Acute Toxicity Study of a Food Additive Combination (Monosodium Glutamate and Tartrazine) in Albino Rats

ACUTE TOXICITY STUDY OF A FOOD ADDITIVE COMBINATION (MONOSODIUM GLUTAMATE AND TARTRAZINE) IN ALBINO RATS

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ABSTRACT:

Objective: To evaluate the Acute toxicity studies of a Food Additive Combination (Monosodium Glutamate and Tartrazine) in Albino Rats. **Methods:** Acute toxicity study was conducted at a dose of 90 mg/kg of Monosodium glutamate and 2mg/kg of Tartrazine. Animals were grouped into 4 groups for acute studies. According to OECD guidelines observation were recorded immediately after 30 min, 1, 2, 4, 8, 24 and 48 h test administration from respectively. **Results:** Change in the behavior of animals were observed in the animals of acute study. Histopathology of liver, Kidney, brain and heart of sub-acute study animals in all three test groups had shown necrosis, fibrosis, inflammation and formation of vacuoles. **Conclusion:** Monosodium glutamate and Tartrazine alone or in combination may lead to mild/severe organ toxicities depends on its exposure or convention.

KEYWORDS:

Acute toxicity studies, Albino Rats, Mosodium Glutamate and Tartrazine

INTRODUCTION:

Health is considered to be the most important value of life". To maintain and achieve health one should have these three basic things and are as follows (Norman sartorlus, 2006).

- Healthy food/ healthy nutrition
- Physical exercise
- Sleep

One of the most important ways to keep health is to have healthy diet/food. A healthy food consists of variety of plant based and animal-based foods that provide nutrients to your body (Norman sartorlus, 2006).

In recent years the food we are getting is mainly consists of various natural and synthetic chemical substance that are added as food additives at various steps in processing and storing of food substances (FSSAI, 2015).

Food additive is a substance or mixture of substances other than the basic food stuff, which is present in a food as a result of any aspects of production, processing, storage or packaging (Pandey et.al., 2012).

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The food additives used in our daily life are showing various side effects and are withdrawn from the market in US, Norway, Australia, Sweden, Denmark, Germany, Japan, France, Switzerland, France, Hungary (Pandey et.al., 2012).

But still some nations are continuing their usage. When consumed alone these chemical substances are producing the effects and adverse effects. When two or more are consumed, it may increase the chances of precipitating severe toxicity over a period (WHO food safety 2015).

This laid to the basis of the present study to evaluate the toxic effects of a combination of food additives, Monosodium glutamate and Tartrazine.

In this study various effects of combination of Monosodium glutamate and Tartrazine. (Most commonly used food additive combination in fast foods in India).

CHEMICAL PROFILE:

Monosodium glutamate:

Monosodium glutamate is the monosodium salt of L-glutamic acid. It is commonly used as a flavor enhancing agent (chemicalland21, 2011).

Tartrazine

Tartrazine is an azo dye that is used as coloring agent in food, drugs and in cosmetics. (chemicalland21, 2011).

AIM & OBJECTIVE

Aim: The aim of the present study is to determine the acute toxic effects of the food additive combination (Monosodium glutamate and Tartrazine) in albino rats.

Objective: The objective of the present study is to evaluate the effects of combination of Monosodium glutamate and Tartrazine on

Behavioral parameters

- ✓ Behavioral changes
- ✓ Neuronal changes
- ✓ Autonomic changes
- ✓ Motor changes
- ✓ Respiratory changes

STUDY PROTOCOL

Acute toxicity study

Animals: The study requires male and female Albino rats of Wistar strain, (150-200gms B W) which will be grouped into five groups (n=3) and maintained under normal laboratory requirements.

Duration of treatment: 24 hrs+ 14 days observation.

MATERIALS AND METHODS:

PROCUREMENT OF TEST COMPOUNDS

The chemicals Monosodium glutamate (GRM681-100G) and Tartrazine (GRM431-100G) certified by Hi-media laboratories Pvt. Ltd.

were procured from Bros Scientifics, Tirupathi. Andhra Pradesh, India. Test kits required for estimation of serum parameters are procured from Andhra Surgical& chemicals, Tirupathi, Andhra Pradesh, India.

PREPARATION OF SOLUTION

Stock solutions of both Monosodium glutamate (MSG) and Tartrazine were prepared in distilled water of concentrations 10mg/ml of MSG and 0.2mg/ml of Tartrazine.

