Histological Changes after Monosodium Glutamate Administration on Reproductive System for Each male and Female Adult Albino Mice

Noor Noori Abid AL-Shemary

Middle Technical University, Institute of technical ,Wasit-IRAQ

Abstract:

Monosodium Glutamate (MSG) is the sodium salt of glutamate (simply glutamate, water and sodium) and is widely used as food additive and as flavoring agent to increase appetite. The present study was to evaluate the effect of daily oral admiration of (MSG) (100mg/kg/day) for 30day on reproductive system of males and females Swiss albino mice. Forty albino mice weighting about (25-30kg) were used in the present study. The animal were divided in two groups: control group (10 males and 10 females)(n=20) and treated group(males and females) n=20.The body weight of the animals detected at the beginning and the end of experiment. At 30 days animals sacrificed and ovaries, uterus, testis were removed and weighted, Samples of ovaries, uterus testis fixed in 10%formaline for histological study. The final result show highly significant increase in the body weight and show significant decrease in the weight of ovaries and testis. The histological study in the treated group of testis show suppression of spermatogenesis, degenerate of seminiferous tubules and congestion of blood vessels. The histological changes in the ovaries such as congested blood vessels of medulla, increased atretic follicles and uterus show necrosis in perimetrium , presence of lacunae in the uterine wall structure .

Conclusions:

Monosodium glutamate has negative effect on reproductive system for each sex. So that will effect on the fertility .Also the increased body weight could lead to increase the incidence of congestive heart diseases and diabetes mellitus.

Keywords:

Monosodium glutamate, male and female mice, testis, ovaries, uterus, Histopathology

Introduction:

MSG is the sodium salt of glutamate (simply glutamate, water and sodium)[1] and is widely used as food additive and as flavoring agent to increase appetite[2] ;due to presence of Na ion and the appetizer effect intensively increased by presence of glutamate ion on gustator nerve[3] .Through its stimulation of oro-sensory receptor and by improving the palatability of meals. MSG effect the appetite positively and induce weight gain [4] ; because the effect of MSG on hypothalamus lead to prevention of sequence signaling of leptin action[5] .

MSG induce side effect in experimental animals even at relatively lower concentration [6] such as numbness, weakness ,flushing , sweating and headache. In addition to this symptom ingested MSG has been alleged exacerbate numerous condition include asthma, atopic dermatitis, neuropathy and abdominal discomfort [7]. Consumption of MSG has been shown to cause metabolicdisorders and oxidative damage of tissues [8] which may possibly beresponsible for the pathophysiology of many diseases like cancer, diabetes, endothelialdysfunction , brain lesion and Coronary Heart Disease [9].

Some studies were done to find the effects of MSG on the tissues resoponsible with reproduction e.g. testis, ovary, uterus etc in the experimental animals (rats mice) [10]. The researchers, though reported reduction in weight of both testes and ovaries [11] and histomorphological changes on ovary like, increased number of atretic follicles, reduced number of Grafian follicles [12] no corpora lutea etc [13] .Fertility rate has been reported to be reduced in both sexes [14].

In addition MSG induced male infertility by inducing testicular hemorrhage and inhibition of spermatogenesis [15] and reduction of sperm count and increase of sperm abnormities [16] . Higher doses of MSG led to damage of the hypothalamic nuclei and subsequently disturbance sin the hypothalamic-pituitary axis [17] ; MSG has been shown to pass through the placenta barrier. Monosodium-L-glutamate given subcutaneously to pregnant rat cause acute necrosis. However embryonic neurons were more sensitive to glutamate which is dose dependent so that MSG increase trans placental poisoning in human.

Materials and Methods:

Chemicals

The salt used in this study is MSG commercially available in the markets of Baghdad. A stock solution was prepared by dissolving known gram (1-5g) of monosodium glutamate crystals in (100mL) of distilled water. The dose scheduled was represent the amountof MSG administrated per animal with respective group weights [18].

Experimental Animals

This study was performed on forty adult Swiss albino mice obtained from animal house of Al-Kut technical institute and their ages between 6-10 weeks and weighting about (25-30gram). The mice were housed under appropriate controlled room in approved cages with the standard temperature (22C-25ºC) , light (12hrs. Light ; 12 hrs Dark), humidity and fed with pellet during experimental period.

