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PROTECTIVE POTENTIAL OF HEMIDESMUS INDICUS ETHANOLIC EXTRACT AGAINST MONOSODIUM GLUTAMATE-INDUCED TESTICULAR ALTERATIONS: HISTOLOGICAL AND ANTIOXIDANT MODULATION IN ALBINO RATS

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Abstract

The issue of infertility stemming from the impact of food additives and enhancers on testicular toxicity has garnered significant attention. In response, herbal medicines are gaining prominence for their potential to counteract these detrimental effects on the testes, owing to the inherent chemical constituents within plants. This study aimed to assess the protective influence of Hemidesmus indicus ethanolic extract against alterations induced by Monosodium Glutamate (MSG) in rat testicular histology and the modulation of antioxidant markers.Twenty-four young adult Wistar male rats, aged 12 weeks, were subject to random allocation into four groups: the Control group (C), the MSG group (MSG), the Hemidesmus indicus ethanolic extract (HIE) group, and the combined Hemidesmus indicus ethanolic extract and MSG (HIE+MSG) group, each comprising six animals. The study extended over a 30-day period. Subsequent to the stipulated duration, the right testis underwent histological evaluation, while the left testis underwent assessment of antioxidant enzymatic activity. Rats exposed to MSG exhibited distinct morphological changes including arrested germ cell maturation, disruption of the basal lamina, cytoplasmic vacuolation, diminished germ cell development, including spermatids, and perturbations in testicular antioxidant levels. Encouragingly, the MSG+HIE treated group displayed a significant, progressive amelioration of these effects. This study underscores the potential of Hemidesmus indicus ethanolic extract as a protective agent against MSG-induced testicular alterations, warranting further investigation for its implications on testicular health and male fertility.

Keywords ; Hemidesmus indicus Extract, Monosodium Glutamate,Testicular histoloy ,seminiferous tubules,Antioxidant enzymes ,SOD,Gluatathione,Vitamin C,catalase .

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1. Introduction

Infertility is major health concern worldwide owing to its psychosocial implications. Several chemicals or food additives have been linked to infertility. Food additives act as preservatives or as palatability enhancers; monosodium glutamate (MSG) is one such food addition that has sparked debate both locally and globally over its safety (Moore, 2003). MSG is a sodium salt of the naturally occurring non-essential L-form of glutamic acid. It is one of the most abundant amino acids found in nature and is employed as a taste enhancer in a variety of food products. MSG is also produced in the body and is necessary for human metabolism; it is a key component of many proteins, including meat, fish, milk, and various vegetables (IFIC, 1994). MSG pleasant taste has led to its inclusion as a flavoring agent in foods. Its excessive inclusion in foods has required its safety evaluation, which has raised the public concerns about its transfer into the blood and thus increasing the brain glutamate levels, thus causing functional disruptions and affected other organs of the body because MSG is a neurotransmitter, this concern originally raised greatly and has led to an extensive series of scientific studies to examine this issue, conducted primarily in rodents.

Despite its taste stimulation and improved appetite enhancement, reports indicate that MSG is toxic to humans and experimental animals (Andrew, 2007). Russel and Blaylock (1994) reported that most taste enhancing additives contain exitotoxins, and the authors went on to say that exitotoxins are widely distributed in our food supply due to the strong food lobby known as the Glutamate Association, which rejects any unfavorable press or reports of research demonstrating the deleterious effects of MSG. According to Samuels (1999), MSG is a neurotoxic substance that damages kidneys and causes retinal degeneration, endocrine abnormalities, and damage to brain and retinal cells. Numerous studies have shown that (MSG), which damages testicles, may play a role in cases of male infertility. Herbal remedies are well known for improving fertility and have been shown to shield the testicles from toxic chemicals and pollutants.

