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THERAPEUTIC EFFECTS OF HEMIDESMUS INDICUS EXTRACT IN ALLEVIATING MONOSODIUM GLUTAMATE - INDUCED TESTICULAR DAMAGE

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Abstract

Much emphasis has been focused to the issue of infertility linked to the impact of food additives and enhancers on testicular toxicity. Because of the natural chemical components found in plants, herbal medications are becoming a viable option to alleviate these detrimental effects on the testes. The purpose of this study was to assess how well Hemidesmus indicus ethanolic extract safeguard rat testicular structure from the damage brought about by monosodium glutamate (MSG) and how antioxidant markers were altered. The study involved Twenty-four (12-week old) male Wistar rats were divided into four groups at random: six animals each for the Control group (C), MSG group (MSG), Hemidesmus indicus ethanolic extract (HIE) group, and paired Hemidesmus indicus ethanolic extract and MSG (HIE+MSG) group. The study was conducted over 30 days. The left testis was evaluated for antioxidant enzyme activity at the conclusion of the time, and the right testis was subjected to a histological examination. The rats that were subjected to MSG showed significant morphological changes, including diminished growth of germ cells (including spermatids), disruption of the basal lamina, cytoplasmic vacuolation, and abnormalities in the levels of antioxidants in the testicles. Positively, there was a noticeable and steady alleviation in these effects in the group that received MSG and HIE treatment. The present study highlights the potential of ethanolic extract from Hemidesmus indicus as a therapeutic measure against testicular alterations caused by MSG, indicating the necessity for additional research on the effects of this extract on reproductive health and male fertility.

Keywords: Hemidesmus Indicus Extract, Monosodium Glutamate, Histoloy of Testis, Antioxidants, Seminiferous Tubules, SOD, Gluatathione.

1. INTRODUCTION

Infertility presents a significant global health challenge due to its profound psychosocial repercussions. Various chemicals and food additives have been associated with infertility concerns. One such additive, monosodium glutamate (MSG), has stirred considerable debate both domestically and internationally regarding its safety (Moore, 2003). MSG, a salt of naturally occurring glutamic acid, serves as a taste enhancer in numerous food products. Despite being a naturally abundant amino acid essential for human metabolism, excessive MSG consumption has raised safety concerns, particularly regarding its potential to increase brain glutamate levels and disrupt normal functioning, given its role as a neurotransmitter (IFIC, 1994).

Although studies have shown that MSG is hazardous to humans and animals, it is prized for its ability to enhance flavor and stimulate hunger (Andrew, 2007). Russel and Blaylock (1994) pointed out that a lot of taste-enriching chemicals have excitotoxins in them, and MSG is a common ingredient in food because of the Glutamate Association's persistent lobbying, which tends to minimize research findings. Samuels (1999) goes on to describe MSG as a neurotoxin that can cause harm to the brain and retinal cells, endocrine changes, kidney damage, and retinal degeneration. Furthermore, a number of research point to a possible connection between MSG and testicular injury, which raises questions about male infertility. Herbal medicines, which are popular for boosting fertility,have been demonstrated to safeguard the testes from dangerous substances and impurities.

Hemidesmus indicus R. Br., a member of the Asclepiadaceae family frequently referred to as"Indian sarsaparilla," is a widely utilized therapeutic plant in India. Traditionally employed to address various ailments such as blood disorders, respiratory issues, skin conditions, and digestive ailments, its root is reputed for its therapeutic properties dating back centuries (Yoganarasimhan SN, 2007). Studies suggest pharmacological attributes including antioxidant, kidney-protective, and hepatoprotective effects (V.G. Khanna et al., 2007). Rich in phytosterols like saponins, hemidesmol, and essential oils, Hemidesmus indicus offers a promising avenue for therapeutic exploration. However, despite its traditional use and pharmacological potential, scientific inquiry into its testicular protective properties remains scarce.

