# EXPOSURE TO METHYL METHACRYLATE UPREGULATES THE EXPRESSION OF INOS IN THE TONGUE TISSUE OF RATS EXPERIMENTAL MODEL

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**Abstract**

Methyl methacrylate is widely used particularly in dental laboratories and its toxicity is a matter of concern. The aim of the present study was to investigate the effects of exposure to MMA through the expression of iNOS in tongue tissue. The present study involved the exposure of Wister rats to 0.2 mg/kg body weight for 30 days. Two groups of animals (N=10 for each group) and assigned as control group and test group. After the end of the experiment, animals were terminated and tongue tissue was used to investigate the expression of iNOS using indirect immunohistochemistry. Slides were assessed using adopy photoshop software. Photos for sections were taken and divided into pixels. The total number of pixels was computed and represented both colours (blue and brown), then the brown colour (the colour of the marker under study) was computed and divided by the total number of pixels. Study findings revealed significant expression of iNOS in test group compared with control group (P 0.000).

Taken together, previous studies targeting the effects of exposure to MMA pointed to some inflammatory conditions without exploring molecular mechanisms underlying these effects. The present study showed toxic effects of exposure to MMA through upregulation of iNOS in tongue tissue of rats exposed to MMA. These findings may be the first to explore this aspect.

**Keywords:** Methyl methacrylate, toxicity, iNOS, tongue, rats, experimental model

## Introduction

Methyl methacrylate (MMA) has certain properties including being colorless liquid with an acrid, fruity odor. MMA as a monomer can be

polymerized under the effect of light, heat, oxygen, ionizing radiation, and catalysts (IARC, 1979; Sax, 1984).

Several studies have shown that the human exposure to MMA to be associated with irritations to skin, eyes, or mucous membranes as well as induction of allergic dermatitis or stomatitis (Fisher, 1954; Pegum and Medhurst, 1971). Other studies demonstrated that using MMA as bone cement in surgery led to severe hypotension in patients, which may be followed by cardiac arrest (Lee, 1974) and even death (Kepes et al., 1972).

Studies conducted on dental technicians who used in their work bare fingers to mold and shape MMA putty demonstrated mild axonal degeneration on the area of contact with MMA (Seppalainen and Rajaniemi, 1984).

Animal studies suggested that the toxic effects of MMA are due to the monomer since the polymer seems to be inert. According to this context, the severity of toxic reactions induced by MMA is thought to be inversely proportional to the degree of polymerization prior to use in the tissues (Bohling et al., 1977).

Studies targeting rats showed that long exposure to MMA vapor caused denuding of the tracheal mucosa cilia, and reducing the number of microvilli on the epithelium (Tansy et al., 1980). In another study conducted by Spealman et al (1945), degenerative changes in the liver were observed in guinea pigs and dogs exposed to MMA vapors.

Several studies reported various outcomes. As an example, one study on rats exposed to MMA (up to 2,000 ppm) in drinking water for 2 years did not show compound-related lesions (Borzelleca et al., 1964). These findings were also confirmed by another study on dogs which were given MMA for 2 years and did not show chemically related lesions (Treon et al., 1949). In another study, dermal application of MMA to rats, 3 days per week for 4 months did not induce local tumors (Oppenheimer et al., 1955).

Nitric oxide (NO) is considered as highly reactive oxygen radical, which is found in both normal and malignant tissues. The levels of NO is significantly higher in inflammatory conditions compared with non-inflammatory conditions as well as in malignant tissues. NO is generated by nitric oxide synthase (NOS). there are three isoforms of NOS; NOS1 or type 1 or nNOS (neuronal), NOS2 or type 2 or iNOS (inducible), NOS3 or type 3 or eNOS (endothelial). iNOS has been found to produce increased amounts of NO and iNOS is expressed in many malignant tumors. NO has various which seems to be concentration dependent (Lechner, Lirk, Rieder, 2005; Fukumura, Kashiwagi, Jain, 2006).

From an anatomical point of view, the tongue is a muscle which is a part of the floor of the oral cavity. The tongue of human has two parts anterior and posterior parts (Drake et al., 2005). Several studies across

literature of Chinese medicine (CM) have shown the importance of tongue as a sign reflecting exposure to toxins (FENG et al., 2011).