The Asclepiadaceae family member Hemidesmus indicus R. Br., also known as "Indian sarsaparilla," is a widely dispersed medicinal plant in India. It is used as a tonic and to treat biliousness, blood diseases, respiratory diseases, skin diseases, diarrhea, syphilis, bronchitis, asthma, fever, eye diseases, epileptic fits in children, kidney and urinary disorders, snake bite, burning sensation, loss of appetite, and rheumatism using the plant's root, which has been used traditionally for centuries(Yoganarasimhan SN ,2007). It is asserted that it has pharmacological properties that include antioxidant, renoprotective, antinociceptive, and hepatoprotective activity.[V.G.Khanna et al.; 2007]It mostly comprises of phytosterols, such as saponins, hemidesmol, and essential oils.Although Hemidesmus Indicus was said to be helpful in treating various disorders, there was no scientific research on the plant in the testicular protective properties .

2. Material And Methods

Chemicals

The substance utilized was Monosodium Glutamate (Purity 99% NT), was acquired from SF Traders in U.P India. 100 grams of MSG crystals were dissolved in 100 cc of distilled water to create a stock solution. The dosing schedule was changed in such a way that the amount of MSG administered to animals matched their individual weights.

Preparation of plant extract

Hemidesmus indicus was procured from a neighborhood herbal source in Marthandam, Tamil Nadu (Power Lab). Identification and authentication were performed by botanist, Dr. Ajith Kumar, Department of Botany, Government College, Trivandrum. The Roots were washed, dried in the shade, and ground. The soxhlet equipment was used to prepare the ethanolic extract for 72 hours (Ingle KP et al 2017). In a vacuum rotary evaporator, the solvent from the plant extract was removed to create the semi-solid materials.

Experimental Designs

Albino male adult Wistar rats weighing approximately 200 grams were obtained from the KMCH College of Pharmacy's central

animal house. The rats were housed in polypropylene cages with rice husk bedding for two weeks to adapt under laboratory settings of (32°C) temperature and (54) humidity. To avoid stress caused by overpopulation, three rats were placed in each cage. The rats were fed ad libitum Laboratory chow and water (Lipton India Ltd. laboratory pellets). The Institutional Animal Ethical Committee's rigorous rules were observed in all experimental operations.

The animals were divided into four Groups, each having six in one group. Namely,

Group-1: Control- received one ml of distilled water orally using oral gavage,

Group-2: MSG- received Monosodium Glutamate (4gm/kg body weight) for 30 days,

Group-3: HIE400- received an ethanolic extract of Hemidesmus indicus (400mg/Kg body weight) orally

Group-4: MSG + HIE400- received Monosodium glutamate (4gm/kg Body weight) plus Hemidesmus indicus extract (400mg/Kg body weight) orally for 30 days.

The animals received treatment every morning at 10.30 AM, For 30 days, MSG (4gm/Kg body weight) were given before the daily HIE dosage. Animals were put to death by euthanasia 24 hours after their last treatment. After the last day of treatment, the laparotomy was performed to expose the reproductive system. The epididymis was carefully separated from the testis, cleaned of any extraneous structures, and weighed. The testes were carefully removed from the epididymis, fixed in fresh alcoholic Bouin's fluid for eight hours, dehydrated in various alcohol concentrations, cleaned with xylol, embedded in paraffin, and cut into five-micrometer slices before being stained with haematoxylin and eosin. The seminiferous tubules in the tissue slices were examined under a light microscope for qualitative alterations.200 tubules were examined for each animal .other side testis were utilized for biochemical investigation .

Preperation of Testicular Homogenate

A part of testis tissues was fixed in 10 % neutral buffered formalin and by applying other processes as described by Gabe .

Assessment of Biochemical Parameters.

Total protein was determined by method (Lowry OH, etal 1951).Determination of the enzymatic antioxidant biomarkers in testis Catalase activity (CAT) was determined by Sinha, A.K (1972). Superoxide dismutase (SOD) was estimated by Kakkar P etal (1984). Glutathione (GSH) was assessed by Bio-Diagnostic kit.

Statistical Analysis: All the data were expressed as mean ± S.D. The data was analyzed for statistical significance using ANOVA followed by Bonferroni multiple comparison tests. a p value less than 0.05 were considered significant.

