

Original Research Article

Effects of Monosodium Glutamate in Ovaries of Female Sprague-Dawley Rats

I. C. Oladipo^{1*}, E. A. Adebayo² and O. M. Kuye³

¹Science Laboratory Technology Department, Ladoke Akintola University of Technology, P.M.B 4000, Ogbomoso, Oyo State, Nigeria

²Department of Science Laboratory Technology, Ladoke Akintola University of Technology, Ogbomoso 210214, Oyo State, Nigeria

³Department of Microbiology, University of Ibadan, Ibadan 200284, Oyo State, Nigeria

**Corresponding author*

ABSTRACT

Keywords

Monosodium glutamate; histopathology; female infertility; ovaries; sprague-dawley rats

Fear has increased in recent years, due to the adverse reactions and toxicity of monosodium glutamate (MSG) which is commonly used as a food additive and there is growing concern that this may play a critical role in both male and female infertility. The effect of analar and food grade monosodium glutamate (MSG) on the ovaries of Sprague-Dawley rats was studied. Fifty female Sprague-dawley rats with an average weight of about 100 – 150g were randomly assigned into 5 groups A, B, C, D and E of 10 rats each. The treatment groups (A, B and C) were given 0.10g/kg, 0.15g/kg and 0.20g/kg body weight of analar grade monosodium glutamate while treatment group D was given 0.20g/kg body weight of commercial food grade monosodium glutamate in 1.5ml of water, respectively on a daily basis. The control group (E) received equal amount of water without monosodium glutamate for fourteen days. The rats were sacrificed on day fifteen of the experiment. The ovaries were carefully dissected out and quickly fixed in 10% formal saline for routine histological procedures. Significant body weight increase was noted in MSG treated rats and the histological findings in the treated groups showed evidence of cellular hypertrophy, degenerative and atrophic changes with more severe changes in the group that received 0.20g/kg of analar MSG. These findings indicate that MSG induced considerable structural changes, including degenerated follicles, oocytes and medulla with vacuoles having congested blood vessels in the ovaries of Sprague-dawley rats; these changes are more severe at higher doses which may contribute to the causes of female infertility.

Introduction

Monosodium glutamate (MSG) is one of the world's most extensively used food additives, which is ingested as part of commercially processed foods. As a flavor

enhancer, MSG increases the sapidity of food. MSG produces a flavor that cannot be provided by other foods. It elicits a taste described in Japanese as umami, which is

translated to “savory” (Schiffman and Gill, 1987). The effect of MSG is attributed to the presence of the sodium ion, although the glutamate ion by itself can intensify the activity of gustatory nerves (Yamamoto *et al.*, 1991).

The average intake of MSG in United Kingdom was 580mg/day for general population individual and 4.68g/day for extreme users (Rhodes *et al.*, 1991). The estimated average daily MSG intake per person in industrialized countries is 0.3-1.0g, but it depends on the MSG content in foods and an individual’s taste preferences (Geha *et al.*, 2000). According to a joint inquiry by the governments of Australia and New Zealand in 2003, a typical Chinese restaurant meal contains between 10 and 1500mg of MSG per 100g (Freeman, 2006). The oral dose that is lethal to 50% of subjects [LD50] in rats and mice is 15.000 – 18.000mg/kg body weight (Walker and Lupien, 2000). Data from the United Kingdom indicates an average intake of 590mg/day, with extreme users (97.5th percentile consumers) consuming 2330mg/day (Rhodes *et al.*, 1991). The optimal palatability concentration for MSG is between 0.2 – 0.8% and its use tends to be self-limiting as over-use decreases palatability. The largest palatable dose for humans is about 60mg/kg body weight (Walker and Lupien, 2000). In Nigeria, most communities and individuals often use MSG as a bleaching agent for the removal of stains from clothes. There is a growing apprehension that its bleaching properties could be harmful or injurious to the body, or worse still inducing terminal diseases in consumers when ingested as a flavor enhancer in food. Despite evidence of negative consumer response to MSG, reputable international organizations and nutritionist have continued to endorse MSG, reiterating that it has no adverse reactions in

humans. Notably of such are the Directorate and Regulatory Affairs of Food and Drug Administration and Control (FDAC) in Nigeria, now NAFDAC has also expressed the view that MSG is not injurious to health (Okwuraiwe, 1992).

