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MONOSODIUM GLUTAMATE (MSG) INDUCED DEVELOPMENTAL DYSFUNCTION IN FEMALE ALBINO RATS *(RATTUS NORVEGICUS)*

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ABSTRACT
Effects of a flavor enhancer, monosodium glutamate (MSG) was evaluated in female albino rats during the gestational period. Different doses of MSG (0.4g/kg and 4g/kg body weight) were administered orally using drinking water as a vehicle for 0 -15th day of gestational period. The control received water only. On completion of the treatment period, the half of the experimental animals were sacrificed under light anesthesia using ether and the other half were allowed to deliver their pups. The body weight, the gravid uterine weight, organ weight, number of implantations, litter size, litter weight, growth rate of the viable off springs etc. were altered after MSG treatment. MSG also induced some abnormal changes in gestation such as abortion and or resorption of fetuses. The present study suggested that MSG adversely affected the fetal development of the pregnant female rats.

INTRODUCTION

Monosodium glutamate (MSG) is one of the commonest food additives in the developed world and it is a commonly used flavour enhancer. MSG can be found in various concentrations in numerous food products. MSG is a sodium salt of glutamic acid, a naturally occurring non-essential amino acid with trade names such as Ajinomoto, Vetsin, Ac'cent and Tasting Powder. Although, MSG could improve the palatability of foods by exerting a positive influence on the appetite centre, it increased body weight (Egbuonu *et al*., 2010) and adversely affected locomotor activities (Eweka and Om'Iniabohs, 2008) and the testis, causing significant oligozoospermia and abnormal sperm morphology in male Wistar rats (Onakewhor *et al*., 1998). It has also been established that MSG may be implicated in cases of male infertility as it causes testicular hemorrhage, degeneration and alteration of sperm cell population and morphology (Oforofuo *et al*., 1997). Olney and Sharpe (1969) reported hypothalamic lesions, stunted skeletal development, obesity, and female sterility, as well as a spate of observed pathological changes found in several brain regions associated with endocrine function in maturing mice which had been given GLU as neonates. Neuronal and endocrine dysfunction or abnormalities in growth and behavior induced by MSG have been noted in number of animal studies, but little is known from when it is administered orally in pregnant rats. Moreover, the great similarity and homology among the genomes of the rodents and of the humans turn the animal models into an important tool for the study of conditions that affect us and that can be simulated in rats. Therefore, the present study was designed to evaluate the effects of oral administration of MSG in the fetuses of female rats during the pregnancy period.

MATERIALS AND METHODS

The study was carried out on healthy, young adult, colony bred pregnant female albino rats (*Rattus norvegicus)* of Charles foster strain. These animals weighed 195g - 200g each. They were reared in the Department's animal house at a temperature of 22 $\pm$ 2ºC and exposed to 10-12 hrs of day light. Different experimental groups of the animals were caged separately and an average of 4 animals per cage was maintained. The control as well as other treated groups of rats was given free access to standard chow and water *ad libitum*. All these animals were housed in polypropylene and provided water in glass bottles.

Experimental protocol

A total of 60 animals, 40 females and 20 males were paired for mating. After 7 days of coupling, we obtained 36 female pregnant rats and were taken as the experimental animals. The experimental animals were divided into 3 groups, each group consisting of 12 rats, 2 groups were maintained as treated and one group was maintained as control. Monosodium Glutamate (MSG) (Chemical formula: $C_5H_8NNaO_4$, Molar mass: 169.111g/mol, Melting point 232ºC) was used for studying the developmental dysfunction in pregnant female rats. MSG dissolved in distilled water at dosage of 0.4g/10mL/kg body weight was given orally using a syringe to the animals of low dose MSG treated groups (Group II).The high dose MSG treated groups(Group III) on the other hand received MSG at a dose of 4g/10mL/kg body weight. The doses were selected after finding out the acute LD_{50} value which was

found to be 16.60g/kg (oral, rat) (Bush, 2003). The animals of control (Group I) received water as *ad libitum*. The treatment was given from 0 to 15th day of gestational period. The bodyweights of all the experimental animals were recorded every five days of interval.