3. Results

Oxidative stress markers

The study revealed that the total protein increased in MSG group than control group and decreased in MSG+HIE group compared to MSG group.SOD increased non significantly in MSG Group comparing the control group.Vitamin C and GSH increased significantly in MSG Group than control,whereas Vitamin C decreased in MSG+HIE group but remained higher in HIE group.

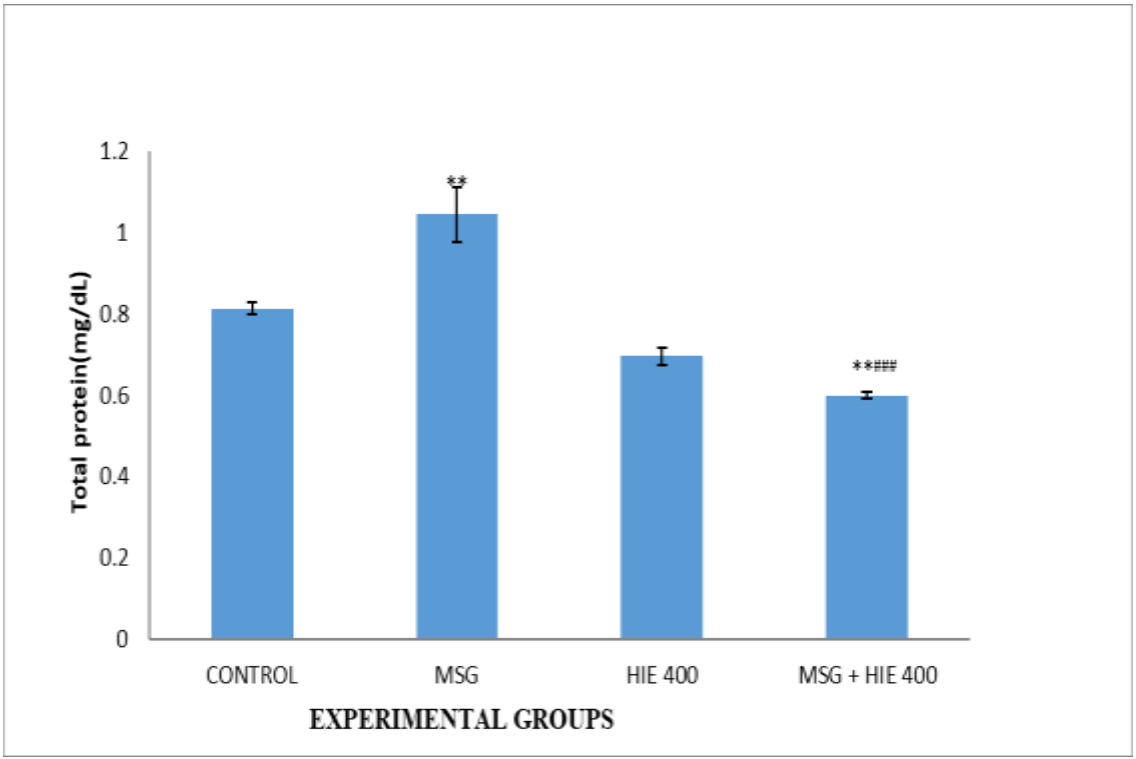

Fig 1: Comparison of Total protein between the animals of different experimental groups.* Significance with Control *<0.05, **<0.01 ***<0.001# Significance with MSG #<0.05, ##<0.01 ###<0.001

