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Effect of ethanolic extract of *Hemidesmus indicus* on spermiogram and sex hormone levels of wistar rats

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ABSTRACT

Male infertility poses an emerging concern in public health circles. While surgical procedures and interventions are available, they come with hefty costs. Given their perceived safety and affordability, herbal remedies have gained popularity in addressing infertility. Hemidesmus indicus (HI), a traditional medicinal plant, is renowned for its purported efficacy in enhancing semen quality. Thus, this study aimed to evaluate the reproductive effects of Hemidesmus indicus (HI) extract on sperm abnormalities elicited by Monosodium Glutamate (MSG). Subfertile rats induced with Monosodium glutamate (MSG) were orally administered the ethanolic extract of Hemidesmus indicus (400mg/Kg body weight) for 30 days. The extract's impact on sperm count, motility, viability, morphology, testis weight, semen pH, was compared with control and MSG-treated groups. The results revealed that Hemidesmus indicus extract significantly boosted sperm count ($p<0.001$), and viability ($p<0.001$) motility ($p<0.001$), compared to the MSG group, which experienced notable declines in these parameters. Abnormal sperm morphology was significantly higher in the MSG group ($p<0.001$) but significantly reduced in the HIE group ($p=0.007$) and MSG+HIE group ($p=0.001$) compared to the MSG group. These findings indicate that Hemidesmus indicus extract mitigated the adverse effects of monosodium glutamate on sperm parameters, suggesting its potential in enhancing fertility and safeguarding the reproductive system against monosodium glutamate-induced damage.

Keywords ; Hemidesmus indicus Extract, Monosodium Glutamate, Spermiogram, sperm count,sperm morphology ,male sex hormones

1. Intoduction.

Male infertility, the incapacity of a sexually proficient male to inseminate a fertile female, stands as a significant issue (Pandruvada et al., 2021), contributing to 40.1–50.2% of infertility cases in humans, affecting 7% of males (Hirsh, A., 2003). Semen quality serves as an alternate measure of male fecundity, as deficits in semen often underlie male infertility. Recent advancements include sophisticated sperm analyses, examining sperm cellular components (Turner et al., 2020).

Infertility is widespread, with an estimated 60–80 million couples globally experiencing it (Calverton, 2004). In India, infertility rates vary among states, with male factors contributing significantly in at least 40% of cases (Sadock BJ et al.).

Male infertility, with its psychological and medical ramifications, having a substantial challenge to healthcare providers and society alike. Concerns have risen regarding declining semen quality in recent years (Fisher JR and Hammarberg K., 2012). Lifestyle factors such as excessive alcohol consumption, obesity, and smoking can negatively impact fertility. Moreover, exposure to environmental pollutants and toxins can directly harm gametes, leading to decreased numbers and quality (Gore AC et al., 2015; Segal TR et al., 2019)

Monosodium glutamate (MSG), a flavor enhancer containing 78% glutamic acid, reamining sodium, and water, is extensively used in food processing, restaurants, and households (Ataseven N et al., 2016). Despite its widespread use, there is concern over MSG misuse due to its presence in unlabeled food products (Egbuonu AC et al., 2009). While MSG enhances meal palatability, leading to increased appetite and potentially weight hike (Rogers PJ et al., 1990), studies indicate its toxic effects on various animal tissues, including damage to hypothalamic neurons and disruption of reproductive hormone secretion (Seo HJ et al., 2014). Additionally, MSG has been linked to liver, kidney, and cerebellum damage in rats, possibly contributing to male infertility (Ortiz GG et al., 2006; El- Meghawry et al., 2013; Hashem HE et al., 2012; Alalwani AD, 2013; Abd-Ella EM et al., 2016). High doses of MSG have also been associated with decline in sperm and sperm morphological defects in male Wistar rats (Onakewhor J et al., 1998) and oxidative stress through reactive oxygen species production (Hemalatha et al., 2003).

Traditional medicinal plants have long been used across cultures for various ailments, including fertility issues. Hemidesmus indicus R.Br. (Indian sarsaparilla), widely used in Ayurveda, Siddha, and Unani medicine, boasts pharmacological properties such as antioxidant, renoprotective, antinociceptive, and hepatoprotective effects (Ravishankara MN et al., 2002; Kotnis MS et al., 2004; Verma PR et al., 2005; Prabakan M et al., 2000). Despite its known medicinal actions, the fertility-enhancing effects of Hemidesmus indicus have not been extensively explored. Hence, this study aimed to assess the fertility effects of Hemidesmus indicus root (HIE) on Monosodium glutamate (MSG)-mediated alterations in sperm quality and sex hormone.

2. Materials and Methods

Chemicals

Monosodium Glutamate (MSG) with a purity of 99% was procured from SF Traders, UP, India. A solution was prepared by dissolving 100gm of MSG in 100 ml of distilled water. The dosage regimen was adjusted to administer an appropriate amount of MSG per animal based on their respective weights.