2. MATERIAL AND METHODS

Chemicals

The substance employed was Monosodium Glutamate (99% purity, procured from SF Traders in U.P India). To prepare the solution, a stock solution was created by dissolving 100 grams of MSG crystals in 100 cc of distilled water. Adjustments to the dosing regimen ensured that the quantity of MSG administered to each animal corresponded to its individual weight.

Preparation of plant extract

Hemidesmus indicus root was acquired in Marthandam, Tamil Nadu, from a nearby herbal source (Power Lab). Botanist Dr. Ajith K. Government College, Department of Botany, carried out the authentication and identification. The roots were cleaned, shade-dried, and ground. After that, a Soxhlet device was used to prepare the ethanolic extract for about 72 hours, following the instructions provided by Ingle et al. (2017). Semi-solid materials were produced when the solvent was extracted from the plant extract using a vacuum rotary evaporator.

Experimental Designs

Male adult albino (200-gram approx.) Wistar rats were obtained from the KMCH College central animal house. Upon arrival, the rats were acclimatized to laboratory conditions for two weeks, housed in p p cages filled with rice husk bedding, and maintained at a temperature of 32°C and humidity of 54%. To prevent overcrowding stress, three rats were housed together in each cage. They had access to standard laboratory feed and water (Lipton India Ltd. laboratory pellets) ad libitum. All experimental procedures strictly adhered to the guidelines outlined by the Institutional Animal Ethical Committee.

There were four groups of animals, each with six rats in them. Specifically,

Group 1: Oral gavage was used to administer one milliliter of distilled water to the control group.

Group 2: For 30 days in a row, the MSG group was given 4 grams of monosodium glutamate per kilogram of body weight.

Group 3: 400 milligrams of Hemidesmus indicus ethanolic extract per kilogram of body weight were given orally to the HIE400 group.

Group 4: MSG + HIE400 group was given 400 mg of Hemidesmus indicus extract and 4 grams of monosodium glutamate per kg of body weight orally for 30 days.

All treatments were administered every morning at 10:20 AM. For the duration of 30 days, MSG (4 grams per kg body weight) was given prior to the daily HIE dosage. Euthanasia was conducted 24 hours after the final treatment. Upon euthanasia, the reproductive system was exposed during a laparotomy. The testis was removed from the epididymis with caution, and it was then cleaned and weighed. After being extracted, the testes were preserved for eight hours in a newly made alcoholic Bouin's solution, dried with different alcohol concentrations, cleaned with xylene, set in paraffin, and sectioned into five-micrometer pieces. Hematoxylin and eosin were used to stain these slices, which were then examined under a light microscope to determine whether the seminiferous tubules had changed qualitatively. For every animal, 200 tubules in all were evaluated. Biochemical analysis was performed on the contralateral testis.

Preparation of Testicular Homogenate

A portion of the testicular tissues were immersed in 10% buffered formalin and underwent further processing following the methods outlined by Gabe.

Evaluation of Biochemical markers

Total protein content was assessed using the method described by Lowry and colleagues in 1951. Enzymatic antioxidant biomarkers in the testis were evaluated as follows: Superoxide dismutase (SOD) levels were estimated according to the method by Kakkar and colleagues in 1984. Glutathione (GSH) levels were measured using a Bio-Diagnostic kit

Statistical Analysis

The data was all presented as mean ± standard deviation. ANOVA was used to assess the data for statistical significance, and Bonferroni multiple comparison tests were then performed. P values below 0.05 were regarded as significant.

3. RESULTS

Biochemical Markers

The study findings indicated that the total protein levels were higher in the MSG group compared to the control group, and lower in the MSG+Hemidesmus indicus group compared to the MSG group. While superoxide dismutase (SOD) levels showed a non-significant increase in the MSG group compared to the control group. Vitamin C and levels of glutathione (GSH) were markedly higher in the MSG group compared to

the control group. However, in the MSG+HIE group, vitamin C levels decreased, although they remained higher than in the control group, whereas GSH levels remained elevated in the HIE group.