Through its stimulation of the oro-sensory receptors and by improving the palatability of meals, MSG influences the appetite positively, and induces weight gain. Despite its taste stimulation and improved appetite enhancement, reports indicate that MSG is toxic to human and experimental animals (Biodun and Biodun, 1993). Studies on MSG has been reported to cause burning sensation at the back of the neck, forearms, chest, facial pressure/tightness, chest pain, headache, nausea, palpitation, numbness in the back of the neck radiation to arms and back, tingling, weakness etc. (Daniel *et al.*, 2013). In addition to this MSG symptom complex, ingestion of MSG has been alleged to cause or exacerbate numerous conditions including asthma, urticarial, atopic dermatitis, ventricular arrhythmia, neuropathy and abdominal discomfort (Geha *et al.*, 2001).

MSG has a toxic effect on the testes by causing a significant oligozoospermia and increase abnormal sperm morphology in a dose-dependent fashion in male Wistar rats (Onakewhor *et al.*, 1998). It has been implicated in male infertility by causing testicular hemorrhage, degeneration and alteration of sperm cell population and morphology (Oforofuo *et al.*, 1997). The ovary, (from Latin: ovarium, literally “egg” or “nut”) is an ovum-producing reproductive organ, often found in pairs as part of the vertebrate female reproductive system. The ovary also functions in the production of various steroids and functions in the reproductive system (Indebir, 2012). Fears has increased in recent years, due to the

adverse reactions and toxicity of MSG, with few and limited literatures regarding the histological studies of the damage in the ovaries treatment of animal with analar MSG. So, the present study is aimed at studying the severity of monosodium glutamate using its analar and commercial food grade on the ovarian tissue of young female rats.

Materials and Methods

Chemicals

The chemicals used in this study were an analar grade MSG ($C_5H_8NNaO_4 \cdot H_2O$) and the commercially available food grade package sold in most open market in Nigeria.

A stock solution was prepared by dissolving known gram (g) of both analar grade and food-grade monosodium glutamate crystals differently in separate bottles in known millilitres (mL) of distilled water. The dose scheduled was so adjusted that the amount of MSG administration per animal was as per their respective group weights.

Experimental Animals

Fifty (50) female Sprague-Dawley rats with an average weight of about 100 – 150g were used for the experiment. The rats were housed under appropriate controlled room in approved cages with the standard temperature ranging between $26.5 \pm 2^{\circ}C$ and maintained under standardized conditions away from any stressful conditions with 12/12 light and dark cycle with free access to humidity and were fed to a dry balanced meal (Grower' mash) for experimental animals, with a constant source of water.

All experimental procedures and animal maintenance were conducted in accordance

with the accepted standards as well as ethical guidelines of animal care unit of the Department of Physiology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

Clinical examination of the experimental animals

The animals were randomly divided into 5 groups of 10 rats each. Group A received 0.10g/kg body weight, Group B 0.15g/kg body weight, Group C 0.20g/kg body weight of analar grade MSG in 1.5ml of water daily while Group D received 0.20g/kg body weight of commercial food grade MSG also in 1.5ml of water daily for fourteen days. Group E served as the control and all grades of MSG were administered orally.

Biochemical analysis of the serum

At the end of treatment period, blood samples of the rats were taken through the ocular route into glass EDTA bottle. Serum was separated by centrifugation at 3000rpm for 10 min.

The serum was collected and used for analysis, which included total protein, blood glucose, albumin, globulin and enzymes activities (AST and ALT).