Preparation of Plant Extract

Identification and authentication were carried out by Dr. Ajith Kumar, a botanist from Government College,Department of Botany. a nearby herbal supplier from Marthandam, Tamilnadu, was the source of Hemidesmus indicus ,After cleaning and drying, the roots were crushed. Using a soxhlet equipment, the ethanolic extract was made for more than 70 hours, providing a 9.8% yield. The resultant extract, which was sticky and dark brown, was chilled until needed.

Experimental Animals

Adult male Wistar rats, weighing approximately 200 grams, were obtained from the central animal house, Kovai College of Pharmacy. Rats were acclimated for two weeks under controlled laboratory conditions ($30\pm2^{\circ}$) temperature and 50±4% humidity) in polypropylene cages with paddy husk bedding. Each cage housed three rats to prevent overcrowding. Ad libitum water and laboratory food were provided to the rats (Lipton India Ltd.). The Institutional Animal Ethical Committee's stringent criteria were followed during every step of the experimental process. Four groups of six rats each were employed to divide the rats: One milliliter of distilled water was administered orally to Group 1 (Control); for thirty days, Group 2 (MSG) received monosodium glutamate (4 grams per kilogram body weight); Group

3 (HIE400) received an ethanolic extract of Hemidesmus indicus (400 mg per kilogram body weight); and Group 4 (MSG + HIE400) received both monosodium glutamate (4 grams per kilogram body weight) and Hemidesmus indicus extract (400 mg per kilogram body weight) orally for thirty days. Every day at 10.30 AM, treatments were given, with MSG given out prior to the daily administration of HIE.

Sacrifice and Dissection

Animals were euthanized 24 hours after the last treatment. Dissections was performed to obtain the reproductive system, followed by careful removal of testes and detachment of epididymis, which were then washed of accessory structures and weighed.

Sperm count and Morphology

The cauda epididymal duct was visible and cut on one side. As soon as possible, the sperm that was dripping from the incision was collected up to 0.05µL in a capillary tube. Following that, phosphate buffer saline was added to dilute it. After complete mixing, the diluted seminal fluid was used for the spermatological analysis. Neubauer's counting chamber was used to count sperm in accordance with established procedures. A portion of the diluted semen was mixed with a drop of eosin yellow, and then a smear was made on a sterile glass slide and left to dry. The morphological anomalies were inspected using an oil immersion or 40X microscope. For morphological evaluation, at least 200 spermatozoa from different fields of each slide were inspected. The percentage of spermatozoa that had aberrant morphology was recorded.

Sperm viability:

As stated by Nayanatara, A.K. et al. (2008), viability was assessed using eosin nigrosine staining. To measure the viability of the sperm, one drop of eosin stain Yellow and one drop of nigrosine are taken to an Eppendorf tube. A Pasteur pipette is used to mix in one drop of semen. At least 200 spermatozoa were found after a drop of the combination was placed on a microscope slide, covered with a coverslip, and stained spermatozoa were considered dead and unstained sperms considered as alive.

(Total sperm/Number of Live Sperm) × 100% = Viability

Semen pH:

The epididymis was punctured with a sterile pin as soon as the dissection was completed. The semen of the pin was rubbed against 4.0–10.0 pH paper. The color variations are read from the paper and correlate with pH.

Estimation of Sex Hormone

To estimate sex hormones, rats were weighed after treatment and blood samples were collected via retro-orbital puncture into tubes. Sera were isolated from blood samples by centrifugation at 3500 rpm for 15 minutes. The electrochemiluminescence immunoassay "ECLIA" was used to assess serum testosterone levels, and the manufacturer's instructions for the "cobas e immunoassay analyzers" kit were followed.

Statistical Analysis: Mean ± S.D. was used to express all the data. ANOVA was used to assess the data for statistical significance, and Bonferroni multiple comparison tests were then performed. A p-value of less than 0.05 was deemed noteworthy.

3. RESULTS:

Results

Changes in testicular weight, semen pH, and spermiogram parameters were recorded and analyzed. Notably, Testicular weight decreased in the MSG group but showed marked increase in the HIE and MSG + HIEgroups. Semen pH increased significantly in the MSG group compared to controls but remained near control levels in the HIE and MSG + HIE groups. Sperm count, motility, viability, and morphology were adversely affected by MSG administration but improved in the HIE and MSG + HIE groups compared to MSG alone

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Weight of the testis

Testicular weight changes were noted and represented in Fig. 2. Testicular weight dropped in comparison to the control group, whereas it increased significantly in the HIE and MSG+HIE groups ($p<0.001$) when compared to the MSG group.

Semen pH

The pH of the semen varied significantly after the treatment of monosodium glutamate (Fig. 3). While the semen pH in HIE and MSG+HIE stayed close to the control value, the semen pH in the MSG treated group increased dramatically ($p<0.001$) in comparison to the control. Compared to the MSG Group, the pH in the MSG+HIE group was considerably lower ($p<0.001$). The pH of the semen varied significantly after the treatment of monosodium glutamate (Fig. 3). While the semen pH in HIE and MSG+HIE stayed close to the control value, the semen pH in the MSG group increased dramatically ($p<0.001$) in comparison to the control. Compared to the MSG Group, the pH in the MSG+HIE group was considerably lower ($p<0.001$).