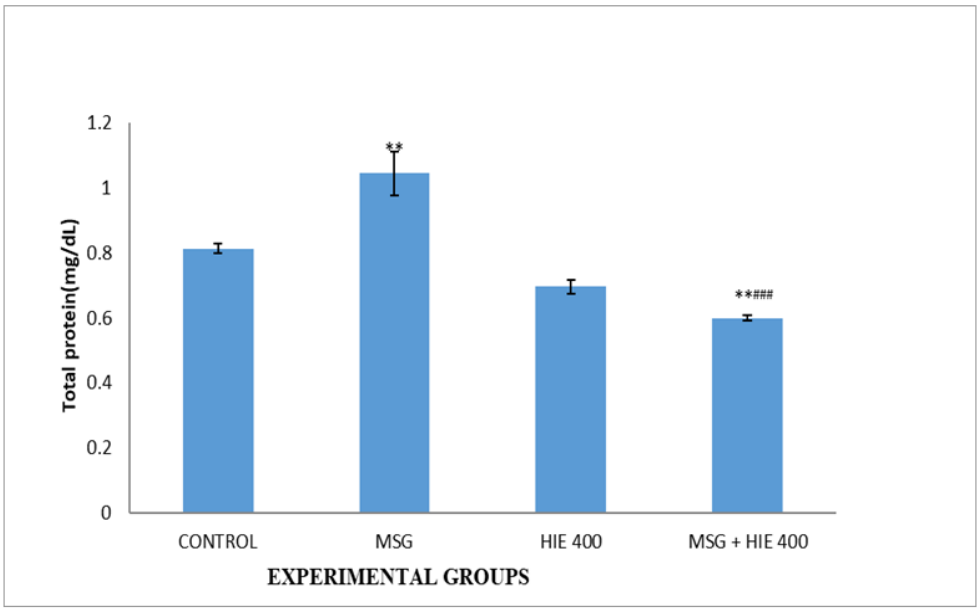

Fig 1: Total protein levels in animals from several experimental groups are compared. * Significance with Control *<0.05, **<0.01 *<0.001# Significance with MSG #<0.05, ##<0.01 ###<0.001 as indicators of significance**