Glucose and cholesterol assay

Glucose was determined by spectrophotometric method as described by Teusch and Richterich (1971) and cholesterol was determined by enzymatic colorimetric reaction according to Siedel *et al.* (1981).

Transaminases assay

ALT and AST was determined by spectrophotometric method as described by Schmidt (1963).

Total serum protein assay

Total serum protein was determined using the biuret method as described by Reinold (1953).

Albumin assay

Albumin was determined using the BGG (Bromocresol green) method as described by Peters *et al.* (1982).

Histopathological analysis of the ovaries

At the end of the experimental period, the animals were sacrificed by cervical dislocation and they were dissected, the ovaries were removed and fixed in 10% formalin solution for routine histological techniques. The tissue pieces were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in melted paraffin wax.

The wax block is then cut on a microtome to yield a thin slice of paraffin containing the tissue. The specimen slice is then applied to a microscope slide, air dried, and heated to cause the specimen to adhere to the glass slide. Residual paraffin was then dissolved followed by rinsing with an acid-alcohol and by rinsing with water to remove the acid-alcohol. The sections were stained with hematoxylin and eosin, and assessed under an Olympus microscope (Olympus Optical Co., GMBH, Hamburg, Germany). Images were captured using Camedia software (E20P 5.0 Megapixel; Hamburg, Germany) at 20X magnification

Statistical analysis

The data gathered were processed using SPSS 17.0 software. Comparison between groups was made using One-way Analysis of variance (ANOVA).

Results and Discussion

Severe diarrhea, frequent urination with a repulsive smell, reduced restlessness, and increased appetite were observed in the groups administered with MSG (both analytical and commercial grade) during the experimental period when compared to control. Body weight of the experimental animals was taken on weekly basis. All groups gained weight but Group C (group treated with 0.20g/kg body weight of analar grade MSG) had highest weight gained after the fourteens days of MSG treatment as shown in Table 1.

Biochemical analysis of the blood serum showed that analar and food grade MSG administration increased aspartate aminotransferase, alanine aminotransferase, albumin, total protein and globulin levels when compared with the control as shown in Table 2. MSG administration also led to a decrease in glucose levels in the sera of all the treated groups. Significant decrease was recorded in the cholesterol levels of groups A, B and D except group C (Table 2).

Histopathology of ovaries of animals administered with 0.1g/kg body weight of analar MSG revealed that the ovary was in an atresic state and there was no matured follicle seen. The medulla was congested and fully developed follicles were seen in the group administered with 0.15g/kg body weight of analar MSG.

Furthermore, the animals administered with 0.20g/kg body weight of analar MSG showed severe ovarian and follicular atresia while animals administered with 0.20g/kg body weight of food grade MSG showed a severe congestion of the medulla with few matured follicles; most are immature or developing (Figure 1).

Table.1 Effects of MSG on body weight of experimental animals

Groups	Acclimatization		Treatment Period
	1 st week	2 nd week	3 rd week
A	117.00 ± 5.96 ^c	118.00 ± 6.04 ^c	130.00 ± 4.64 ^c
B	130.00 ± 6.52 ^d	132.60 ± 1.79 ^d	142.40 ± 4.00 ^d
C	142.25 ± 6.70 ^e	145.25 ± 8.14 ^e	162.50 ± 9.68 ^e
D	107.20 ± 4.55 ^{a,b}	110.20 ± 3.96 ^b	118.00 ± 5.94 ^b
E	100.00 ± 7.48 ^a	105.00 ± 6.88 ^a	1109.75 ± 13.55 ^a