On the completion of the respective experimental period, half of the pregnant animals were autopsied on the 20th day and the other half were allowed to complete their term and deliver their pups. From the autopsied animals, the organs were quickly excised, cleared off from the adhering fat and blotted free of blood. The absolute weights of the adrenals, kidney, spleen and liver of different experimental animal groups were recorded to the nearest milligram. The gravid uterus was dissected out from each animal, taken its weight and counted the number of implantations, resorptions and live pups. The number of corpus lutea were counted on the right and left sides of the ovary. The weights of the pups were noted to the nearest milligram. The body weights of the new born viable pups were recorded from the delivered female rats. The growth rate of the viable off springs was recorded from the delivered females

A minimum of 6 replicates were taken for each parameter and data was analyzed statistically using student's 't' test.

RESULTS AND DISCUSSION

Table 1 represents body weight, absolute body weight and gravid uterine weight of control and treated groups of female albino rats. The body weight of low dose and high dose MSG treated female albino rats was significantly ($p < 0.05$) decreased on the 20th day, due to increased number of resorptions after MSG treatment, when compared to control (Table 1). However, the absolute body weight (body weight devoid of gravid uterus) was insignificantly increased in MSG treated groups when compared to control and this increase was accordance with earlier findings. Researchers suggest that flavoring agent MSG at concentrations that only slightly surpass those found in every day human food, exhibits significant potential for damaging the hypothalamic regulation of appetite and thereby determines the propensity of world – wide obesity (Hermanussen *et al.*, 2006). The model of neonatal monosodium glutamate rats is of special interest regarding the development of obesity. Neonatal treatment with MSG has been shown to destroy hypothalamic arcuate nucleus neurons, resulting in several endocrine disturbances (Onley and Sharpe, 1969), stunted growth and obesity (Ribeiro *et al.*, 1997). There is a considerable experimental and clinical evidence that an altered body composition before and during pregnancy produces altered metabolism in the offspring; unbalanced maternal nutrition or over weight is associated with changes in metabolic control in the offspring, which then have a greater propensity to develop obesity (Gluckman *et al.*, 2007). The gravid uterine weight significantly ($p < 0.01$), ($p < 0.05$) decreased in MSG treated rats (Fig. 2 and 3) when compared to control (Fig. 1, Table 1). The decrease in the gravid uterine weight resulted from reduced litter size and increased number of resorptions after MSG treatment.

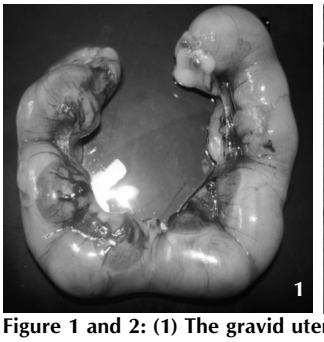

Figure 1 and 2: (1) The gravid uterus of control female albino rats. Uterus with normal number of embryos; (2) The gravid uterus of low dose MSG treated female albino rats. Uterus with reduced number of embryos and resorptions were seen

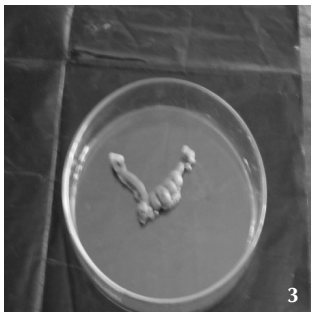

Figure 3 and 4: (3) The gravid uterus of high dose MSG treated female albino rats resorptions were seen and no normal embryos were found; (4) The litter weight of MSG treated control female albino rats

Table 2 represents number of corpus lutea, number of implantations and resorptions in control and MSG treated female albino rats. The number of corpus lutea did not show any significant change in MSG treated groups when compared to control. Implantations significantly ($p < 0.05$) decreased after MSG treatment. The decrease in the implantations resulted from the embryo as well as foeto toxicity induced by MSG.