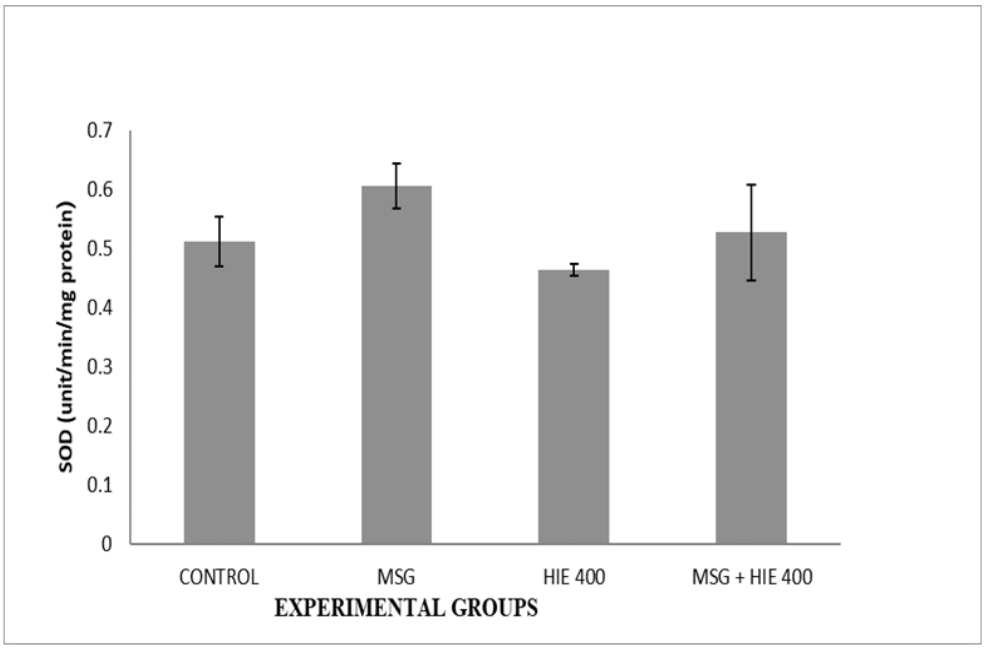

Fig 2: Comparing the SOD levels of the animals in several experimental groups

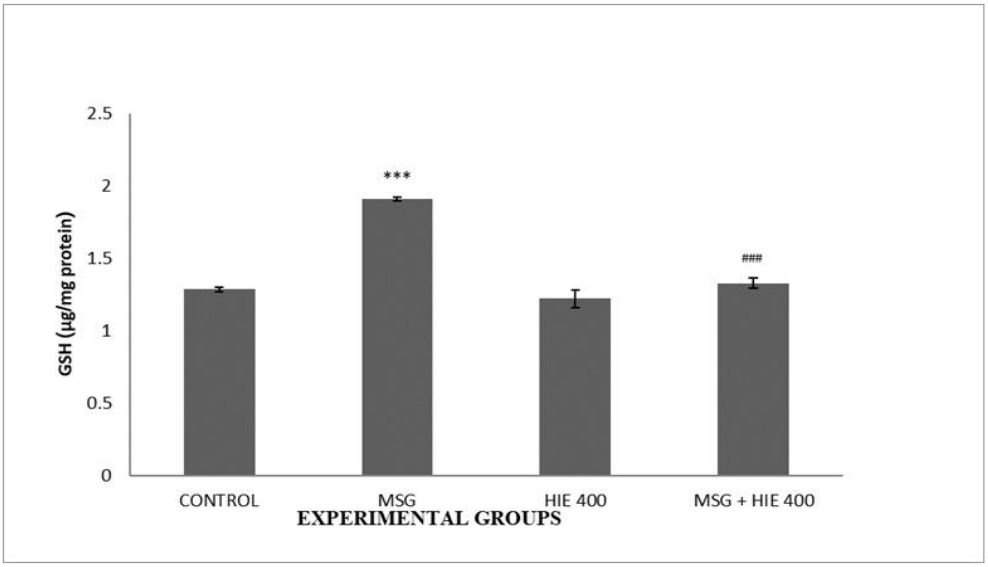

Fig 3: Testicular GSH levels across animals from several experimental groups are compared