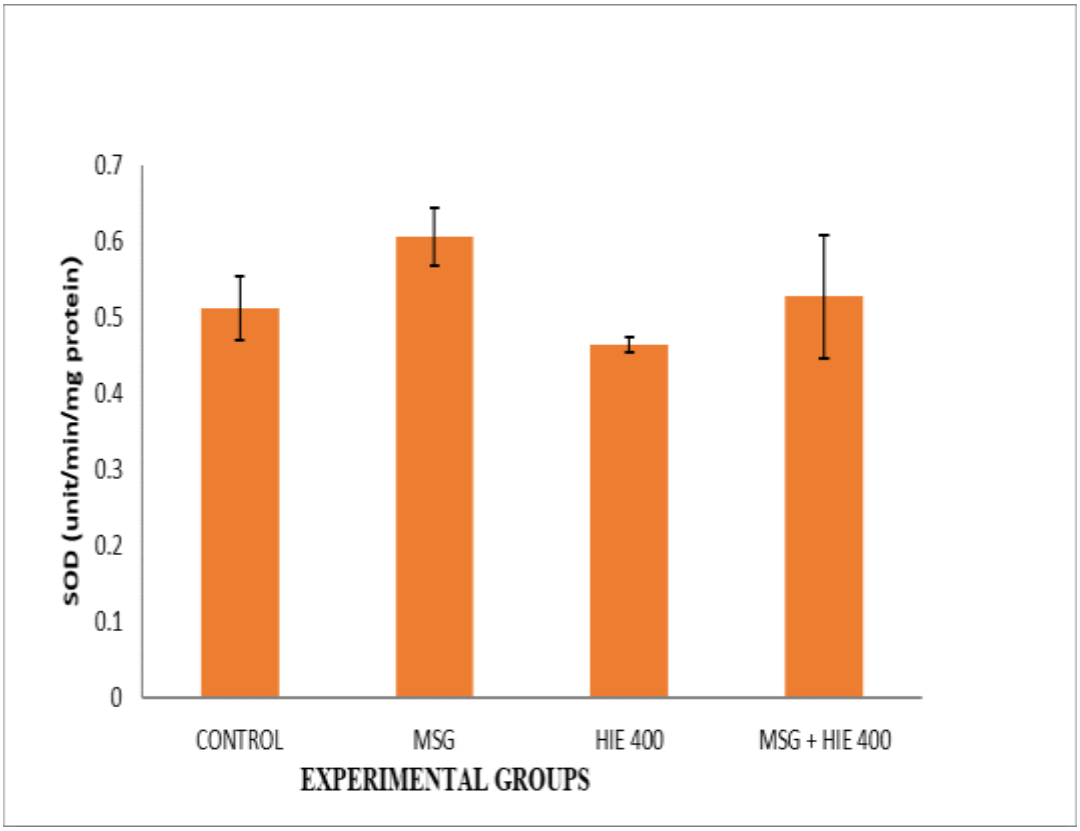

Fig-2**:** Comparison of SOD between the animals of different experimental groups