Clinical examination of the experimental animals:

The mice were randomly divided into two equal groups (control group and treated groups):1-Control group C. (n = 20) (10male +10female) received distilled water for 30 day. 2-Treated group. (n=20)) (10male +0female) were treated with MSG administrated orally by cavage needle for 30 days. The body weight of each animals was determined at the beginning of treatment and before begin sacrificed, by electrical balance. At the end of the experimental period, the animal were sacrificed by cervical dislocation and they were dissected, ovaries, uterus ,testes weighted by sensitive balance then preserved in 10% formalin buffer solution until preparation of histopathological section. Tissue was cut at 7-8µm and embedded in paraffin and takes sections of ovaries, uterus ,testes were stained with hematoxylin–Eiosin stain (H&E) for histopathological study [19, 20].

Statistical analysis:

Data from treated and control groups were expressed as mean + standard error (M + SE), and analyzed by using the students' – test. P value ($p<0.05$) was considered significant while ($p<0.01$) considered highly significant [21].

Results:

1- Body Weight and reproductive organs weight:

The effect of oral treatment with monosodium glutamate (MSG) on animal body weight of both sex (males and female) and reproductive organ weight were recorded for all treated animals they were presented in (Table 1 and Figure 1, 2, 3, 4). After twenty day of treatment at dose 1oomg/kg/day the result showed highly significant ($P<0.01$) increase in the body weight in both sex and weight of ovaries when compare with control group ;while the result showed significant ($P<0.05$) increase in the weight of testis in comparison with control group.

Table (1): Effect of Monosodium glutamate (MSG) on the body weight (male+ female) of the adult mice and weight of testis, ovaries.

Variable / Groups	Male Body Weight (gm)	Testis weight (gm)	Female Body Weight (gm)	Ovaries weight (gm)
Control	05017± 31.920	0.3250 ± 0.1128	27.290 ± 0.542	0.2620 ± 0.056
Treated	** 38.260± 0.4012	* 0.0440 ± 0.00636	** 37.130 ± 0.825	** 0.03048 ± 0.077

The value represent Mean(gram) + Standard Error ; Significant* ($p<0.05$), highly Significant**($p<0.01$).

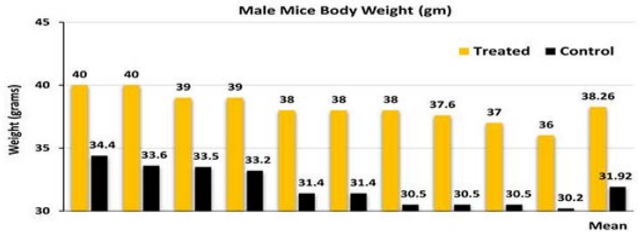


Figure 1: Mean difference in body weight of male treated mice vs. control (*t-test: -9.869, df: 18, p-value <0.01*).

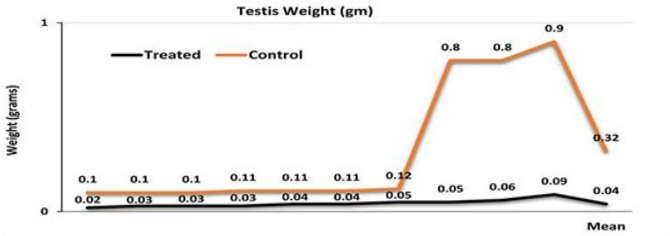


Figure 2: Mean difference in testis weight of treated mice vs. control (*t-test: 2.251, df: 18, p-value <0.05*).

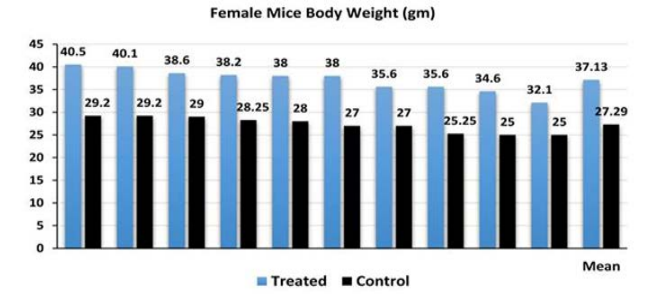


Figure 3: Mean ± S.D. differences in body weight of female treated mice (37.13 ± 2.61) vs. control (27.29 ± 1.71), (*t-test: -9.960, df: 18, p-value <0.01*).