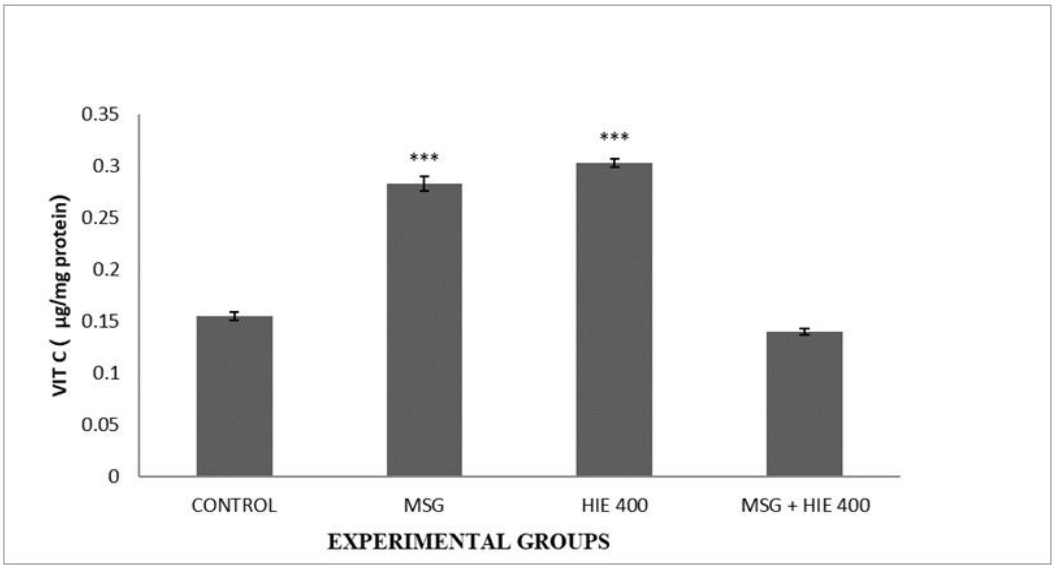

Fig 4: Vitamin C levels in animals from several experimental groups are compared

Testicular histology

The testicular histology of the group control exhibited a usual structure, characterized by abundant sperms and well-organized spermatogonia stacked in multiple layers along the seminiferous tubules (Fig. 5A). However, exposure to MSG led to structural variations in the testes, including a reduced number of spermatids and disorganization of the germinal layer, with few parts showing degeneration (Fig. 5B). Notably, the MSG+HIE group displayed evident improvement, with partial restoration of germinal cells and an increased presence of spermatozoa, along with multiple layers of spermatogenic cells (Fig. 5E). The HIE-treated group exhibited Seminiferous tubules in a healthy state, along with Leydig cells (Fig. 5C).

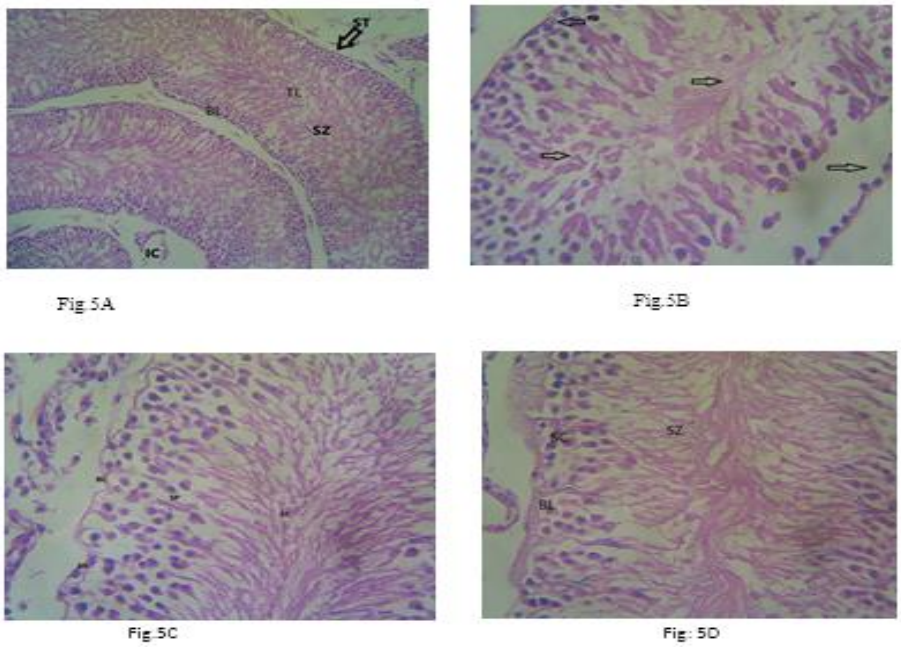

Figure 5: presents the histological examination of testicular tissue. Subfigure 5A displays the histology of the normal control group at 100X magnification. Subfigure 5B depicts the histology of the testis in the monosodium glutamate (MSG) treated group at 400X magnification. Subfigure 5C exhibits the testis section in the group treated with herbal extract (HIE) at 400X magnification. Lastly, Subfigure 5D showcases the microscopic view of rat testis treated with a combination of MSG and HIE at 400X magnification. The annotations indicate various structures: ST for seminiferous tubules, BL for Basal lamina, TL for Tubular lumen, IC for interstitial cells, SZ for Spermatozoa, SP for spermatids, SC for Sertoli cells, and SG for Spermatogonia