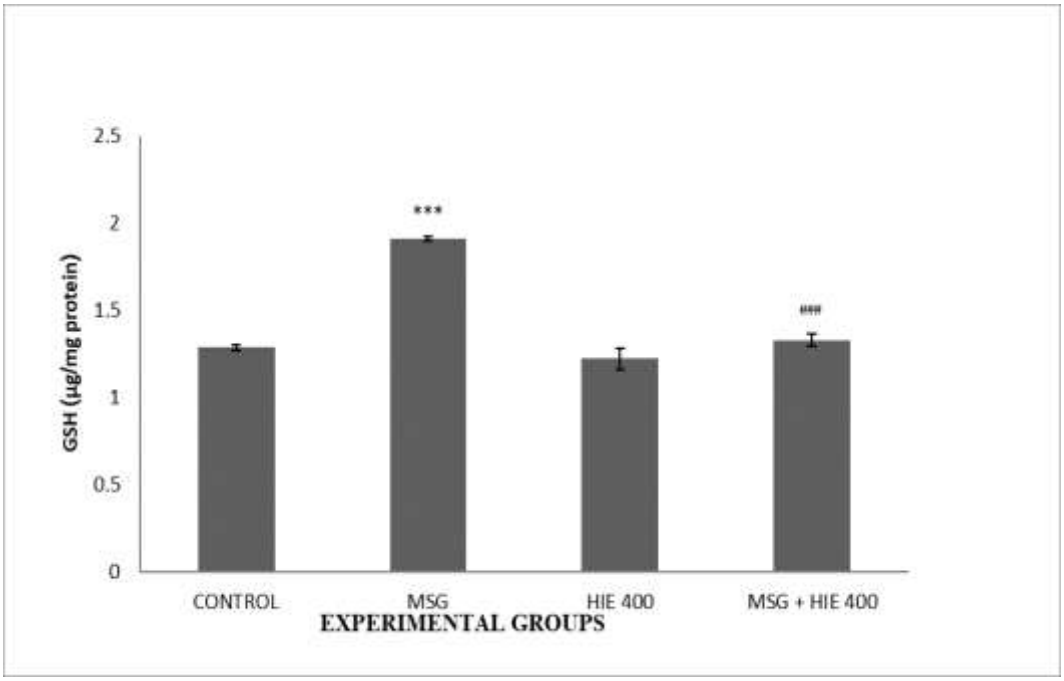

Fig 3: Comparison of GSH of testis in animals of different experimental groups

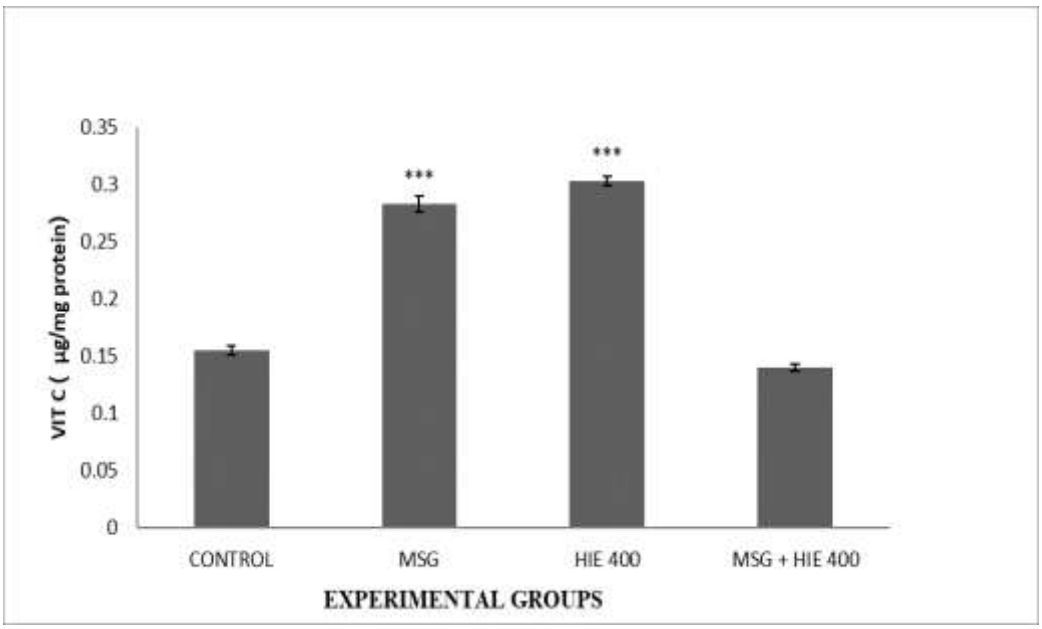

Fig 4; Comparison of Vitamin C between the animals of different experimental groups

Testicular histology

The control group's testicular tissues were normal in structure with plenty of sperms (Fig. 5A) showed cells with normal spermatogonia stacked in several layers on seminiferous tubule. MSG induced testicular structural variations by appearance of few numbers of spermatids showing disorganized germinal epithelium with some degenerated parts (Fig. 5B). The clear improvement was noticed in MSG+HIE and group where there was some restoration of the germinal cells and some spermatozoa with plenty layers of spermatogenic cells (Fig.5E). The seminiferous tubules with normal structure with Leydig cells were found HIE treated group (Fig. 5C).