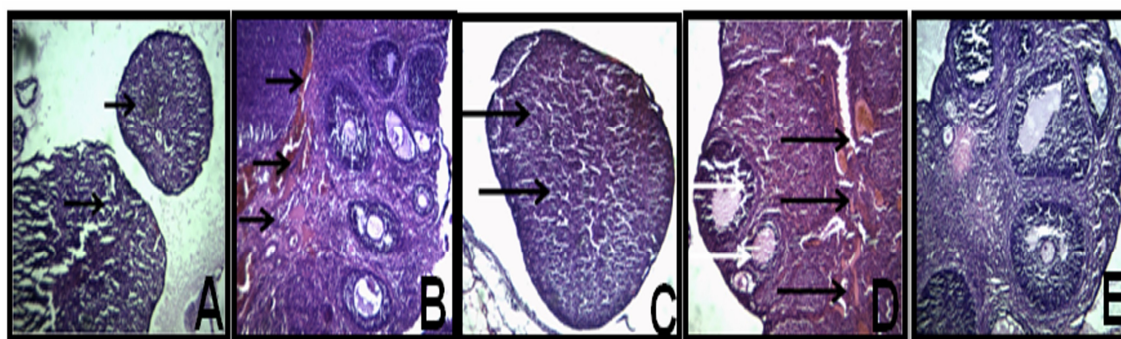
Each value is a mean of 3 replicates ± standard deviation; values accompanied by identical superscript letters are not significantly different (p≤ 0.05). A = Group administered with 0.10g/kg MSG; B = Group administered with 0.15g/kg MSG; C = Group administered with 0.20g/kg MSG; D = Group administered with 0.20g/kg Food grade MSG; E = Control group.

Table.2 Biochemical analysis of the blood serum of experimental animals

Parameters	A	B	C	D	E
AST	1.53 ± 0.40 ^a	1.56 ± 0.19 ^a	1.64 ± 0.17 ^a	1.68 ± 0.14 ^a	1.41 ± 0.11 ^a
ALT	0.37 ± 0.05 ^a	0.37 ± 0.01 ^a	0.39 ± 0.06 ^a	0.41 ± 0.07 ^{a,b}	0.28 ± 0.07 ^b
ALB	5.23 ± 0.21 ^{a,b}	5.67 ± 0.39 ^{b,c}	5.73 ± 0.30 ^c	5.59±0.17 ^{a,b,c}	5.12 ± 0.48 ^a
TP	74.75±4.87 ^{b,c}	74.96±9.44 ^{b,c}	76.94 ± 2.12 ^c	66.42±5.30 ^{a,b}	59.60±8.74 ^a
GLOB	35.82±7.92 ^{b,c}	37.44±3.94 ^{b,c}	38.65±5.27 ^c	27.82±5.42 ^a	24.25±10.97 ^a
CHOL	67.18±16.37 ^{b,c}	69.93±7.89 ^{a,b,c}	84.74±11.32 ^c	54.66±2.26 ^a	77.10±11.99 ^{b,c}
GLU	3.47±0.95 ^a	3.77±0.95 ^a	4.21±0.29 ^a	4.26±1.73 ^a	4.41±0.79 ^a
BIL	6.29±2.81 ^a	7.65±1.45 ^a	8.86±7.83 ^a	5.71±1.94 ^a	5.90±0.90 ^a

Each value is a mean of 3 replicates ± standard deviation; values accompanied by identical superscript letters are not significantly different (p≤ 0.05). Aspartate aminotransferase (AST-μKat/L); Alanine aminotransferase (ALT-μKat/L); Albumin (ALB-μmol/L); Total protein (TP-g/L); Globulin (GLOB-g/L); Cholesterol (CHOL-mg/dL), Glucose (GLU- mmol/L); Bilirubin (BIL-μmol/L). A = Group administered with 0.10g/kg MSG; B = Group administered with 0.15g/kg MSG; C = Group administered with 0.20g/kg MSG; D = Group administered with 0.20g/kg Food grade MSG; E = Control group.

