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# EFFECT OF DOOSHIVISHARI AGADA (DVA) OVER MONOSODIUM GLUTAMATE (MSG) INDUCED FEMALE REPRODUCTIVE TOXICITY WSR TO UTERUS AND ESTROUS CYCLE

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## ABSTRACT

**Background:** MSG makes food yummy but along with cause many bad effects on body. MSG is proved to cause infertility. *Dooshivisha* is a state of latent poisoning that occurs due to low potency poison. *DooshivishariAgada* is told in context of treatment of *Dooshivisha*. **Material and Methods:** 24 female wistar rats were divided in four groups. Control group was fed on laboratory diet and other groups; MSG was given for 14 days. In third group DVA was given and in fourth group no intervention was given afterwards. **Result:** significant difference was found at $p<0.05$ in LH value and Diameter of uterus. Estrous cycle was daily noted. Diestrous phase was significantly lower in MSG treated animal at $p<0.05$.**Discussion:** MSG affects reproductive system by increasing oxidative stress and by altering hormonal balance. DVA contains *Tagar, Pippali* which are neuroprotective, might neutralise the effect of MSG. *Yastimadhu* reduce oxidative stress by its anti-oxidant activity.

**Keywords:** *Dooshivisha,* oxidative stress, anti-oxidant

## INTRODUCTION

It's very true-"You are what u eat". A healthy diet is very important aspect of being healthy because eating is just not about satisfying hunger, it's all about our body and mind. If one is eating fast food and junk food, it will certainly affect bodily organs and lead to several physical ailments as well as mental conditions. MSG gained attractiveness in first half of twentieth century as taste enhancer but at the same time doubts were raised about safety of MSG and as a causative agent of Chinese restaurant syndrome. So many researches were carried out on different types of animals including human to clear the doubts.[1]

MSG is added in many packed foods like noodles, sauce etc. to make it tasty but at one side it make food yummy and on another side it have very bad effect on body system. So many studies have shown that it causes degenerative change in cells of brain, liver, kidney, spleen and pancreas, reproductive organs and also cause hormonal imbalance.[2]A K Nayantara et al (2008) found that MSG cause prolongation of proestrus phase in estrus cycle.

*Dooshivisha* is a unique concept told in *Samhita*. *Dooshivisha* is told to be *viryaalpabhava* (less potent) and *Svabhav* (by nature its self) or after treating improperly with anti-poisonous treatment and it is not completely expelled from body, It remains in body. When it gains aggravating factor like *dushitadesha, kala, diwaswapana* etc., it vitiate *dhatus* and cause long term disease.[3]

In the quote of *dooshivisha* it is said that *kshapayetchashukram*, i.e. the poison acts directly on *shukra* and *shonita* and does *dusti* which leads to *garbhadushti*. It can happen in acute exposure when *dooshivisha* have affinity towards *shukra* and *shonita*. It may affect directly on *shukra* and *shonita* and produce *beejadushti* or may act after conception and lead to *garbhadushti*.

Reproductive toxicity is said to be an abnormality which disturbs normal conception.

All *rasadidhatudushti* takes place and lead to *shukra* and *shonitadushti*. There will be denaturing of *stree* and *purushabeeja*. MSG comes under organ specific toxin because it causes direct toxicity to gametes.

From previous research done on MSG it can be concluded that it does damage to the reproductive system and leads to infertility so it falls under the category of *Dooshivisha*. So this study was done to evaluate the effect of *DooshivishariAgada* (DVA) in MSG induced Female reproductive toxicity.

## MATERIAL AND METHODS-

The chemicals used in this study was MSG ($C_5H_8NNaO_4.H_2O$) purchased from SDFCL company, Mumbai.

Ethical clearance was taken from IAEC (BMK/IAEC/Res-14/01/2015)

24 adult female wistar rats (150-180 g. average weight) randomly divided into four groups A (control group), B (MSG) and C (DVA) and D (auto recovery) with 6 rats in each group. Animals were housed in cages at standard room temperature maintained on 12 hour light/dark cycle. Rats were fed to a dry balanced meal (Grower mash) for experimental animals, with a continuous source of water. Rats of Group A were fed on laboratory diet for 14 days. In Group B, C, D along with laboratory diet 0.5 ml of distilled water containing Monosodium Glutamate (MSG) 0.20gm/kg body weight/day was given by oral gavage tube at 9 am daily for 14 days. In group C, from $15^{th}$ to $42^{nd}$ day *DooshivishariAgada* (DVA) 216mg/200gm was given and in group D no intervention was given; only normal food and water was given to see the auto recovery from toxicity.

On $15^{th}$ day control group and MSG group and on $42^{nd}$ day DVA, Auto recovery group were anesthetized with diethy ether. In semi-conscious stage, 2ml blood was withdrawn and on complete death animals were sacrificed; uterus were removed and preserved in 10 per cent formalin.

**Estrous cycle:**

**Sample collection-** Every morning between 9:00 and 10:00 a.m. vaginal smear was taken. Vaginal secretion was collected with a plastic pipette filled with 10 µL of normal saline by inserting the tip into the rat vagina, but not deeply. This vaginal fluid was placed on glass slides. Different glass slides were used for each animal. One drop was collected with a clean tip from each rat. Unstained material was observed under a light microscope, with 10 and 40 x objective lenses.