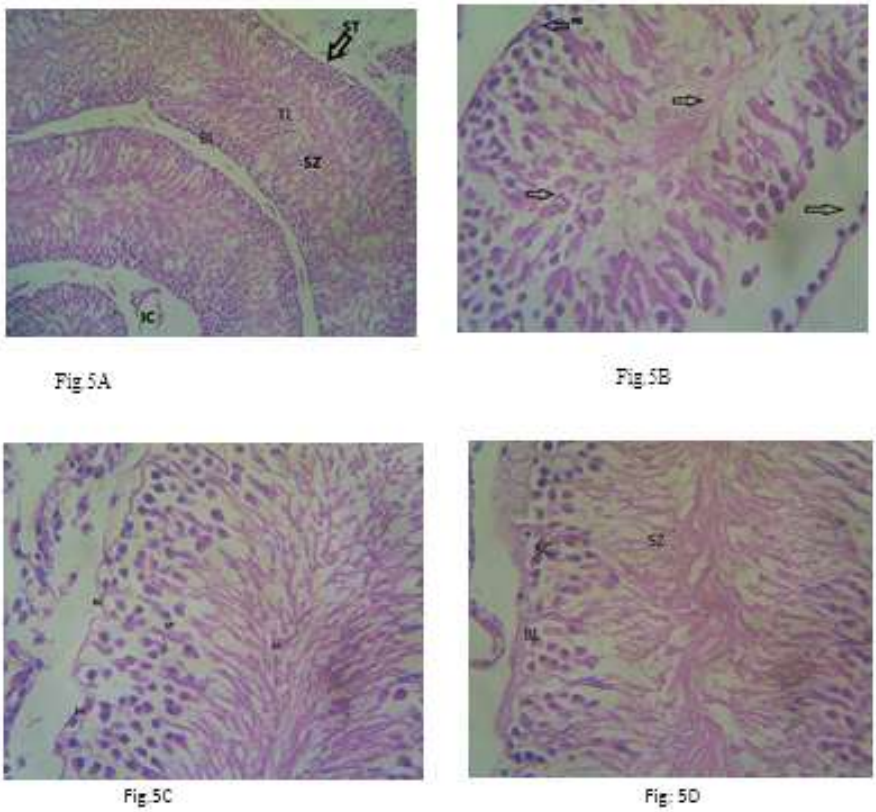

Fig 5 :showing testicular Histology 5A Histology of Normal control group (100X) ,5B Histology of testis in MSG treated group (400X).5C shows the testis section in HIE treated group (400X).5D shows the microphotograph of rat testis treated with MSG+HIE(400X).ST;seminiferous tubules.BL:Basal lamina,TL:Tubular lumen,IC;interstitial cell ,SZ:Spermatozoa ,SP;spermatids ,SC;sertoli cells ,SG;Spermatogonia

4. Discussion

In the present study, rats treated with MSG showed changes in the seminiferous tubular cytoarchitecture compared with age matched control rats that showed significant improvement with group treated with MSG+HIE . These changes included decrease in spermatids ,appearance of vacuoles in the cytoplasm and sloughing of basal lamina of germinal epithelium and disorganized germinal epithelium .The findings are in accordance with earlier studies Oforofuo et al (1997) mentioned that administration of MSG induced oligozoospermia and testicular changes and Atallah suggested that these histological changes may be due to either local effect of the chemical or indirectly caused by imbalance in gonadotrophic hormones .

The maturation arrest seen in this study was explained by (.E.M. EL-zayat 1988), who linked it to testosterone inhibition, which halted spermatogenesis. Glutamate receptors can be found in a variety of tissues, including the hypothalamus, spleen, thymus, liver, and kidneys. (Previous research demonstrated the presence of functioning glutamate transporters and receptors in rat testes (Takarada, et al.: 2004).As a result, the testes are considered a target organ for MSG. One of the reasons may be a direct effect of MSG on the epithelial cells of the seminiferous tubules via glutamate receptors and transporters. Other researchers (R.J. Aitken et al.; 1989) established the second mechanism, stating that MSG has neurotoxic effects on the function of the hypothalamus-pituitary-gonadal system. The consequences of such toxins on male reproduction may be anatomical or physiological.

