INTRODUCTION

The term "food additive" is inevitable in present day lifestyle as global food industry grown exponentially with the use of food flavors to attract consumers globally. In Japan, initial production patent of Monosodium glutamate (MSG) was obtained in 1908 by Professor Kikunae Ikeda [1]. MSG (C₅H₈NO₄Na) appears as a white crystalline chemical which is the sodium salt of L-glutamic acid popularly known as Ajinomoto [2]. Most of the packaged foods such as chips, Jelly, Pastry, Candy, Biscuit, Chocolate, French-fries, Pizza, and Noodles contain MSG, which imparts "Umami" or "Savory" or "Broth-like" or "Meaty taste" [3]. The regular daily consumption of MSG in industrialized country is about 0.3–1g/day, which is alarmingly increasing many times in developing countries [4]. The increased consumption of MSG around the globe is proportional to its increased production, especially major share of production by countries of Asia [5]. The controversies regarding the safety of MSG dietary consumption still sparks the debate among scientists even though FDA announced "MSG as safe for human consumption" [6]. The free radicals constitute highly reactive, unpaired electrons produced during cellular metabolism, which tries to form pairs with other electrons and one such group of free radicals are Reactive Oxygen Species (ROS) [7]. ROS is composed of superoxide, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. The oxidative stress is the imbalance between ROS and body's antioxidant defense system which causes lipid peroxidation, oxidative DNA damage, disrupts signal transduction in living cell [8]. The liver is a vital accessory gastrointestinal organ which plays a pivotal role in carbohydrate, protein and fat metabolism. The liver not only detoxifies waste products, but also secretes bile, synthesizes lipoprotein, produces plasma proteins including clotting factors, and many more functions. The high metabolic activity of the liver attributes to its increase in free radical production naturally, which is counteracted by the endogenous antioxidant system [9]. Oxidative stress in the liver induced by MSG can be explained by exhausting endogenous antioxidants namely Catalase (CAT), Superoxide dismutase (SOD), and reduced glutathione [10,11]. Most of the animal studies concerned with MSG fail to clarify the comparative human dosage along with the route of administration [12]. Therefore, we tried to explore whether chronic consumption of MSG at three different doses (low, mid, and high) induces oxidative stress in liver of Wistar albino rats based on human consumption of MSG.

METHODS

Animals

The study was conducted with twenty four adult Wistar albino rats of either sex (n=24). The approval for the study was granted by the Institutional Animal Ethical Committee (IAEC) with reg no: VIMS/IAEC/2016/03. The Institutional animal house facility was well maintained with 23±1°C and 12 h light/12 h dark cycle. The rats were provided with surplus standard laboratory chow and drinkable water. The animals recruited for the study were acclimatized before the inception of the study. Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines were pursued for animal handling as well as procedural protocols for the experiment.

Experimental design

The acclimatized animals for the study were randomly chosen and grouped (n=6 per group) for the experimentation conducted for a chronic period of 120 days. The Wistar albino rats were organized into four groups (n=6 per group) as follows:

a. Group I (Control) – The rats were fed with distilled water
b. Group II (Low dose) – The rats were given MSG of 180 mg/kg body wt
c. Group III (Mid dose) – The rats were given MSG of 360 mg/kg body wt
d. Group IV (High dose) – The rats were given MSG of 720 mg/kg body wt.
The food grade MSG was administered to the experimental rat’s daily using oral gavage for a chronic period (120 days) as per the design of the study.

**Oxidative stress markers assessment**

After the end of the chronic experiment period, animals were euthanized using cervical dislocation and abdomen of animal was dissected out to harvest liver. One part of the liver sample from each animal was homogenized, centrifuged at 12000 RPM (2°C) for 10 min and the supernatant was collected for oxidative stress marker analysis. The enzymes for oxidative stress, namely SOD, Glutathione Peroxidase (GPx), CAT, and Myeloperoxidase (MPO) were estimated using Enzyme Linked Immunosorbent Assay (ELISA) plate method. The double-antibody sandwich ELISA technique was employed for the oxidative stress marker assay to get higher specificity and increased sensitivity.

**Statistical analysis**

The statistically analyzed data were expressed as mean±standard deviation (SD). GraphPad Prism software 9.1.2 was adopted for data analysis statistically. The data were subjected to One-way ANOVA analysis and followed by Tukey’s post hoc test. After analysis, the results were considered significant if p<0.05. The p-values in the present experiment were illustrated as * for p<0.05, ** for p<0.01, *** for p<0.001, **** for p<0.0001.

**RESULTS**

The study results found significant differences (p<0.05) in oxidative stress markers between control and MSG induced groups. Administration of MSG significantly (p<0.05) escalated MPO in MSG induced groups when compared with control group (Fig. 1). MPO activity drastically increased up to 77.30% in high dose MSG while low and mid dose MSG induced groups showed 24.11% and 35.94%, respectively. The activities of SOD in MSG induced groups significantly (p<0.05) decreased contrasting to control group (Fig. 2). The present recording of SOD activity showed 21.41%, 29.12%, and 41.23% decreased in low, mid, and high dose MSG groups compared to control group. The CAT activity significantly (p<0.05) decreased in MSG induced group when analyzed with control group (Fig. 3). The determined 13.48%, 23.67%, and 29.79% reduction in CAT for low, mid, and high dose MSG groups, respectively, were noted during the analysis. The GPx showed 14.91%, 22.76%, and 30.35% reduced in low, mid, and high dose MSG induced groups compared to control group significantly (p<0.05) (Fig. 4).