Table 1: The body weight, absolute body weight and gravid uterine weight (g) of control and treated groups of female rats during the gestational period (0-20 days)

Parameter	Days	Control(g)	Low dose MSG treated(g)	High dose MSG treated (g)
Body weight(g)	0	200 ± 3.14	196 ± 2.92	197 ± 1.22
	5	209 ± 2.26	202 ± 3.39	216 ± 4.30
	10	215 ± 1.64	208 ± 4.35	228 ± 6.04
	15	220 ± 3.67	212 ± 8.15	220 ± 8.66
	20	235 ± 1.28	* 226 ± 11.11	*215 ± 10.95
*Absolute body weight (devoid of gravid uterus) (g)	20th day	202±1.24	204 ±3.2	*214 ± 5.2
Gravid uterine weight(g) (uterus with embryos)	20th day	33.33 ± 0.57	*22.14 ± 4.79	**11.35 ± 5.077

Values are mean ± S.E; *Significant ($p < 0.05$), **Significant ($p < 0.01$);* Absolute body weight on 20th day = body weight of 20th day - gravid uterine weight - body weight of first day

Table 2: Number of corpus lutea, number of implantations and resorptions in control and MSG treated female albino rats

Parameter	Control			Low dose MSG Treated			High dose MSG Treated		
	Right	Left	Total	Right	Left	Total	Right	Left	Total
Number of corpus lutea	4.21± 0.47	5.66± 0.54	9.87 ± 0.71	4.3 ± 0 .55	5.4 ± 0.68	9.7 ±1.03	4.2 ± 0.80	5.3± 0.37	9.6± 0.92
Number of implantations	4.3 ± 0.47	5.4 ± 0.47	9.7 ± 0.81	3.2 ± 0.75	3.1 ± 0.55	*6.3 ± 0.93	3.3 ± 0.69	4.2 ± 0.73	*7.5 ± 0.47
Resorptions	0	0	0	1 ± 0.08	1 ± 0.06	2 ± 0.05	*2 ± 0.05	*2± 0.03	*4 ± 0.04

Values are mean ± S.E; *Significant ($p < 0.05$)

MSG was found to cause infertility problems in test animals. Male rats fed MSG before mating had less than 50% success rate (5 of 13 animals), whereas male rats not fed MSG had over 92% success rate (12 0f 13 animals). Reports suggest that rabbits received 2.5mg/kg bodyweight, 25mg/kg and 250mg/kg of L-glutamic acid hydrochloride at 70h post coition and 192h post coition showed the corpora lutea, the resorbed, implanted, normal and deformed fetuses (Gottschewski, 1968). Resorptions were significantly ($p < 0.05$) increased in MSG treated rats when compared to control. According to some researchers, in pregnant rabbits, there were some abnormal changes in gestation such as abortion or resorption of foetuses, with an incidence of 21 per cent in the glutamic acid hydrochloride group, of 25 per cent after administration of monosodium glutamate. There were no external and skeletal malformations in the aborted fetuses (Yonetani, 1967). MSG has been shown to cross the placental barrier in rats and new studies suggest that in cases where human mothers who suffer from intrauterine infection are at risk to Glutamate causing excitotoxic prenatal brain injury to the fetus. Monosodium-L-glutamate given subcutaneously to pregnant rats caused acute necrosis of the acetyl cholinesterase-positive neurons in the area postrema. The same effect has been observed in the area postrema of fetal rats. The process of neuronal cell death and the elimination of debris by microglia cells proved to be similar in pregnant animals and in their fetuses (Gao *et al*., 1994). However, embryonic neurons were more sensitive to glutamate as judged by the rapidity of the process and the dose (John Erb Report on Monosodium Glutamate Presented to the WHO August 2006) response relationship. These observations raise the possibility of transplacental poisoning in human fetuses after the consumption of glutamate-rich food by the mother.