PROCUREMENT OF ANIMALS

Healthy albino rats of Wistar strain of either sex body weighing about 150-220 g were used for the study. The rats were purchased from Sri Venkateswara Agencies, Bangalore. The animals were caged individually and kept in air-conditioned room, at the temperature of 22±24 with 50%±10% relative humidity with 12 hours light and dark cycle. Throughout the study the animals were maintained at normal laboratory conditions. Animals were maintained at standard rat pellet diet and drinking water ad libitum.

After acclimatization animals were selected randomly and were divided into 5 groups (n=6). All the experimental protocols were approved by the Institutional Animal Ethical Committee (IAEC) of Sri Padmavathi School of Pharmacy (SPSP: 1016/PO/E/S/ CPCSEA/2016/003). All the guidelines and principles of the protocol were following the guidelines and principles laid down by the committee for the purpose of control and supervision of experiments on animals (CPCSEA), Govt. of India, New Delhi.

INSTRUMENTS

UV-Visible spectrophotometer (analytical systems, model no: AUV 2060), electronic balance (Shimadzu, Model no: DS-852 J), homogenizer (Ever shine, Model no: 607), auto analyzer (Mispa excel, Version: 14e) and Cooling centrifuge (Remi, Model.no: C-24 BL).

CHEMICALS

Diagnostic kits for estimating SGPT, SGOT, ALP, total proteins, Albumin, Creatinine and Bilirubin (Total and direct bilirubin) diagnostic kits were procured from span diagnostics Ltd, India and Excel diagnostics Ltd, India.

DOSE SELECTION

The doses of MSG and Tartrazine for the present study are based on the daily human consumption. The data is collected from various fast-food centers.

EXPERIMENTAL PROTOCOL

Table 3: Treatment schedule for assessing the acute toxic effects of a food additive combination (Monosodium glutamate and Tartrazine)

S. No	GROUPS	TREATMENT	PURPOSE OF THE STUDY
1.	NORMAL	Vehicle (distilled	Serves as

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		water)	normal
2.	TEST-1	MSG (90 mg/kg)	To study the effect of MSG
3.	TEST-2	TARTRAZINE (2mg/kg)	To study the effect of Tartrazine
4.	TEST-3	MSG + TARTRAZINE (90mg/kg+ 2mg/kg)	To study the effect of combination of MSG + Tartrazine

HISTOPATHOLOGY

The liver, kidney, that is collected after sacrificing the animals was blotted free of blood and other tissue fluids using the filter paper and stored in 10% formal saline solution before determining the histopathology.

To determine the histopathology, the tissue was fixed with bovine fluid (picric acid, formalin and acetic acid in the ratio of 75:52:5) and after 24 hours it was washed thoroughly in 70% alcohol and subjected to dehydration in ascending grades of alcohol (70% and 100%) followed by treatment of the tissue with the mixture of xylene and toluene of equal proportion in a successive manner of 10%, 50%, 70%, 90% paraffin wax in toluene and finally with 100% paraffin wax at a temperature of 60-70◦C. Finally, the tissue was embedded in the wax (Pithayanukul et al., 2009).

STATISTICAL ANALYSIS

All the data was expressed as mean ± SEM. Statistical significance between more than two groups was tested using one-way ANOVA followed by Bonferroni multiple comparison test using the computer based fitting program (Prism, Graph pad 5). The significance level was set at $P<0.001$ for all tests.

RESULTS:

ACUTE TOXICITY STUDY:

The acute toxicity study was performed for MSG, Tartrazine and Both MSG & Tartrazine at the dose of MSG-89mg/kg, Tartrazine 2mg/kg (based on human consumption). No animals had shown mortality at the given dose of MSG, Tartrazine and their combination (MSG+Tartrazine). But, shown some abnormal changes in behaviour, neurological, autonomic, motor and Respiratory functions at the given doses by oral route (p.o).

Behavioural changes:

The behavioural changes were observed form the time of administration upto 24hrs of administration for all the three groups MSG, Tartrazine and its combination. The rats in every group had shown various behavioural changes like grooming, irritability, restlessness and were lacking alertness up to 8hrs from the time of administration and grooming alone was observed up to 24hrs. It was observed that the given substances alone and in combination affected behavioural profile of the rats when compared with normal rats.