**DISCUSSION**

The present study investigated the effects of MSG at different doses inducing oxidative stress in the rat liver tissue with the spotlight on the human corresponding dosage of MSG at low, mid, and high dosage chronically viewing the consumption in developing Asian countries. The previous research findings did show oxidative stress changes in various organ systems of animal model but failed to corroborate definitive conclusions as those study design had MSG doses varied from human consumption and differed in route of administration [12].

The current study investigations expressed that there were abnormal variations in oxidative stress markers of liver tissue after administration of MSG chronically. The significant abnormal increase was recorded in MPO activity in high dose MSG group and marked increase in low and mid dose groups. The MPO is the crucial oxidative stress marker and marker

**Fig. 1: Effect of Monosodium glutamate on Myeloperoxidase in liver homogenate. (Values are expressed as mean±SD. *Significantly different from the control group at p<0.05. **Significantly different from the control group at p<0.01. ***Significantly different from the control group at p<0.001. ****Significantly different from the control group at p<0.0001)**

**Fig. 2: Effect of Monosodium glutamate on Superoxide Dismutase in liver homogenate. (Values are expressed as mean±SD. *Significantly different from the control group at p<0.05. **Significantly different from the control group at p<0.01. ***Significantly different from the control group at p<0.001. ****Significantly different from the control group at p<0.0001)**

**Fig. 3: Effect of Monosodium glutamate on Catalase in liver homogenate. (Values are expressed as mean±SD. *Significantly different from the control group at p<0.05. **Significantly different from the control group at p<0.01. ***Significantly different from the control group at p<0.001. ****Significantly different from the control group at p<0.0001)**
for inflammation [13]. The MSG has adversely affected liver tissue by
inducing oxidative stress at all doses in our current study. The present
investigation findings show that SOD levels were significantly declined
in all MSG induced group as a result of MSG administration [11,14]. The
deprecated SOD levels may indicate an increase in ROS such as superoxide
radical and hydrogen peroxyde which further speculate that these ROS
damage the DNA and lipid bound biological membrane in liver
cells [15]. The CAT activity in cellular redox status is crucial for converting
hydrogen peroxyde into water. The CAT in the current study decreased
substantially in monosodium induced groups which may be suggestive of
definitive imbalance in the redox status of liver cell [16]. The altered
SOD and CAT levels in liver tissue may be detrimental in the oxidative
stress pathophysiology by disrupting signal transduction, cellular
mechanisms in the cell leading to apoptotic changes in liver [17,18].
The GPX endogenously scavenges hydrogen peroxyde in liver tissue
and declined levels of GPX in MSG induced groups in the present
study may indicate an alarming increase of ROS damaging the hepatic
cellular inclusions [19]. The redox status imbalance would have either
decreased vital endogenous antioxidant enzymes by increasing ROS in
MSG administered groups [5,20]. The results can also be corroborated
glycation of the endogenous antioxidant enzymes by MSG may also
make these antioxidant enzymes inactive [21]. The DNA damage in liver
cells may be attributed to the modifications in purines and pyrimidine,
DNA repair enzymes, and DNA polymerases leading to mutation in
DNA. The oxidative stress is leading cause of several hepatic diseases
that has to be addressed clinically to improve the overall hepatic tissue
health [17,22]. The increase in ROS and oxidative stress can also trigger
the apoptosis in the cells [23,24]. The present study may speculate
that oxidative stress induced by MSG after chronic administration
may lead to a variety of hepatic diseases such as Non-Alcoholic Fatty Liver
Disease, Non-Alcoholic Steatohepatitis if not addressed with healthy
food supplements containing exogenous antioxidants.

CONCLUSION

The present study findings show that MSG induces oxidative stress in the
liver of Wistar albino rats on chronic consumption. The safety concern
of MSG must be reconsidered by the food industry and government
agencies to culminate the progression of liver diseases by its chronic
usage. The healthy lifestyle changes involving physical exercises and
regular consumption of foods rich with antioxidants may reduce the
oxidative stress. The future perspective of MSG research should focus on
various genetic and epigenetic changes induced by MSG at the molecular
level in liver tissue to understand clear pathogenesis of MSG.

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AUTHORS’ CONTRIBUTION

Surendra babu Thangachi formulated the present study, executed data
collection, data analysis, and penned the manuscript. Varsha Srimat
Mokhasi was instrumental in guiding throughout the study and edited
the manuscript. Agy Ammar Murtuzu was constructive in helping for
manuscript writing.

CONFLICTS OF INTEREST

The authors announce that there were no conflicts of interest in current
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