Three types of cells could be recognized: round and nucleated ones are epithelial cells; irregular ones without nucleus are the cornified cells; and the little round ones are the leukocytes. The proportion among them was used for the determination of the estrous cycle phases.[4]

Data was analysed using SPSS-20and expressed as Mean ± standard deviation (SD). Comparisons of the variables were made using the ANOVA followed by post-hoc Bonferrony test. *p≤0.05* was considered as statistically significant.

**Results –**

**Body weight**

Body weight of all animal was recorded for every 15th day with the help of electronic weighing machine. There was gradual increase in weight of all animal throughout the experimental period.

**Table 1:** Body weight of animals

| **Body wt(gm)** | **0th day** | **14th day** | **28th day** | **42nd day** |
|---|---|---|---|---|
| Control | 171.2±4.400 | 182.5 ± 7.395 | ------ | ----- |
| MSG | 155.7 ± 7.312 | 171.5 ± 16.158 | ----- | ----- |
| MSG+DVA | 156.8 ± 11.461 | 170.3 ± 17.154* | 175 ± 18.165 | 180.3 ± 24.508 |
| MSG A | 165.7 ±13.750 | 174.8 ± 11.178* | 178.5±12.029 | 180.7±15.474 |
| F value | 2.217 | | | |
| P value | *p>0.05* | | | |

(*significant compared to 0th day at *p<0.05*, wt= weight, gm=gram)

LH and diameter of uterus was significant at *p<0.05*. Others parameter were non-significant.(table no.2)

**Table 2:** Showing result of LH hormone and Uterus parameters

| **Groups** | **Control** | **MSG** | **MSG+DVA** | **MSG A** | **F value** | **P value** |
|---|---|---|---|---|---|---|
| LH | 0.200 ± 0.089 | 0.150 ± 0.083 | 0.300 ± 0.063 | 0.233 ± 0.103 # | 3.202 | *p<0.05* |
| Weightof uterus | 1.176 ± 0.392 | 1.226 ± 0.091 | 1.075 ± 0.382 | 1.058 ± 0.581 | 0.243 | *p>0.05* |
| DM of uterus | 3.466 ± 1.754 | 2.283 ± 0.222 | 2.000 ± 0.282 | 2.116 ± 0.231 | 3.369 | *p<0.05* |
| Myometrium thickness | 0.366 ± 0.121 | 0.316 ± 0.098 | 0.250 ± 0.083 | 0.233 ± 0.051 | 2.680 | *p>0.05* |
| Endometrium thickness | 0.450 ± 0.207 | 0.466 ± 0.186 | 0.500 ± 0.154 | 0.516 ± 0.172 | 0.169 | *p>0.05* |

(*significance at *p<0.05*, compared to control group, #Significance at *p<0.05* compared to MSG group, LH-Leutenising Hormone, DM= diameter)

**Estrous cycle –**

Estrous phase were noted daily. 14 days data was compared with next 14 days data. As control group and MSG group were sacrificed on 15th day, same data was kept for comparison in other group data. Proestrous and estrous phase data was non-significant but diestrous phase was significant at *p<0.05*

**Table3:** showing repeated measure analysis (ANOVA) of Proestrous phase

| **Proestrous phase** | **MEAN ± SD** | **MEAN ± SD** | **MEAN ± SD** |
|---|---|---|---|
| Days interval | 0-14 days | 15-28 days | 29-42 days |
| Control | 3.333 ± 0.516 | 3.333 ± 0.516 | 3.333 ± 0.516 |
| MSG | 4.833 ± 1.833 | 4.833 ± 1.833 | 4.833 ± 1.833 |
| MSG+DVA | 4.666 ± 1.211 | 4.00 ± 1.264 | 4.1666 ± 2.401 |
| MSG A | 4.666 ± 1.211 | 3.833 ± 1.940 | 2.666 ± 1.366 |
| F-Value | 1.869 | | |
| P-Value | *p>0.05* | | |

**Table 4:** showing result of repeated measure analysis (ANOVA) of Estrous phase

| **Estrous phase** | **MEAN ± SD** | **MEAN ± SD** | **MEAN ± SD** |
|---|---|---|---|
| Days interval | 0-14 days | 15-28 days | 29-42 days |
| Control | 3.500 ± 0.547 | 3.500 ± 0.547 | 3.500 ± 0.547 |
| MSG | 5.166 ± 1.602 | 5.166 ± 1.602 | 5.166 ± 1.602 |
| MSG+DVA | 5.00± 1.264 | 4.333± 1.505 | 3.333 ± 1.366 |
| MSG A | 5.500 ± 1.643 | 3.8333± 1.940 | 3.666± 1.211* |
| F-Value | 2.972 | | |
| P-Value | *p>0.05* | | |