4. DISCUSSION

Rats treated with MSG in this study showed variations in their cytostructural organization of seminiferous tubules when compared to age-matched control rats; however, the MSG+HIE group displayed a notable amelioration. The reduction in spermatids, the emergence of cavities in the cytoplasm, the shedding of the germinal epithelium's basal lamina, and the disarray of the germinal epithelium were among these alterations. These results are in line with earlier research; Oforofuo et al. (1997) found that MSG administration caused sperm paucity and testicular alterations, and Atallah proposed that the chemical's local effect or gonadotrophic hormone imbalances may be indirectly responsible for these histological changes. E.M. EL-zayat (1988) provided an explanation for the maturation arrest seen in our study, relating it to testosterone suppression that stops spermatogenesis. MSG specifically affects the testes because they contain glutamate receptors, which are found in a variety of tissues. Previous studies showed that the testes of rats contained functional glutamate transporters and receptors (Takarada et al., 2004). Therefore, MSG's direct activity via glutamate receptors and transporters may be one explanation for its effect on seminiferous tubule epithelial cells. A second mechanism, put up by other researchers (R.J. Aitken et al., 1989), suggests that MSG induces neurotoxicity within

the hypothalamus-pituitary-gonadal system., which may have anatomical or physiological ramifications for male reproduction.

Infertility may result from testicular damage brought on by oxidative stress, with lipid peroxidation serving as the main mechanism for oxidative damage. It is commonly known that MSG produces reactive oxygen species, which emphasizes the significance of antioxidant enzymes in reducing MSG toxicity (R.J. Aitken et al., 1989). Because of the high concentration of unsaturated fatty acids in their plasma membranes and the low quantities of antioxidants in their cytoplasm, spermatozoa are especially sensitive to oxidative stress (R. Jones et al., 1979). In the testes of rats received MSG, our investigation revealed a considerable rise in GSH, and vitamin C activities. This increase may have been caused by the rats' adaptability to the oxidant therapy and ability to deal with oxidative stress in the testis. This increase in enzyme activity may also be due to increased production of enzymes as a result of enzyme induction. Increased amounts of lipid peroxidation can influence spermatozoa growth, modify membrane functions, cause oxidative damage to sperm DNA, and impede motility. The elevated generation of free radicals in the rat reproductive system may be connected to the adverse impact of MSG on physiological and biochemical processes. The equilibrium of the enzymatic antioxidant pathway is mostly determined by antioxidant enzymes including GPx, SOD, and CAT, which are highly active in the testis, sperm, and seminal plasma, among other reproductive organs (M.M. Aruldhas et al., 2005). As a first line of defense against oxidative stress, the SOD-CAT system eliminates active oxygen species by converting superoxide radicals to hydrogen peroxide, which is then transformed into water (Makker et al., 2009).

5. CONCLUSION

The findings of this study demonstrated that oral treatment of monosodium glutamate caused damage to the seminiferous tubular epithelium, but that this damage may be lessened by administering Hemidesmus indicus root extract potentially indicating the therapeutic potential of the plant. Furthermore, the research demonstrated that albino Wistar rats saw an adaptive rise in antioxidant markers within a 30-day period.

References

1) Andrew OE. Histological studies of the effects of monosodium glutamate on the kidney of adult Wistar rats. Internet J Health. 2007;6(2).

2) Tezcan E, Atmaca M, Kuloglu M, Ustundag B. Free radicals in patients with posttraumatic stress disorder. Eur Arch Psychiatry Clin Neurosci. 2003; 253:86–91.

3) Jihen EH, Imed M, Fatima H, Abdelhamid K. Protective effects of selenium (Se) and zinc (Zn) on cadmium (Cd) toxicity in the liver of the rat: effects on oxidative stress. Ecotoxicol Environ Saf. 2009; 72:1559–1564.

4) El-zayat EM. Biochemical, histochemical and histopathological studies on the role of zinc in regulating testicular function in male albino rats [M.Sc. Thesis]. Zoology Department, Faculty of Science, Cairo Univ; 1988.

5) Ellman GL. Tissue sulphydryl groups: Archives in biochemistry and biophysics. 1959;82(1):70-77.

6) Gong SL, Xia FQ, Wei J, Li XY, Sun TH, Lu Z, et al. Harmful effects of MSG on function of hypothalamus–pituitary–target gland system. Biomed Environ Sci. 1995; 8:310–7.