Testicular damage from oxidative stress leads to infertility. One of the primary mechanisms of oxidative damage, lipid peroxidation is crucial to the toxicity of several xenobiotics. The production of reactive oxygen species by MSG is well recognized. Antioxidant enzymes

may therefore be extremely important in the development of MSG toxicity (R.J. Aitken ET AL.; 1989) .According to earlier research, spermatozoa are vulnerable to oxidative stress because they have a high concentration of unsaturated fatty acids in their plasma membranes and a very low level of antioxidants in their cytoplasm (R. Jones et al.;1979).

Our findings on testicular antioxidants showed that the activities of GSH, catalase, and vitamin C have significantly increased in the testes of rats treated with MSG. It is possible that the increase in enzyme activity is a response to oxidant treatment and an adaptation to cope with oxidative stress in the testis. In addition, it may be due to the increased synthesis of enzymes resulting from enzyme induction.

Previous studies reported that the increased lipid peroxidation led to oxidative damage to sperms DNA, alter membrane functions, impair motility and possibly have a significant effect on the development of spermatozoa. Possibly, the toxic effects of MSG on the spermatozoa physiological and biochemical parameters might be related to the increased production of free radicals in the rat reproductive organs. There is a defense system, which consists of antioxidant enzymes such as GPx,

SOD and CAT

These enzymes are also thought to be crucial indicators of the first and second steps of the enzymatic antioxidant pathway's balance [E.H. Jihen et al.; 2009]. High levels of antioxidant enzyme activity can be found in the testis, epididymis, sperm, and seminal plasma [M.M. Aruldhas et al.; 2005]. CAT transforms hydrogen peroxide into water, whereas SOD catalyzes the conversion of superoxide radicals to hydrogen peroxide [Makker, ET AL.; 2009]. Since these enzymes cooperate to remove active oxygen species, the SOD-CAT system serves as the first line of defense against oxidative stress

5. Conclusion

Present study reveaed that Hemidesmus indicus root extract has ameliorated the damage in the seminiferous tubular epithelium induced by Monsodium glutamate oral administration and showed an adapative elevation in antioxiant markers in 30 days of study in albino wistar rats.

6. References

1. Andrew, O.E. (2007) Histological studies of the effects of monosodium glutamate on the kidney of adult Wistar rats. The Internet Journal of Health, 6(2).
2. E. Tezcan, M. Atmaca, M. Kuloglu, B. Ustundag, Free radicals in patients with posttraumatic stress disorder, Eur. Arch. Psychiatry Clin. Neurosci. 253 (2003) 86–91.
3. E.H. Jihen, M. Imed, H. Fatima, K. Abdelhamid, Protective effects of selenium (Se) and zinc (Zn) on cadmium (Cd) toxicity in the liver of the rat: effects on the oxidative stress, Ecotoxicol. Environ. Saf. 72 (2009) 1559–1564.
4. E.M. EL-zayat, Biochemical, histochemical and histopathological studies on the role of zinc in regulating the testicular function in male albino rats. M.Sc. Thesis, Zoology Department, Faculty of Science, Cairo Univ. 1988
5. E.M. EL-zayat, Biochemical, histochemical and histopathological studies on the role of zinc in regulating the testicular function in male albino rats. M.Sc. Thesis, Zoology Department, Faculty of Science, Cairo Univ. 1988
6. Ellman G.L: Tissue sulphydryl groups: Archives in biochemistry and biophysics: 1959: vol 82 (1):pp 70-77).
7. 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
8. Hermanussen, M., & Tresguerres, J. A. (2003). Does high glutamate intake cause obesity? Journal of Pediatric Endocrinology and Metabolism, 16(7),965–96.
9. IFIC (Institute of Food Technologists'). (1994) Review of Monosodium Glutamate, Examining the Myths. IFIC, 41(5), pp. 143-145