Neurological changes:

Form the time of administration up to 24hrs changes in neurological profile were observedin all the three groups MSG, Tartrazine and its combination. The rats in group II, III, IV had shown various changes such as tremors, twitches, sedation from 30min upto 8hrs of administration. Rats in group II has not shown any Twitches when compared with the group I rats which served as normal group.

Autonomic profile:

Changes in the autonomic profile were observed from the time of administration upto 24hrs of administration in all the three groups MSG, Tartrazine and its combination. Rats in all the three groups possessed mydriasis followed by miosis and others like lacrimation, urination, defecation were normal/no marked effect. This shows that the given substances are having effect on autonomic system when compared with that of normal group.

Motor Activity:

The test groups II, III and IV were shown Changes in motor activity from the time of administration upto 24hrs.The rats in each test group shows abnormalities like altered body posture and righting reflex. Among these three-group group-III shows abnormal gait when compared with the normal control group.

Respiratory effects:

Changes in the respiratory system were observed from the time of administration up to24hrs administration in all the three test groups. The rats in every group had shown Apnoea and Dyspnoea except test group-II. This shows that test compounds altered the function of respiratory system when compared with the group-I which serves as normal control.

DISCUSSION:

Monosodium glutamate and Tartrazine are the most widely used food additives in various fast-food items. Monosodium glutamate is used as flavour enhancer, while Tartrazine is used as a colorant in variety of foods prepared at homes, restaurants and by food processors. The use of food additives in food products has increased in recent times. Food processors are using various chemical substances as food additives.

Monosodium glutamate is used as flavour enhancer, it increases the sapidity of food. It is used in dry soups, canned goods and cheese spreads. It has shown various adverse effects on liver, kidney, reproductive organs and shows effects on neurons. It also shows various pathological conditions such as endocrine disorders, retinal degeneration, stroke, schizophrenia, anxiety, Parkinson's disease, Alzheimer's disease (veronica et.al., 2013).

Tartrazine an azo dye is used as a colorant. It is used to dye sweets, chewing gums, juices, jams, sodas, mustards, cosmetics and also in drugs. The metabolite of Tartrazine is an aromatic amine "sulphanillic acid", it is highly sensitizing in nature. It shows various effects like chromosomal aberrations and various allergic reactions (Imane Himri et.al., 2011)

Acute toxicity study performed on MSG, Tartrazine and combination of both MSG and Tartrazine. From the results the test groups administered with MSG, Tartrazine and combination of MSG and Tartrazine has not shown any mortality at given doses. They were shown behavioral changes like Grooming, irritability, restlessness and lack of alertness; Neurological changes like tremors, twitches and sedation; Autonomic changes like mydriasis followed by miosis, urination and defecation; Motor activity changes like abnormal gait and postural changes; changes in respiratory functions like Apnea and Dyspnea. All these changes were observed when the test substance was administered in single oral dose (p.o).

The change in body weights were observed in the animals of the three groups administered with the MSG, Tartrazine and combination of MSG and Tartrazine. The body weights are increased in all the three groups when compared with the control group. Increase in the body weights may be because of MSG on palatability of food, by disrupting the hypothalamic signaling cascade of leptin action on adipose tissue and effect of Tartrazine on the fat deposition in adipose tissue (veronica et.al., 2013).

CONCLUSION:

Monosodium glutamate and Tartrazine are the most widely used food additives in fast foods as flavour enhancer and colorant. In the present study acute and sub-acute toxicity studies of MSG, Tartrazine and combination of MSG & Tartrazine were performed. The acute toxicity results shows that MSG, Tartrazine, and combination of MSG & Tartrazine administered group shows effects on behavioral changes, neuronal changes, autonomic changes, changes in motor activity, respiratory effects were observed and no lethality was observed when they are administered in single dose and observed for 14 days.

Although it is a chemical phenomenon to be able to make a food stay preserved longer than it should be, and be able to enhance the flavor and color of our foods, but at the cost of our healthy life? I feel the public lacks the knowledge about what is really in our foods. After learning so much about effects of consuming mixture of food additives I know I will be thinking twice about the foods I choose to eat. In addition, I believe other people would also be more cautious if they

were aware about what they were really putting in their bodies. Collectively, if we took a stand together we could radically change the way the food industry operates.

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Source of Support: Nil. **Conflicts of Interest:** None