

## Original Research Article

# In vitro Study on the Effects of Garlic and Mustard Extracts on Antioxidative Enzymes in Mouse Stomach

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## ABSTRACT

### Keywords

Garlic and mustard extracts, Antioxidative enzymes, Nitric oxide synthase-Superoxide dismutase

Garlic and mustard are found to contain organosulphur compounds which modulate the activities of antioxidative enzymes, such as nitric oxide synthase (NOS), superoxide dismutase (SOD) and glutathione-s-transferase (GST). Thus Garlic and mustard can boost immunity in an individual by reducing the risk of infection, inflammation and tumor formation by arresting reactive oxygen species. NOS, SOD and GST enzyme's activities were found to increase, both in presence of garlic and mustard extracts alone and in combination. NOS and SOD gave maximum increase in activity in presence of garlic and mustard extracts in combination, that is 164.92% and 140.31% respectively, whereas GST gave maximum increase in activity in presence of mustard extract alone, that is 392.50%.

## Introduction

Medicinal properties of Indian spices have been widely known and used since ancient times as they possess multiple beneficial effects such as antimicrobial, anti-inflammatory, anti-tumor and many more and thus help boost overall immunity of the body. These beneficial effects are accomplished by modifying the activity of important cellular enzymes by the bioactive principles of Indian spices. Garlic and mustard are two such important spices which contain potent organosulphur compounds that can potentially reduce the risk of microbial infection, inflammation, tumor growth (Hasler, 1998) etc. by modulating the activities of phase II

biotransformation enzyme glutathione-s-transferase (GST), cellular signalling enzyme nitric oxide synthase (NOS) and antioxidant enzyme superoxide dismutase (SOD) (Ibrahim, *et al*, October 2008).

The main purpose of this study was to assess the in vitro level and rate of activities of GST, NOS and SOD enzymes in presence and absence of garlic and mustard extract, both individually and in combination in stomach tissues obtained from mice under normal condition.

GSTs play an important physiological role in initiating and promoting the

detoxification of xenobiotic compounds by catalyzing the conjugation of glutathione via a sulfhydryl group to electrophilic centers on a variety of substances making the compounds more soluble which can be further metabolized and are excreted from the body (Kou, *et al*, 2013). Nitric oxide synthase catalyzes the production of NO from L-Arginine. NO has many biological functions in the human body which includes regulation of blood flow, neurotransmission and mediated cytotoxicity in microbes and tumor cells (Kanwar, *et al*, 2009). Superoxide dismutase enzymes (SOD) physiological function is to provide a defence against the potentially damaging activities of the superoxide radical by catalyzing their dismutation into oxygen and hydrogen peroxide and decomposition of hydrogen peroxide to water and molecular oxygen and thus acting as an important antioxidant defence (Larry *et al*, 1979). Active principles of garlic and mustard are known to enhance the activities of GST, NOS and SOD enzymes (Sheen, *et al*, 1999 and Hofmann, 2009).

## Materials and Methods

1-chloro-2,4-dinitrobenzene (CDNB), Reduced Glutathione (GSH), phenyl methane sulfonyl fluoride (PMSF) were obtained from Sisco Research Laboratory Pvt. Ltd. (SRL). 2-mercaptoethanol and Hepes buffer from HIMEDIA. Arginine monochloride, and potassium ferricyanide were purchased from Loba Chemie Pvt. Ltd. and Qualigens Fine Chemicals respectively. Pyrogallol was obtained from BDH chemicals. The other compounds and chemicals were readily available in the laboratory.

Cloves from fresh raw garlic was peeled, washed with distilled water and by using mortar and pestle made into fine paste with sterile water. Garlic paste was then weighed

and dissolved in sterile water at the ratio of 4:5 (wt/vol) following the method of Giles, *et al*, 2000, with slight modifications. Following the same method mustard paste was prepared at the ratio of 1:10 (wt/vol) containing 1% DMSO. Both were magnetically stirred for 2 hours separately. They were collected over filtration and then centrifuged for 15 minutes at 2000 rpm. The supernatants were collected separately for further use and final concentrations were determined which was 800 mg/ml for garlic extract and 100 mg/ml for mustard extract respectively.

BALB/c mice weighing 80-100 g were purchased and were housed in cages at 28±2°C, at departmental animal house. They were fed with standard laboratory food and water. The mice were sacrificed using mild chloroform anaesthesia and whole stomach from each mice were respectively excised and immediately kept on ice for processing further and use in the enzymatic assay.

All the stomach samples were washed with cold PBS and were separately homogenized using a homogenizer with PBS containing 2-mercaptoethanol and PMSF. 10% of the tissue extract of each sample was taken separately with 90% of PBS buffer and centrifuged for 45 minutes at 4°C in a cooling centrifuge at 10000 rpm following the method of Skehel 2004 with slight modifications. The supernatant of each sample was collected and utilized as whole enzyme source. They were grouped into four – i) S1 (Control) ii) S2 (Experimental with garlic extract) iii) S3 (Experimental with mustard extract) iv) S4 (Experimental with both garlic and mustard extracts)

The protein contents of the samples were quantified by standard Folin-Ciocalteu method. The absorbance was measured at 660 nm.

### Statistical analysis

The data was analysed using Standard Deviation formulae. The results were expressed as mean  $\pm$  S.D of 5 mice.

### Results and Discussion

NOS and SOD enzyme's activities were measured spectrophotometrically at 420 nm after 2 hours of incubation of the tissue homogenate with prepared extracts at room temperature, following the methodology of Sarkar, *et al*, 2015, with slight modifications.

GST enzyme activity was measured in the same way at 340 nm using the method of Habig, *et al*, 1974, with slight modification.

