $\alpha$ -Amylase inhibitory activity of acarbose and monoammonium glycyrrhizinate ( $n=3$ )**

Sample	Concentration ( $\mu\text{g/ml}$ )	% Inhibition	$IC_{50}$ ( $\mu\text{g/ml}$ )
Acarbose	1	11.16	59.63
	3	18.14	
	5	24.41	
	10	30.04	
	30	35.22	
	50	40.26	
	100	72.93	
Monoammonium glycyrrhizinate	1	21.34	80.51
	3	24.12	
	5	33.36	
	10	38.94	
	30	41.83	
	50	46.01	
	100	51.07	

## CONCLUSION

The results indicate that MAG lacks  $\alpha$  - amylase inhibitory activity and this data can be used as one component of phytopharmacological database.

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