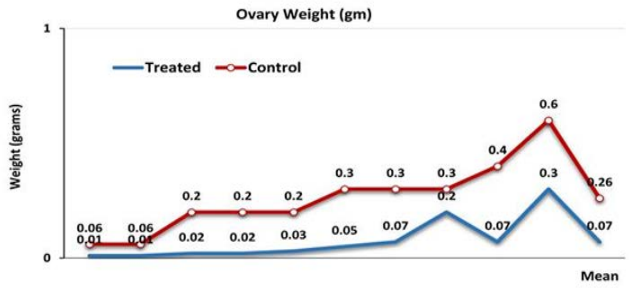
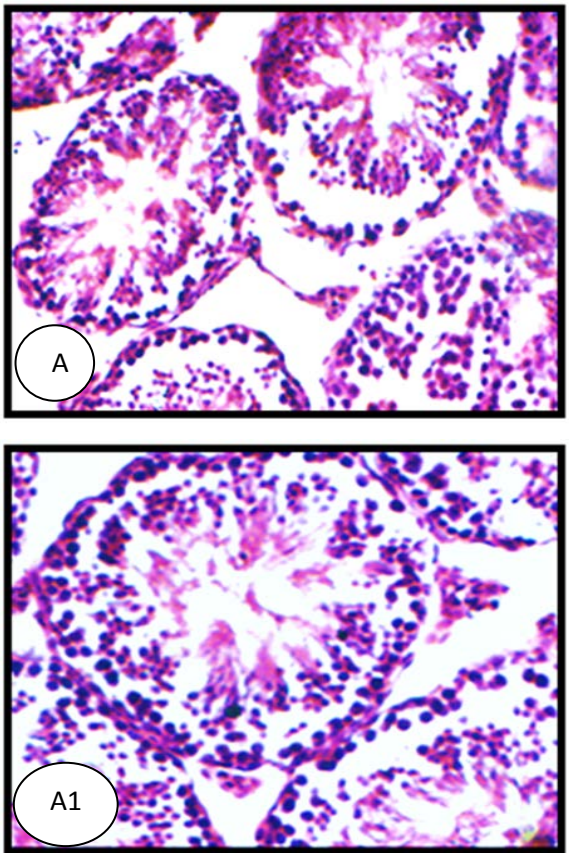


Figure 4: Mean ± S.D. differences in ovary weight of treated mice (0.07 ± 0.096) vs. control (0.26 ± 0.160), (*t-test: 3.130, df: 18, p-value <0.05*).



Plate(1): Section in Testis of Group I (control group) in mice showing normal histological structure of the seminiferous tubules and showing spermatogonia, primary spermatocyte, spermatide and Sertoli cell. (H&E) A (10X)A1 (40X).

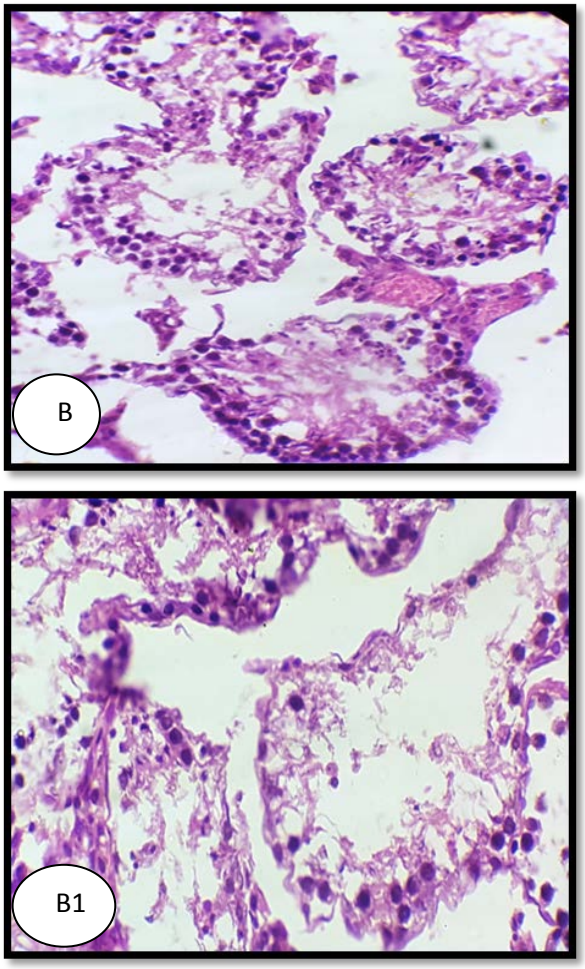


Plate (2) : Section in Testes of treated groupII (treated group with MSG 100mg/kg/day for 20 day) in mice showing suppression of spermatogenesis, Reduced numbers of spermatozoa in the lumen, degenerate of seminiferous tubules, congestion of blood vesseles and vaculation of spermatogonia (H&E) B (10X), B1 (40X).