Figure.1 Histology of the ovaries of experimental rats after MSG treatment. A = Group administered with 0.10g/kg MSG; B = Group administered with 0.15g/kg MSG; C = Group administered with 0.20g/kg MSG; D = Group administered with 0.20g/kg Food grade MSG; E = Control group



Monosodium Glutamate (MSG) is a substance widely used as a flavoring agent in the whole world. However, its safety has been questioned (Biodun and Biodun, 1993) and it was discovered to be possibly harmful to the body organs including the ovaries (Eweka and Om'iniabo, 2011). Monosodium glutamate is a widely used flavor enhancing that may be present in packaged foods without appearing on the label (Adrienne, 1999). Consumption of MSG has been shown to cause metabolic disorders and oxidative damage of tissues (Dinz *et al.*, 2005) which may possibly be responsible for the pathophysiology of many diseases like cancer, diabetes, endothelial dysfunction brain lesion and Coronary Heart Disease (Dinz *et al.*, 2005; Mallick, 2007).

After administration of analar and food grade MSG, the rats showed significant increase in body weight, this agrees with the previous work of Oluba *et al.* (2011) who reported that consumption of MSG increases body weight gain. There was a significant increase in the activity levels of the aspartate amino transferase, alanine amino transferase, albumin, total protein, globulin and bilirubin dose-dependently in the serum of the rats administered with MSG when compared to the control group, this is in agreement with the significant elevation of the serum marker recorded by Ajibade *et al.* (2013) which was also confirmed by Nayira *et al.* (2009) who reported a significant increase in AST and ALT level upon MSG administration. Decrease in the activity of glucose in MSG administered rats is suggestive of decrease glucose utilization through the glycolytic pathway (Diniz *et al.*, 2004). Diniz *et al.* (2004) had suggested a possible deterioration of glucose tolerance in rats following administration. The reduced glucose tolerance could be attributed to decreased cellular sensitivity even under conditions of hyperinsulinemia observed in

animals administered with MSG (Macho *et al.*, 2000).

Female infertility is a very real medical problem. The female reproductive system is very sensitive to different harmful environmental factors (Bojanic *et al.*, 2009). A variety of environmental chemicals, industrial pollutants and food additives have been implicated as causing harmful effects. Most food additives act as either preservatives or as enhancers of palatability (Moore, 1999). One of such food additive is monosodium glutamate (Zerasky, 2010). Many investigators have studied the effect of MSG on the female reproductive system. They found that MSG plays a critical role in the pathogenesis of anovulatory infertility (Bojanic, 2009; Eweka and Om'iniabo, 2011) but the mechanism of MSG action has not been explained well yet.

In this study, ovarian sections of rats treated with MSG for 14 days showed considerable structural changes, including degenerated follicles, oocytes, degenerated medulla with vacuoles having congested blood vessels. These findings were in agreement with those of Bojanic and colleagues (Miskowiak *et al.*, 2000; Bojanic *et al.*, 2009; Eweka and Om'iniabo, 2011) who reported cystic degeneration of the ovary and degenerative atrophic changes within the oocytes in rat ovaries after MSG treatment. The ovaries contained many atresic follicles with no corpora lutea. Furthermore, the severe atresic state reported in this study may be indicative of the high dose of analar MSG used.

The degeneration of ovarian follicles and their oocytes detected in this study may be due to oxidative stress caused by MSG. This agrees with the results of Ismail and colleagues (Ismail, 2012; Al-mosaibih, 2013) that attributed ovarian pathologies

after MSG administration to oxidative damage. This could be explained by the fact that MSG leads to the generation of oxygen-derived free radicals and related reactive oxygen species (ROS). These substances are dangerous for biological systems as they react with DNA, proteins and lipids, leading to cellular damage as previously shown by Singh and Ahluwalia, (2003).

Congestion of blood vessels within the ovarian medulla may be due to the inhibition of prostaglandin synthesis, as these compounds are known to be involved in the regulation of blood flow. This commensurate with the results reported by Ismail, (2012) who demonstrated congestion of the testicular blood vessels following MSG treatment of male rats. Abnormality in ovarian function usually leads to anovulatory infertility, which constitutes a major problem (Eweka and Om'iniabo, 2011).

Conclusively, these findings showed that monosodium glutamate consumption caused severe damage to the ovaries of female Sprague dawley rats at higher dosage. The relationship between glutamate-induced atresia and its functions is recommended for further studies on a long-term exposure basis.