Table 3: Male litter size, female litter size and total litter size of control and treated groups

Parameter	Control	Low dose MSG treated	High dose MSG treated
Male litter size	4 ± 0.47	* 2 ± 0.12	*1 ± 2.11
Female litter size	5 ± 0.47	*2 ± 1.45	*2 ± 2.18
Total litter size	9 ± 0.81	*4 ± 2.15	*3 ± 2.14

Values are mean ± S.E; Significant ($p < 0.05$)

Table 4: The total male litter weight, the total female litter weight and total litter weight (g) of control and treated groups

Parameter	Control	Low dose MSG treated	High dose MSG treated
Total Male litter weight	15.12±0.05	8.14±0.04	*2.61±0.05
Total Female litter weight	14.15±0.04	*9.18±0.04	*6.12±0.07
Total litter weight	29.27±0.09	*17.32±0.08	**8.73±0.12

Values are mean ± S.E.; **Significant ($p < 0.01$); *Significant ($p < 0.05$)

Table 5: The organ weight (g) of control and treated groups of female albino rats

Parameter (Organ weight)	Control	Low dose MSG treated	High dose MSG treated
Liver(g)	5.35 ± 0.12	7.71 ± 0.35	6.48 ±0.73
Kidney(g)	5.35 ± 0.12	1.23 ± 0.024	1.50 ± 0.09
Spleen(g)	0.38 ± 0.04	0.057 ± 0.024	0.59 ± 0.056
Adrenals (mg)	0.08 ± 0.01	0.066 ± 9.26	0.075 ± 0.022

Values are mean ± SE

Table 3 and 4 represents litter size and litter weight of control and MSG treated female rats respectively. Litter size and litter weight (Fig. 4) significantly ($p < 0.05$) decreased in MSG treated rats when compared to control. The decrease in the litter size resulted from increased number of resorptions after MSG treatment. Researchers suggested that female rabbits (n = 10) received orally 25mg/kg body-weight of glutamic acid during pregnancy showed two animals with delayed pregnancy, the uterus containing degenerate foetuses. Two others had abortions of malformed foetuses. Two animals delivered at the normal time but the pups with reduced body weight and had various limb malformations. Four animals did not conceive. The pups did not become pregnant during seven months and showed limb deformities, decreased growth and development compared with controls. The histopathology showed scattered atrophy or hypertrophy of different organs (Turgrul, 1965).

Table 5 represents the organ weight of control and MSG treated female albino rats. The organ weights did not show any significant change in MSG treated groups when compared to control. Also the organ weights of glutamic acid treated female rabbits were not different from those found in controls (Turgrul, 1965). Table 6 represents the growth rate of control and MSG treated female albino rats. The growth rate of off springs was insignificantly increased in low dose and high dose MSG treated rats when compared to control. A consensus on MSG from 2007 states that the placentary barrier controls the passage of glutamate from the maternal plasma to the fetus metabolizing it before it reaches the foetal circulation (Beyreuther *et al*., 2007). Oral administrations of MSG to pregnant rats influence

Table 6: Growth rate(g) of pups of control and treated groups of female albino rats

Days	Control (g)	Low dose MSG treated (g)	High dose MSG treated (g)
0	4.88±0.14	4.73 ± 0.07	2.61 ± 0.16
5	9.45±0.23	9.12 ±0.65	6.04 ± 0.39
10	15.12±0.34	16.12 ±0.54	15.74 ± 0.34
15	20.15±0.32	21.32 ±0.76	22.34 ± 0.27
20	25.10 ± 0.45	26.17 ± 0.54	26.12 ± 0.24

Values are mean ± S.E

the body weight of the offspring that received MSG during prenatal life via maternal feeding. When MSG is administered orally in female pregnant rats, it might as well cause alterations in the hypothalamus of the offspring and increased growth rate in later life (Olney and Sharpe, 2003).

The glutamate industry would like us to believe that MSG is not a problem for humans and animals because the brain is protected from MSG by the blood-brain barrier. However, that is not true. The blood-brain barrier is not fully developed in newborns, and although there is some evidence that it is not fully developed in some children until puberty, when it reaches full maturity is unknown (Blaylock and Russel,1995). It is definitely not fully developed in any fetus. Furthermore, throughout life, certain regions of the brain, known as the circumventricular organs, lack a blood brain barrier, (Broadwell and Sofroniew, 1993) and the blood brain barrier can be damaged from, among other things, high fever, stroke, trauma to the head, seizures, repeated ingestion of MSG, and the normal process of aging (Toth and Lajtha, 1981) . The developing fetus is at particular risk since the placental barrier is not impervious to MSG (Frieder and Grimm, 1984). Therefore, in the present study it is found that MSG induces embryo toxicity and foeto toxicity in female rats during the gestational period.

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