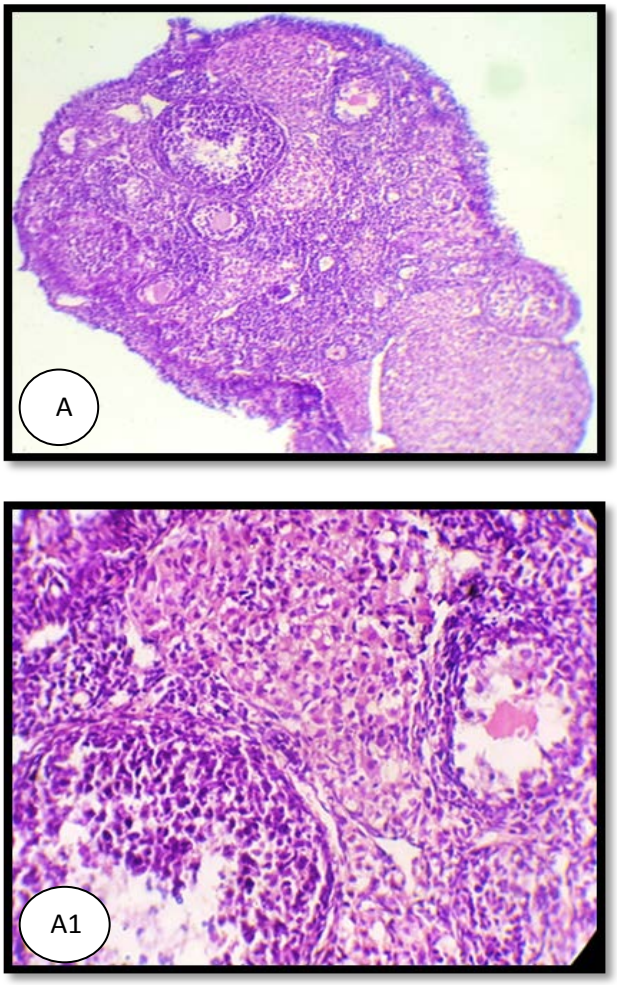
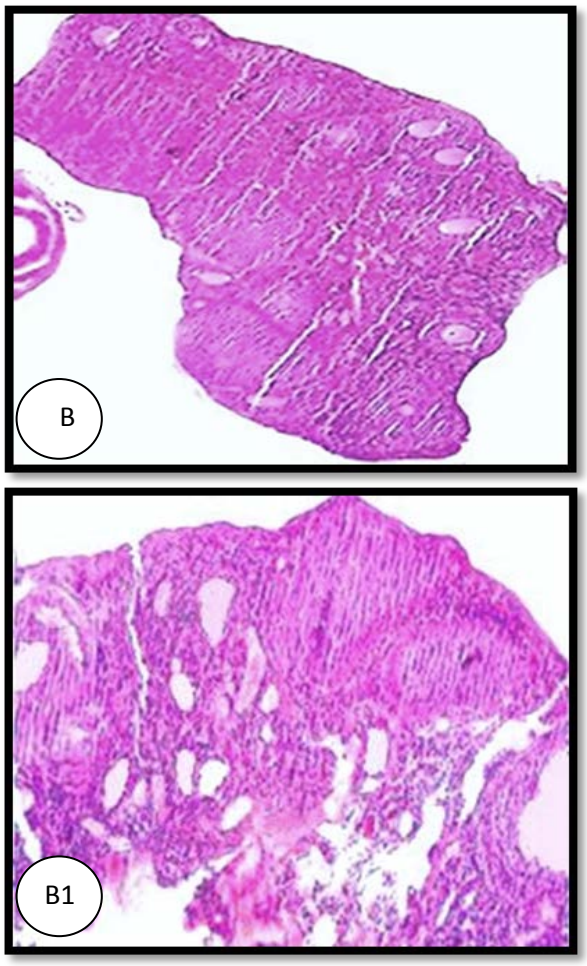
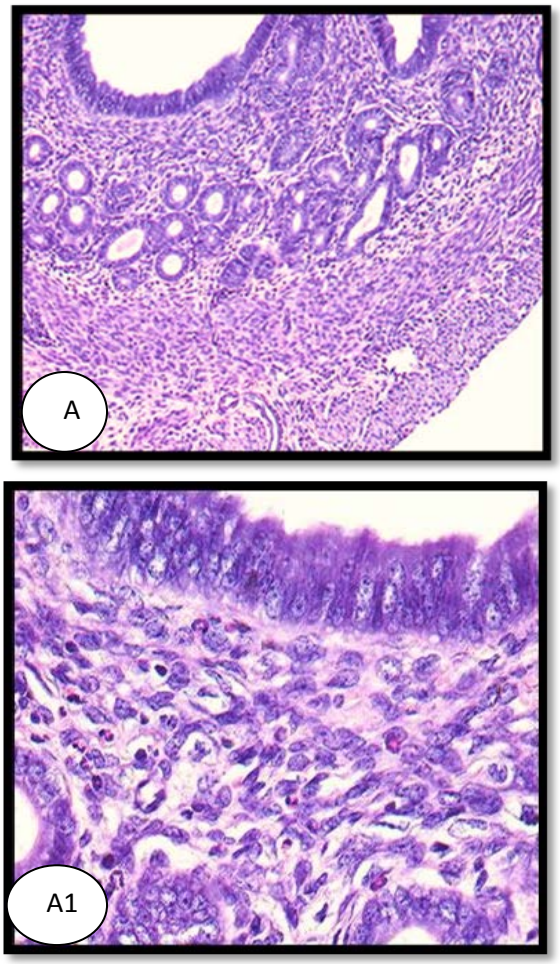