*= compare to 0-14 days

**Table 5:** showing result of repeated measure analysis (ANOVA) of Diestrous phase

| **Diestrous phase** | **MEAN ± SD** | **MEAN ± SD** | **MEAN ± SD** |
|---|---|---|---|
| Days interval | 0-14 days | 15-28 days | 29-42 days |
| Control | 7.00 ± 0.894 | 7.00 ± 0.894 | 7.00 ± 0.894 |
| MSG | 4.166 ± 2.228 | 4.166 ± 2.228 | 4.166 ± 2.228 |
| MSG+DVA | 4.500± 1.048 | 5.666± 1.505 | 6.500± 2.588 |
| MSG A | 4.00± 0.632 | 5.833± 1.722 | 7.666± 1.211*** |
| F-Value | 3.798 | | |
| P-Value | *P<0.05* | | |

(*** compare to 0-14 days at *p<0.00)*

## DISCUSSION

MSG caused reproductive toxicity in two ways- by increasing oxidative stress and by altering the H-P-O axis. MSG excite the cell and allow the entry of Calcium ion ($Ca^{2+}$) into the cell, it activates various molecules that are capable of degrading essential proteins and cellular membranes, increasing the number of free radicals inside the cell[5]

In vertebrates, sex hormone profiles are harmonized by the H-P- Gonadal axis. MSG cause arcuate lesion in hypothalamus and Decrease catecholamine which are involved in LHRH.[6]The glutamate receptor are present in hypothalamus, liver, kidney, endocrine system, ovaries, uterus.[7]MSG directly affect the neuron cell by the mechanism of excitotoxicity.[5] Thus MSG disturbed Neuroendocrine function[7] which results in Disturbed H-P-O axis.[8]Thus MSG, by disturbing H-P-O axis cause hormonal imbalance.

**Body weight-**

During MSG intervention in DVA and Auto-recovery group, body weight was increased at $p<0.05$ and this is in agreement of MuktiMondal et al (2014), Olubaet al. (2011) who reported that consumption of MSG increases the body weight.

In DVA and Auto-recovery group from 15-28$^{th}$ day and 28- 42$^{nd}$ day, body weight gradually increased but was not statistically significant, whereas in DVA group body weight was more compared to Auto-recovery group. It might be due to DVA has *deepana* and *pachana* drugs like *Pippali* which make *Ahara rasa* properly and nourishes all *dhatu*.

**Weight of Uterus**

MuktiMondal et al (2014),found that MSG increases uterus weight. In this study, no significant difference was found in uterus weight. It shows that MSG does not cause increase organ weight.

**LH hormone**

LH activates ovulation and development of the corpus luteum. The concentration of LH is controlled by H-P-O axis. In present study, it is significant at level of $p<0.05$. In MSG group LH concentration is lower compared to control group but statistically insignificant. It might be because MSG alters the neural control that happens via H-P-O axis as glutamate is the main neurotransmitter in hypothalamus and MSG have tendency to damage neuronal cell by the mechanism of excitotoxcity.[5]In DVA group LH hormone is significantly increased at the level of $p<0.05$. As DVA contain flavonoids which are proved for protecting the nerve cell death, that had taken place due to glutamate toxicity.[9]*Pippali*&*Tagara*which are ingredients of DVA have neuroprotective effect on nerve cell.[10,11]

**Estrous cycle**

Alteration in endocrine rhythm, among hypothalamus, pituitary and ovary and the compound which destroy or disturbed the growth of ovarian follicle can have marked effect on estrous cyclicity.[12] MSG is known to alter the H-P-O axisso it have direct effect on estrous cycle. In first 14 days, MSG cause increased duration of proestrous and estrous phase and significant decrease in diestrous duration. In next following days, In Auto-recovery group, estrous cycle had come to normal but not up to the control group but in DVA estrous cycle has come to normal. DVA contain *Tagara* which has neuroprotective effect[11]and *yastimadhu* have antioxidant effect.[13]So these properties might neutralised the effect of MSG. From 29-42 day, estrous phase was reached up to normal. It shows that DVA had corrected the H-P-O axis that's why estrous cycle came to normal. As DVA contain

*Lodhra*, which is proved to correct female reproductive changes that are caused due to oxidative stress.[14]

## CONCLUSION

Many previous researches on MSG haveshowed that it disturbed H-P-O axis by increasing oxidative stress andby direct effect on neuronal cell which result in hormonal imbalance and disturbe destrous cycle. In present study, MSG lowers the LH concentration in animals. It causes increased duration of proestrous phase and estrous phase and lowers the diestrous phase which shows that MSG had caused reproductive toxicity. *DooshivishariAgada* had corrected LH hormone and estrous cycle and normalise the estrous cycle. *DooshivishariAgada* contain *Kustha, Jatamamsi, Chandana,* Tagar which show Antioxidant activity and decrease the increased oxidative stress caused by MSG. *Lodhra* having property to correct the female reproductive system. It proves that *DooshivishariAgada* decrease MSG induced toxicity and corrects the estrous phases. According to Ayurveda, DVA has *Tikta* rasa which is having *Vishaghana* property and counteract the toxicity of MSG. DVA had property of *Deepana*, *pachana* which correct the *Agni* and balance all the *Dhatu*. Auto-recovery also showed good effect but not up-to control group. In one aspect it also establishes the *NidanaParivarjana* concept.

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