From the data analysed it can be said that the activities of NOS, SOD and GST enzymes increased upto a great level in presence of both garlic and mustard extracts, individually and in combination at the concentration of 800 mg/ml and 100 mg/ml respectively. The increase in activities of NOS and SOD enzymes were found to be the highest in presence of both garlic and mustard extracts together, that is 164.92%

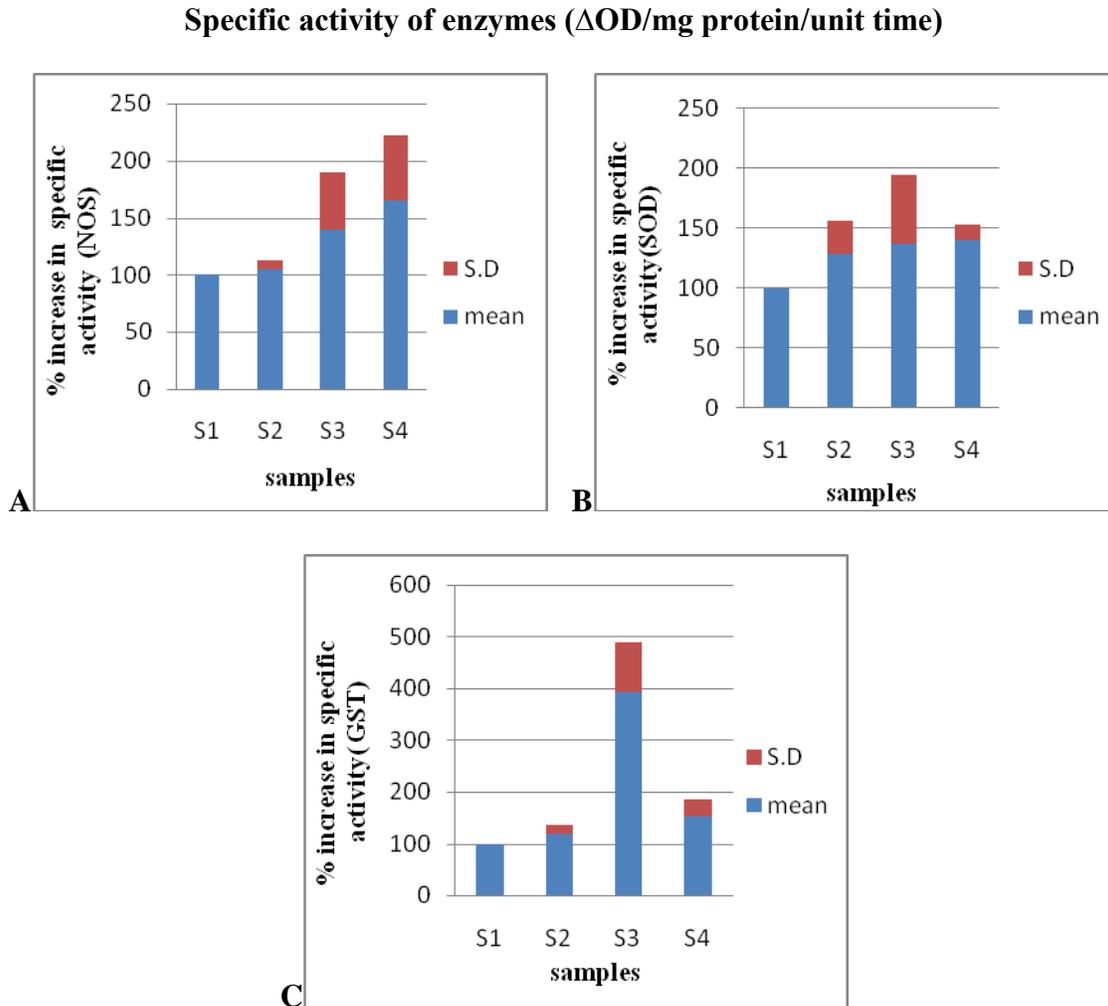
and 140.31%, respectively, whereas the activity of GST enzyme was found to be maximum in presence of mustard extract only, that is 392.50%. GST enzyme's activity was found to decrease (153.13%) when both garlic and mustard were used but it was greater than the activities given by normal tissue and tissue in presence of garlic extract alone.

Thus it can be inferred that these two spices can give protection and prevention for any damaging oxygen free radicals discomfort in stomach. It has been reported that *H.pylori* infection and stomach cancers like adenocarcinomas, lymphomas, gastrointestinal stromal tumors etc. can increase the production of oxygen free radicals. Through research it is observed that garlic can prevent colorectal cancer and several studies revealed that diallyl disulfide is a major component responsible for this action. Phenethyl isothiocyanate and sulforaphane present in mustard inhibit carcinogenesis and tumorigenesis (Hasler, 1998) This in vitro studies on mice can be extrapolated to above mentioned diseases and for human cases by further research.

**Table.1** S1 represents tissue homogenate as control, S2 - tissue homogenate with garlic extract (800 mg/ml), S3 - tissue homogenate with mustard extract (100 mg/ml) and S4 - tissue homogenate with both garlic and mustard extract

Treatment	NOS activity		SOD activity		GST activity	
	Average increase of specific activity in percentage	Standard deviation	Average increase of specific activity in percentage	Standard deviation	Average increase of specific activity in percentage	Standard deviation
S1 (Control)	100	0	100	0	100	0
S2 ( Garlic)	105.15	8.029	129.23	27.25	118.88	16.46
S3 (Mustard)	139.81	50.30	137.64	57.23	392.50	95.46
S3(Garlic+Mustard )	164.92	57.56	140.31	13.13	153.13	30.94

**Figure.1** Percent increase in specific activity of (A) nitric oxide synthase, (B) superoxide dismutase, (C) glutathione-s-transferase.



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