Plate (3): Section in ovary of group I (control group) shows. medulla, primary follicle, secondary follicle ,mature follicle, germinal epithelium, corpus luteum (H&E) A, A1 (10X)



Plate(4): Section in ovary of Group II (treated group with MSG 100mg/kg/day for 20 day), shows congested blood vessels of medulla, vacuolated cells, increased atretic follicles. (H&E) B (10X), B1 (40X)



Plate(5): Section in uterus of group I (control group) in mice showing normal histological structure of uterus (H&E) A (10X), A1 (40X).

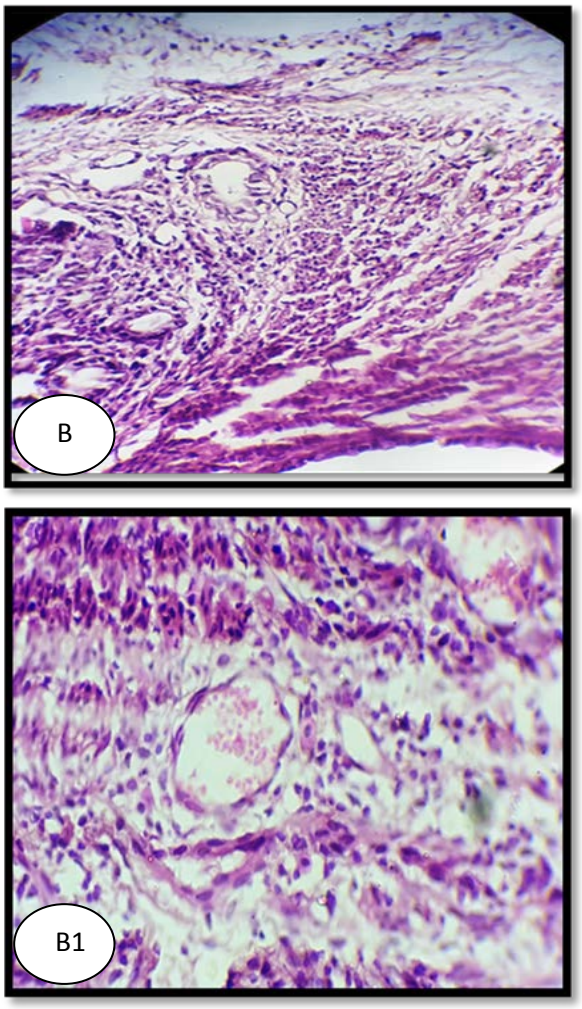


Plate (6): Section in uterus of group II (treated group with MSG 100mg/kg/day for 20 day) in mice showing degenerative changes and mild inflammatory cells, congestion of blood vessels, degenerative changes and necrosis in perimetrium, presenseof lacunae in the uterine wall structure. (H&E) B1 (10X), B2 (40X).