10. Kakkar P. Das,. B. and Viswanathan .P.N. A modified spectrophotometic assay of SOD, Ind. J. Biochem. Biophy. 1984:21:130-132.).
11. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ, 1951. Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265).
12. M.M. Aruldhas, S. Subramanian, P. Sekar, G. Vengatesh, G. Chandrahasan, P. Govindarajulu, M.A. Akbarsha, Chronic chromium exposure-induced changes in testicular histoarchitecture are associated with oxidative stress: Study in a non-human primate (Macaca radiata Geoffroy), Hum. Reprod. 20 (2005) 2801–2813.
13. Makker, Kartikeya; Agarwal, Ashok; Sharma, Rakesh. Oxidative stress & male infertility. Indian Journal of Medical Research 129(4):p 357-367, April 2009.)
14. Mohamed. Aml A., Hayam Z. Thabet, and Amal M. Abdel-hafez. "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 17.1 (2017): 129-146. https://dx.doi.org/10.21608/ejfsat.2017.46106.
15. Moore, K. L. (2003). Congenital malformations due to environmental factors. In Developing humans (2nd ed) (pp. 173–183). W. B. Saunders Company Ltd.
16. O. Atallah, Experimental nitrate, nitreite and hydroxylamie toxicosis in the Guinea pig. Ph.D. Thesis. Department of Pathology. Michigan State Univ., USA, 1966.
17. Petrelli G, Mantovani A. Environmental risk factors and male fertility and reproduction☆. Contraception. 2002;65(4):297-300.
18. R. Jones, T. Manna, R.J. Sherina, Peroxidative breakdown of phospholipids in human spermatozoa: spermicidal effects of fatty acids, oxidized peroxides and protective reaction of seminal plasma, Fertil. Steril. 32 (1979) 257–265
19. R.J. Aitken, J. Clarkson, S. Fisher, Generation of reactive oxygen species, lipid peroxidation and human sperm function, Biol. Reprod. 40 (1989) 183–197
20. G. Trease and Evans. Pharmacognosy, A Physician's Guide to Herbal Medicine. 1989;13:912.
21. Rogers, P. J., & Blundell, J. E. (1990). Umami and appetite: Effects of monosodium glutamate on hunger and food intake in human subjects. Physiology and Behavior, 48(6), 801–804.
22. Russell, L., and Blaylock, F. (1994) Excitotoxins-The Taste That Kills. Santa Fe, Health Press.
23. Russell, L.D., Ettlin, R.A., Sinha- Hikim, A.P., and Clegg, D. (1990) Histological and histopathological evaluation of the testis. Clearwater, Cache River Press.
24. Samuels, A. (1999). The toxicity/safety of MSG; A study in suppression of information. Accountability in Research, 6(4), 259–310. https://doi.org/10.1080/08989629908573933
25. Samuels, A. (1999). The toxicity/safety of MSG; A study in suppression of information. Accountability in Research, 6(4), 259–310.
26. Sinha, A.K. Colorimetric assay off catalase Anal. Biochem. 1972: 47: 389-394).
27. Stanley T.Omaye., J.David Turnbull.,
28. Howerde E.Sauberlich; determination of ascorbic acid in animal cells, tissues, and fluids. Methods in Enzymology Vol 62, 3-11, 1979.
29. Takarada, T., Hinoi, E., Balcar, V., Taniura, H., & Yoneda, Y. (2004). Possible expression of functional glutamate transporters in the rat testis, Journal of Endocrinology, 181(2), 233-244. Retrieved Jul 10.
30. V.G.Khanna and K. Kannabiran. Larvicidal effect of Hemidesmus indicus, Gymnema sylvestre and Eclipta prostrate against Culex quinquifaciatus mosquito larvae. Afr J Biotechnol 6(3): 307-311 (2007).
31. Yoganarasimhan SN. Medicinal Plants of India: Tamil Nadu: Cyber Media; 2000. 275-6 p.