7) Hermanussen M, Tresguerres JA. Does high glutamate intake cause obesity? Journal of Pediatric Endocrinology and Metabolism. 2003;16(7):965–96.

8) IFIC (Institute of Food Technologists'). Review of Monosodium Glutamate, Examining the Myths. IFIC. 1994;41(5):143-145.

9) Kakkar P, Das B, Viswanathan PN. A modified spectrophotometic assay of SOD. Ind J Biochem Biophy. 1984; 21:130-132.

10) Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951; 193:265.

11) Aruldhas MM, Subramanian S, Sekar P, Vengatesh G, Chandrahasan G, Govindarajulu P, et al. Chronic chromium exposure-induced changes in testicular histoarchitecture are associated with oxidative stress: Study in a non-human primate (Macaca radiata Geoffroy). Hum Reprod. 2005; 20:2801–2813.

12) Makker K, Agarwal A, Sharma R. Oxidative stress & male infertility. Indian Journal of Medical Research. 2009;129(4):357-367.

13) Mohamed AA, Thabet HZ, Abdel-hafez AM. Toxicity of monosodium glutamate on male rat reproductive system and effect of curcumin and propolis co-administeration. The Egyptian Journal of Forensic Sciences and Applied Toxicology. 2017;17(1):129-146. https://dx.doi.org/10.21608/ejfsat.2017.46106.

14) Moore KL. Congenital malformations due to environmental factors. In: Developing humans. 2nd ed. W. B. Saunders Company Ltd; 2003. p. 173–183.

15) Atallah O. Experimental nitrate, nitreite and hydroxylamie toxicosis in the Guinea pig [Ph.D. Thesis]. Department of Pathology. Michigan State Univ., USA; 1966.

16) Petrelli G, Mantovani A. Environmental risk factors and male fertility and reproduction. Contraception. 2002;65(4):297-300.

17) Jones R, Manna T, Sherina RJ. Peroxidative breakdown of phospholipids in human spermatozoa: spermicidal effects of fatty acids, oxidized peroxides and protective reaction of seminal plasma. Fertil Steril. 1979; 32:257–265.

18) Aitken RJ, Clarkson J, Fisher S. Generation of reactive oxygen species, lipid peroxidation and human sperm function. Biol Reprod. 1989; 40:183–197.

19) Trease G, Evans W. Pharmacognosy, A Physician's Guide to Herbal Medicine. 1989; 13:912.

20) Rogers PJ, Blundell JE. Umami and appetite: Effects of monosodium glutamate on hunger and food intake in human subjects. Physiology and Behavior. 1990;48(6):801–804.

21) Russell L, Blaylock F. Excitotoxins-The Taste That Kills. Santa Fe, Health Press; 1994.

22) Russell LD, Ettlin RA, Sinha- Hikim AP, Clegg D. Histological and histopathological evaluation of the testis. Clearwater, Cache River Press; 1990.

23) Samuels A. The toxicity/safety of MSG; A study in suppression of information. Accountability in Research. 1999;6(4):259–310. https://doi.org/10.1080/08989629908573933.

24) Samuels A. The toxicity/safety of MSG; A study in suppression of information. Accountability in Research. 1999;6(4):259–310.

25) Sinha AK. Colorimetric assay off catalase. Anal Biochem. 1972; 47:389-394.

26) Omaye ST, Turnbull JD.

27) Sauberlich HE. Determination of ascorbic acid in animal cells, tissues, and fluids. Methods in Enzymology. 1979; 62:3-11.

28) Takarada T, Hinoi E, Balcar V, Taniura H, Yoneda Y. Possible expression of functional glutamate transporters in the rat testis. Journal of Endocrinology. 2004;181(2):233-244.

29) Khanna VG, Kannabiran K. Larvicidal effect of Hemidesmus indicus, Gymnema sylvestre and Eclipta prostrate against Culex quinquifaciatus mosquito larvae. Afr J Biotechnol. 2007;6(3):307-311.

30) Yoganarasimhan SN. Medicinal Plants of India: Tamil Nadu. Cyber Media; 2000. p. 275-6.