Discussion:

Monosodium glutamate (MSG) which is one of the main flavour enhancer used in the food products ,it has toxic effect on different organs [22] . These effects include the nervous system, retina endocrine glands involvedovary ,testis etc.. [23] .

The effect of monosodium glutamate on the body weight of both sex (males and females) after adminstration (100mg/kg/day for 30 day) showing a highly significant increase in the body weight of both sexes in comparison with control group.This increment may attributed to many reasons ; one reason obesogenic properties of MSG by inhibition of leptin (protein hormones responsible for regulation the appetite and metabolism) and insulin which effect the amount of food consumption [24] . The amount of consumpted MSG play roles in weight gain;because glutamate relays singling between digestive system and nervous system. Some studies suggest that the MSG cause scaring of the arcuate nucleus of the hypothalamus lead to increase adiposity and body mass in human and rats [25], the second reason MSG administration may cause increase in the levels of ALT and AST lead to reduce glucose tolerance therefor lead to body weight gain [26] . This result is in agreement with Oluba,. etal 2008 who notice increase the body weight after administration of MSG.

The results in table (1) show significant decrease in the weight of the testis in mice after admiration of MSG that can be explained by many mechanisms.First mechanism the neurotoxic effect of MSG on the hypothalamus – pituitary– gonads axis lead to decrease in the testosterone secretion that effect on spermatogenesis (decrease) [27] ; the second mechanism the effect of MSG on glutamate receptor and transporter in testis cause reduction in the levels of testosterone which caused decreasedthe spermaogensis followed by a testicular weight reduction [28] ; third mechanism MSG effect on vitamin C (antioxidant) levele in testis lead to oxidative damaged in testis that influence on the testosterone that cause reduction in

the weight of testis [29] ; similar result by Saber and Jamal, 2013 who notice decrease in the weight of the rats testis after a treatment of MSG. The histological section of testis after treatment of MSG showed suppression of spermatogenesis because the MSG cause damage to the nerve cells of the hypothalamus it may alter the neural control of reproductive hormone(FSH, LH, Testosteron) lead to suppression of spermatogenesis this result in agreement with Aisha,(2013) [30] ; who noticed inhibiton of spermatogensis after administration of MSG. The results of the testis is showed reduction in spermatocytes, degenerate of seminiferous tubules of the group treated with MSG compared to the control the vacuolations observed may be due to MSG interference [30] . These results were in accordance with Ihab (2012) [37] ; who study similar histopathological changes in the testis after administration of MSG.

The result in the table (1) show significant decrease in the weight of ovaries after administration of MSG (100mg/kg/day for20 day) because the MSG effect on the hypothalamus – pituitary –gonads axis causing a reduction in level of FSH and LH leading to inhibition of follicles and hypogonadism [10] .The histological section of ovaries show increase of follicular atresia, degeneration of oocytes , vacuolated granulosa, and congestion of blood vessels, were observed. These results were in accordance with Oforofuo *et al*., (2015) [15] who study similar follicular damage in rat ovaries after MSG adminstration. Al-mosaibih (2013) [32] attributed the MSG-related degeneration of ovarian follicles lead to increase of oxidative stress. Vacuolation of the granulosa cells after MSG-administration may assimilate a type of cellular defense against toxic agent such as MSG [33] ; therfore vacuolation represent origin of accumulating toxic agents interfering with its biological interactions in cell metabolism [34] . Congestion of ovarian blood vessels may attributed that the MSG cause inhibition of prostaglandin synthesis which is necessary for maintaining blood flow [15] this result agree with Snoor *etal*.,(2015) [38] . Who showed similar histological changes on ovaries after administration of MSG.