References

- Adrienne, S. (1999). The toxicity of MSG, a study in suppression of information. *Accountability Res.* 6(4): 259-310
- Ajibade A. J., Fakunle P. B., Olayemi O. T. and Adetogun A. A. (2011). Some morphological observations on the heart of adult wistar rats following exposure to aqueous extract of *Ocimum gratissimum*. *International Journal of Current Research* 3(12): 100-104.
- Al-Mosaibih M. A. (2013). Effects of monosodium glutamate and acrylamide on the liver tissue of adult Wistar rat. *Life Sci J* 10: 35
- Biodun D. and A. Biodun, "A Spice or Poison? Is Monosodium Glutamate Safe for Human Consumption?" National Concord, 4 January 1993, p. 5.
- Bojanic, V., Bojanic, Z., Najman, S., Savic, T., Jakovljevic, V., Najman, S. and Jancic, S. (2009). "Diltiazem Prevention of Toxic Effects of Monosodium Glutamate on Ovaries in Rats," *General Physiology and Biophysics* 28: 149-54.
- Diniz, Y.S., Fernando, A.A., Campos, K.E., Mani, F., B. Ribas, D., and Novelli, E.L. (2004). "Toxicity of Hyper Caloric Diet and Monosodium Glutamate: Oxidative Stress and Metabolic Shifting in Hepatic Tissue," *Food and Chemical Toxicology* 42: 319-325.
- Diniz, Y.S., Faine, L.A., Galhardi, C.M., Rodrigues, H.G., Ebaid, G.X., Burneiko, R.C., Cicogna, A.C. and Novelli, E.L.B. (2005). Monosodium glutamate in standard and high fiber diets: metabolic syndrome and oxidative stress in rats. *Nutrition* 21: 749-755.
- Eweka, A. O. and Om'iniabo, F. A. E. (2011). "Histological Studies of the Effects of Monosodium Glutamate on the Ovaries of Adult Wistar Rats" *Annal of Medical & Health Sciences Research* 1(1): 37-44.
- Freeman, M. (2006). "Reconsidering the Effects of Monosodium Glutamate: A Literature Review," *Journal of the American Academy of Nurse Practitioners* 18(10): 482- 486.
- Geha, R. S., Beiser, A., Ren, C., Patterson, R., Greenberger, P. A., Grammer, L. C., Ditto, A. M., Harris, K. E.,