Up to the best knowledge of the researcher, the present study may be the first study in literature that investigated the expression of iNOS in tongue tissue as a consequence of exposure to MMA.

**Study hypothesis**

Reviewing literature gives different outcomes about the exposure to MMA. Accordingly, we hypothesize that toxic effects of MMA are long term effects and can be detected through investigation of the expression of iNOS which, in turn, leads to various pathologic outcomes including inflammation.

**Study objectives**

The main objective of the present study is to investigate the effects of exposure to MMA through the expression of iNOS in tongue tissue.

**Methodology**

Twenty rats (Wister rats) , average weight 180 g were randomly assigned into two groups test group (N=10) and control group (N=10). Animals were placed in two cages, one cage for each group. All animals were placed in one room for one week before starting the experiment and offered standard diet and water. Prior to initiating the experiment, all animals were labeled in each group using "C' for control and 'T' for test group. Within each group, each animal was given a special number consisted of 'C' or 'T' in addition to a number, i.e., C1-C10 and T1-T10.

Methyl methacrylate (MMA) was freshly prepared in a concentration of 0.2 mg/kg body weight and introduced daily for animals in test group for one month .

At the end of the experiment, all animals were terminated using special champers with ethylene environment. Tongue tissues from all animals were removed from the oral cavity and placed in 10% formalin containers for 24 hrs to achieve good fixation process. Tongue tissues were processed using automatic processor to have tissues infiltrated by paraffin. Further, tissues were embedded by paraffin and sectioned using rotatory microtome (3 micrometer thickness) on charged slides.

Immunohistochemical staining was carried out in tongue tissues through a series of steps starting from deparaffinizing tongue tissues by heating slides in an oven at 60C for 60 minutes, then through passing tongue sections through xylene and decreasing concentration of Alcohol (from 100% -70% ethanol) to distilled water.

Slides were treated with 3% hydrogen peroxide in methanol for 10 minutes to treat endogenous peroxidase activity. In a further step, tongue tissues were treated with 1% albumin to delete or reduce non-specific reactions for 10 minutes. Heat retrieval treatment was carried out by placing tongue sections in coplin jars filled with reveal solution (Biocare medical) for 4 cycles using microwave, 2 minutes each cycle to avoid the effects of formalin fixation. After that, slides were washed using phosphate buffered saline (PBS, pH 7.2).

Antibody against iNOS, Santa cruz biotechnology, was diluted 1:100 and applied for 1 hr in a humid chamber. After that, slides were treated with universal detection system consisting of biotinylated secondary antibodies for 30 minutes followed by streptavidin conjugated with horseradish peroxidase enzyme for 30 minutes. Following washing by PBS, chromogen (diaminobenzidine) was added for about 3 minutes to detect the color of reaction as brown at antigenic sites .

Slides were assessed using adopy photoshop software. Photos for sections were taken and divided into pixels. The total number of pixels was computed and represented both colours (blue and brown), then the brown colour (the colour of the marker under study) was computed and divided by the total number of pixels ( Khatib, 2013).

The expression of iNOS was compared between groups using T test. P value $< 0.05$ was considered statistically significant.

## Results

The mean expression rate of iNOS in control group was 0.0532, which was increased significantly (P 0.000) in test group (0.3124). The expression patterns of iNOS in study groups is shown in table 1, and the mean expression of iNOS in study groups is shown in figure 1 .

Table 1: Expression rate of iNOS in rat tongue of study groups

| Animal No | Control group | Test group |
|---|---|---|
| 1 | 0.01 | 0.11 |
| 2 | 0.074 | 0.23 |
| 3 | 0.03 | 0.32 |
| 4 | 0.056 | 0.19 |
| 5 | 0.06 | 0.267 |
| 6 | 0.07 | 0.301 |
| 7 | 0.022 | 0.421 |
| 8 | 0.09 | 0.532 |
| 9 | 0.08 | 0.654 |
| 10 | 0.04 | 0.099 |
| Mean | 0.0532 | 0.3124 |