The histological section of uterus after administration of MSG (100mg/kg/day for 20 day) showingdegenerative changes; mild inflammatory cells infiltrate and degenerative changes with focal necrosis in perimetrium and congestion of blood vessels and lacunae in the wall of uterus. The results of the histopathological studies; suggest that MSG may depress the function of the uterus by inducing structural degenerations in the wall structure by oxidative stress and lipid peroxidation in uterine tissue [35] ; this result in agreement with Muki *etal* .,(2015) [36] , who notice hisyological changes on uterus after adminstration of MSG.

Conclusions:

1-Monosodium glutamate has negative effect on reproductive system for each sex. So that will be as a mimic for an effect on the fertility of human.

2-Also it can mimic for increase the body weight of human ,so increase the incidence of congestive heart diseases and diabetes mellitus.

References:

1. Filer, L.J. & Stegink, L.D.(1994). Report of the proceedings of the glutamate

workshop, August 1991 Crit. Rev. Food Sci. Nutr., 34(2):159-174.

2.Schiffman S.S. and Gill J.M. (1987) Psychophysical andneurophysiological tastee and purinergic compounds responses toglutamate andpurinergic compounds.Inc; 271 288.

3.Yamamoto T, Matsoo R, Fujimoto Y,Fukanoga I, Miyasaka A and Imoto(1991). Electrophysiological andbehavioral studies on the taste ofumami substances in the rat. PhysiologicalBehav. 49(5): 919 925.

4.Biodun D (1993) Is monosodium glutamatesafe for human consumption. Natl Concord 1993;4:5

5. LianchengK Z.(2008): Association of Monosodium Glutamate Intake With overweight inChinese Adults: The intermapstudy. Obesity; 1875-1880.6.

6. Egbuonu A, Obidoa, O., Ezeokonkwo, C., Ezeanyika, L., Ejikeme, P. (2009). Hepatotoxic effects of low dose oral administration of monosodium glutamate in male albino rats. Afr J Biotechnol. 8:3031–5

7. Geha R, Beiser A, Ren C, Patterson R, Grammer L, Ditto A (2001). Review of allergic reaction Monosodium glutamate and outcome of a multicenter double blind placebo- controlled study. J Nutr;130:1032S–8S.

8. Diniz Y.S, Faine L.A, Galhardi C.MRodrigues, H.G., Ebaid, G.XBurneiko, R.C., Cicogna, A.C. andNovelli, E.L.B. (2005). Monosodium glutamate in standard and high fiber diets: metabolic syndrome and oxidative stress in rats. Nutrition 21 749-755.

9.Mallick H. N. (2007). Understanding safetyof glutamate in food and brain. Ind. JPhysiol. Pharmacol. 51: 216-234.

10. Das R.S and Ghosh SK (2011) . Long term effects in ova-ries of the adult mice following exposure to Monosodium glutamate during neonatal life a histological study.NepalMed Coll J 13(2): Pp77-83.

11. Olney JW, Sharpe LG, Feigin RD.(1972)" Glutamate in-duced brain damage in infant primates. J Neuropa-thol Exp Neurol; 31: 464-87.

12. Miskowiak B, Kęsa B, Limanowski A, Patryka M, Filipiak B. (2000).Long term effect of neonatal monosodium glutamate (MSG) treatment on reproductive system of the female rat. Folia Morphol.58:105–13.

13.Rodriguez-Sierra JF, Sridaran R, Blake CA.(1980): Monosodium Glutamate disruption of behavioral and endocrine function in the female rat. Neuroendo-crinol 31: 228-35.

14. Pizzi WJ, Barnhart JE. (1977:Monosodium glutamate administration of newborn reducesreproductive ability in female and male mice. Sci 1977; 196: 452-4.

15.Oforofuo O, Onakewhor J, Idaewor P.(1997): The effect of chronicadministration of MSG on the histology of the adult Wistar rat testes. Biosci Res Commun;9:2.

16.Onakewhor J, Oforofuo I, Singh S.(1998): Chronic administration ofmonosodium glutamate induces oligozoospermia and

glycogenaccumulation in Wistar rat testes. Afr J Reprod Health;2(2):190–7

17.Seo, H.J., Ham, H.D., Jin, H.Y., Lee, W.H., Hwang, H.S., Park, S.A., Kim, Y.S., Choi, S.C., Lee, S., Oh, K.J., Kim, B.S., Park, B.R., Lee, M.Y.(2010). Chronic administration of monosodium glutamate under chronic variable stress impaired hypothalamic-pituitary-adrenal axis function in rats. Korean J Physiol. Pharmacol. 14:213-21.