- Shaughnessy, M. A., Yarnold, P. R., Corren, J. and Saxon, A. (2000). "Review of Alleged Reaction to Monosodium Glutamate and Outcome of a Multicenter Double-Blind Placebo-Controlled Study," *The Journal of Nutrition* 130(4S Suppl): 1058S-62S.
- Geha, R.S., Beiser, A., Ren, C., Patterson, R., Grammar, L.C., Ditto, A.M., and Harris, K.E. (2001). "Review of Allergic Reaction to Monosodium Glutamate and Outcome of a Multicenter Double Blind Placebo-Controlled Study," *Journal of Nutrition* 130:1032S-1038S.
- Indebir S. (2012). General structure of ovary. In: Textbook of Human Histology. 5th edition. Jay peebrother medical publisher, New Delhi India. 286 – 292.
- Ismail N. H. (2012). Assesment of DNA damage in testes from young Wistar male rat treated with monosodium glutamate. *Life Sci J.* 9: 930 – 939.
- Macho, L., Fickova, M., Jezova, D., Zorad S. (2000). Late effects of postnatal administration of monosodium glutamate on insulin action in adult rats. *Physiol. Res.* 49 (Suppl.1) S79-S85.
- Mallick H. N. (2007). Understanding safety of glutamate in food and brain. *Ind. J. Physiol. Pharmacol.* 51: 216-234.
- Miskowiak, B., Kesa, B., Limanowski, A., Partyka, M. and Filipiak B. (2000). Long-term effect of neonatal monosodium glutamate (MSG) treatment on reproductive system of the female rat. *Folia Morphol* (Praha) 58: 105-113.
- Moore, K. L. (1999). Congenital Malformations due to environmental factors. 2nd Edn., Developing Humans W.B Saunders Co. Ltd. Philadelphia, pp:173-183.
- Nayira, A., Abdel B., Azza M. M. and Faddah, L.M. (2009). Protective effect of N-acetyl Cysteine and /or Pro vitamin A against Monosodium Glutamate Induced Cardiopathy in Rats. *Journal of pharmacology and Toxicology* 4(5): 178-193.
- Oforofuo, I. A. O., Onakewhor, J. U. E. and Idaewor, P. E. (1997). "The Effect of Chronic Administration of MSG on the Histology of the Adult Wistar Rat Testes," *Bioscience Research Communications*.9: 2
- Okwurawie P. E. (1992). The role of food and Drug Administration and control (FDA&C) in ensuring the safety of food and food ingredients: A symposium held at Sheraton Hotel, Lagos. Sep 1st 1992 pp. 16–15
- Oluba, O. M., Onyeneke, E. C., Idonije, B. O. and Eidangbe, G. O. (2011): Effect of Soy Protein on Monosodium Glutamate (MSG)-induced Obesity in Rats. *Asian J. Pharm. Biol. Res.* 1 (1): 8-14.
- Onakewhor, J.U.E., Oforofuo, I.A.O and Singh, S.P (1998)"Chronic Administration of Monosodium Glutamate In- duces Oligozoospermia and Glycogen Accumulation in Wistar Rat Testes," *Africa Journal of Reproductive Health* 2 (2): 190-197.
- Peters, T., Biamonte, G.T. and Doumas, B.T. (1982). Protein [total protein] in serum, urine and cerebrospinal fluid; albumin in serum. *Selected method of clinical chemistry.* Vol. 9.
- Reinold (1953). Standard methods of clinical chemistry. ED. Reiner Acad Press, New York. 88.
- Rhodes, J., Titherley, A. C., Norman, J. A., Wood, R. and Lord, D. W. (1991). "A Survey of the Monosodium Glutamate Content of Foods and an Estimation of the Dietary Intake of Monosodium

- Glutamate,” *Food Additives and Contaminants*, 8(5): 663-672.
- Schiffman S.S. and Gill J.M. (1987). Psychophysical and neurophysiological taste responses to glutamate and purinergic compounds. In: Kawamura, Y., Kare, M. *Reviews. Umami: A basic taste*. New York: Marce Dekker Inc; 271 – 288.
- Schmidt, E., and Schmidt, F.W. (1963). Enzym-Bestimmungen im Serum bei Leber-Erkrankungen. Funktions-Muster ala Hilfsmittel der Diagnose. *Enzym. Biol. Clin.* 3:1.
- Siedel, J., Schlumberger, H., Klose, S., Ziegenhorn, J., Wahlefeld, A.W. (1981). Improved reagent for the enzyme determination of serum cholesterol. *J. Clin. Chem. Clin. Biochem.* 19: 838-839
- Singh K. and Ahluwalia P. (2003). Studies on the effect of monosodium glutamate (MSG) administration on some antioxidant enzymes in the artresial tissue of adult male mice. *J Nutr SCi Vitaminol* (Tokyo) 49: 145-148.
- Richterich R. (1962). Determination of serum cholinesterase by means of an indicator pape]. *Schweiz Med Wochenschr.* 92: 263–265.
- Walker, R. and Lupien, J. R. (2000). “The Safety Evaluation of Monosodium Glutamate,” *Journal of Nutrition*, 130(4S Suppl): 1049S- 1052S.
- Yamamoto T, Matsoo R, Fujimoto Y, Fukanoga I, Miyasaka A and Imoto. (1991). Electrophysiological and behavioral studies on the taste of umami substances in the rat. *Physiol. Behav.* 49(5): 919 – 925.