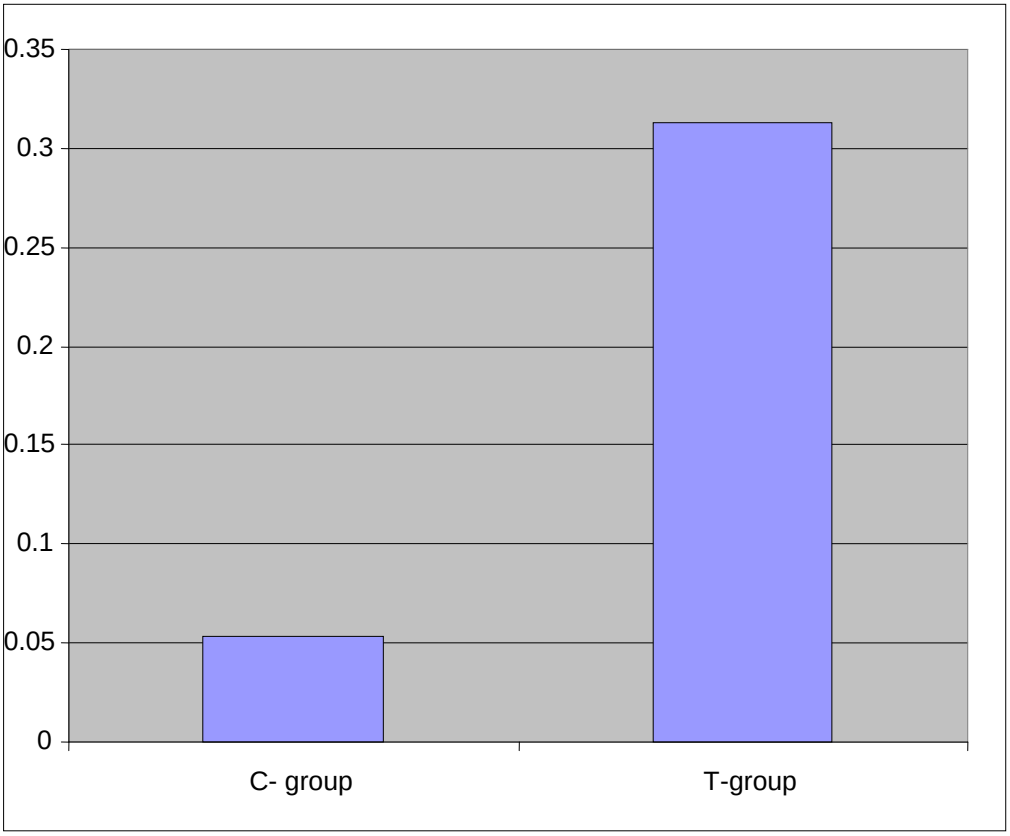


Figure 1: The mean expression of iNOS in study groups due to the exposure to MMA.

**Discussion**

The present study showed for the first time the consequences of exposure to MMA in tongue tissue of rats exposed to MMA. The data of the present study has revealed significant expression of iNOS in tongue tissue of rats exposed to MMA.

Although, there are no previous studies identified in literature to compare our results with, but it is plausible to depend indirectly in other reported studies. Inflammatory reactions take different forms among which are irritation to skin, eyes, or mucous membranes as well as allergic reactions as dermatitis or stomatitis, all these forms have been described upon exposure to MMA (Fisher, 1954; Pegum and Medhurst, 1971).

Findings of the present study supported previous studies reported in literature in which it has been demonstrated that using MMA as bone cement in surgery was associated with severe hypotension in patients, which may be followed by cardiac arrest (Lee, 1974) and even death (Kepes et al., 1972).

Other studies did not point to the effect of iNOS directly in which dental technicians who used in their work bare fingers to mold and shape

MMA putty demonstrated mild axonal degeneration on the area of contact with MMA (Seppalainen and Rajaniemi, 1984).

**Conclusion**

Previous studies targeting the effects of exposure to MMA pointed to some inflammatory conditions without exploring molecular mechanisms underlying these effects. The present study showed toxic effects of exposure to MMA through upregulation of iNOS in tongue tissue of rats exposed to MMA. These findings may be the first to explore this aspect.

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