18.Tyl R, and Friedman ,(2003). Effects of acrylamide on rodents reproductiveperformance. Reprod Toxicol;17(1):1–13

19. Bancroft J.D, Gamble M.(2002) Theory and practice of histologicaltechniques. 5th ed. Edinburgh: Churchill Livingstone Pub. Pp. 172–172.

20.Hummdi L.A.(2012) Histopathological and ultrastructural changes in renal corpuscle of female rats topical application by P-phenylene diamine. Int J Zool 8(3):106–20

21. Sorlie D. G. (1995): Medical biostatistic and epidemiology: examination and board review. (1st ed). Appleton and Lang, Norwalk, Connecticut. Pp: 47 –8.

22. Walker R. and Lupien, J. R. The Safety Evaluation of Monosodium Glutamate, Journal of Nutrition 2000; 130(4S Suppl): 1049S- 1052S

23.Garattini S.(1974). Evaluation of neurotoxic effects of Glutamic acid Nutrition and the Brain, Raven Press New York NY: P:79-124.

24.Matyskova R, Maletinska L, etal.,(2008) comparison of the obsity phenotype related to monosodium glutamate that effect on arcuatenucleus and /or high fat diet feeding in C57BL/6 and NMRI MICE" Physiological research ,57(5):727-34.

25. Pepino M.Y, Finkbeiner S, Beauchamp G.K, Mennella J.A,(2010)."Obese women have lower Monosodium glutamte tast sensitiviyu and prefer higher concentrationthan do normal obsityWeight. 18:959-965.

27. Gill S, Barker M., Pulido O. Neuroexcitatory tar-gets in the female reproductive system of the non-human prinates (Macca fascicularis). Tox-icol.Pathol 2008; 36: 478-484.

28.Giovambattista A, Suescun M, Nessralla C, Franca L, Spinedi E, Calandra R.(2003): Modulatory effects of leptin onleydig cell functionof normaland hyperleptinemic rats. Neuroendocrinology 78:270–9.

29 . Farmobi E, Onyema O. Monosodium glutamate-induced oxidative damage and genotoxicity in the rat modulatory role of vitamin C, vitamin E and quercetin. Hum Exp Toxicol 2006;25:251–9.

30. Aisha D.(2014). Alalwani. Monosodium glutamate in-duced testicular lesions in rats (histological study).Middle East Fertility Society Journal; 19, 274–280.

31.Onakewhor J, Oforofuo I, Singh S.(1998): Chronic administration ofmonosodium glutamate induces oligozoospermia and glycogenaccumulation in Wistar rat testes. Afr J Reprod Health;2(2):190–72.

32. AL-Mosaibih M.A.(2013). Effects of Monosodium Glutamate and Acrylamide

on the LiverTissue of Adult Wistar Rats. Life Science Journal., 10(2):35-42.

33.AliA, El-Seify, El Haroun,H, Soliman.,M..(2014). Effect of monosodium glutamate on the ovaries of adult female albino rats and the role ofgreen tea. Menoufia Med. Journal., 27:793–800

34. Cheville N.F.(2009): Ultrastructural Pthology: The com-parative cellular basis of disease.2nd ed.USA. Wi- ley-Blackwell. A John Wiley of Sons Inc;-1,1-9:(8).

35. Noori S.(2012): An overview of oxidative stress and antioxidant defense system Sci Rep 1(8):1-9..

36. Mukti M, Kaushik S, Partha P and Goutam P.(2015) Monosodium glutamate depresses the function of female reproductive system in rat by prmoting oxidative stress in the structure of uterus. Int. J. Pharm. Sci. Rev. Res., 45(1): 238-241.

37.Ihab K.(2012):The Effects of Oral Dosage of MSG Applied for Short-and Long-terms on the Histology and Ultrastructure of Testes of the Adult Rats.J Anim.Vet.Adv. 11(1):124-133.

38.Snoor J,Hadeel A, Bushra H, and Ali I.(2015)Effect of Monosodium Glutamate on Mice ovaries and the possible protective role of Vitamin C. Annals of Applied Bio-Sciences, Vol. 2; Issue 4: ISSN: 2349-6991