Spermiogram

The control and experimental groups' sperm counts, motility, viability, and abnormalities were noted and shown in Table 1.

When comparing the MSG group with the control, the sperm count drastically decreased ($p<0.001$). On the other hand, when compared to the MSG group, the count was significantly higher in the HIE group ($p<0.001$) and the MSG+HIE group ($p<0.001$), respectively. When compared to the control group, the MSG group's sperm motility dramatically decreased ($p<0.001$). By comparison, the HIE group ($p=0.020$) and MSG+HIE400 group ($p=0.002$) showed a substantial increase in motility in comparison to the MSG group.

When comparing the MSG group to the control, the sperm viability drastically decreased ($p<0.001$). On the other hand, compared to the MSG group, the viability was considerably higher in the HIE group ($p=0.017$) and the MSG+HIE group ($p<0.001$), respectively. When comparing the MSG group to the control, there was a substantial increase in sperm abnormalities ($p<0.001$). By comparison, the HIE group ($p=0.007$) and MS+HIE group ($p=0.001$) showed a substantial decrease in abnormalities as compared to the MSG group.

Testosterone concentration

Sex hormonal changes of the rats were noted and represented in Fig. 3,When comparing the MSG group to the control , there was no apparent difference in the testosterone levels ($p=0.26$). When compared to the MSG treated group, however, it demonstrated a substantial rise in the HIE group ($p=0.001$) and MSG+HIE ($p=0.011$).

TABLES AND FIGURES:

Table-1: Sperm analysis (spermiogram) of both control and treated experimental animals, with a sample size of 6 in each group.

	CONTROL	MSG	HIE	MSG + HIE
Sperm count ($x10^6$/mL)	62.67 ±0.65	41.72 ±0.54 ∗ p= 0.018	76.39 ±1.42 # p<0.001	69.1 ±4.38 ¶ p<0.001
Motility (%)	77.84 ±0.32	62.44 ±0.75 ∗ p=0.020	69.68 ±0.42 #p=0.004	72.38 ±3.39 ¶ p=0.035
Viability (%)	86.15 ±0.96	64.2 ±1.23 ∗ p=0.01	72.98±1.25 #p=0.001	78.66 ±3.11 ¶ p=0.041
Abnormalities (%)	21 ±2.15	38.66 ±0.88 ∗ p=0.01	28.7 ±0.48 #p=0.002	24.72 ±2.69 ¶ p=0.029

Note: ∗Control compared to MSG, # MSG compared to HIE, ¶ MSG compared to MSG + HIE

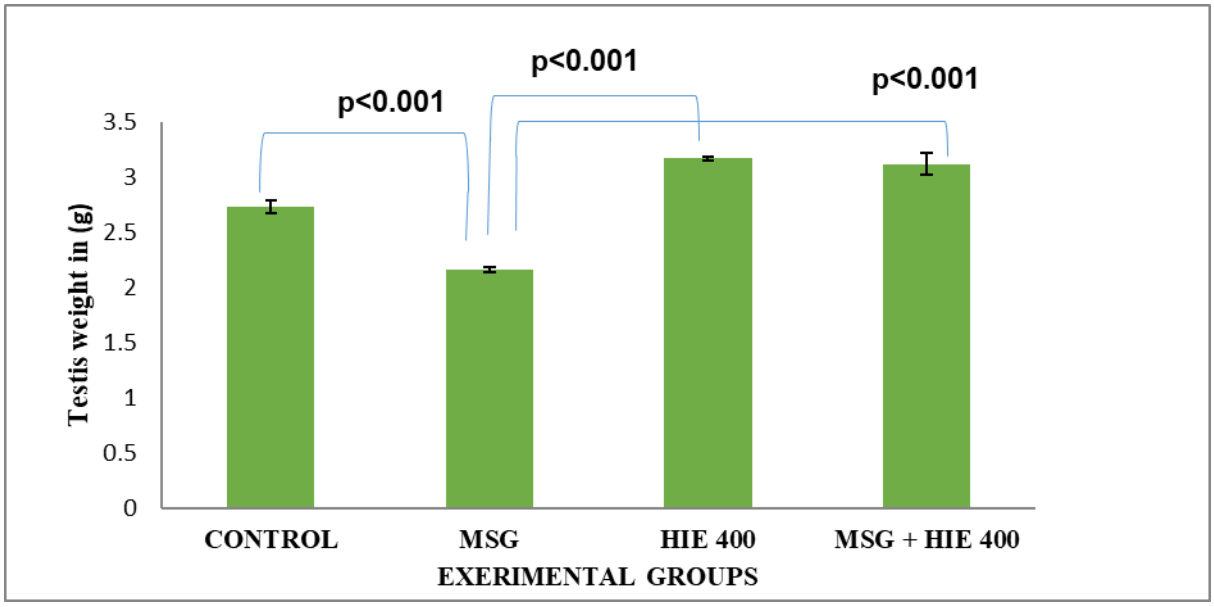

Fig-1: Testes weight comparison among animals in various experimental groups: Control compared to MSG ($p<0.001$); MSG compared to HIE400 ($p=0.005$); MSG compared to MSG+HIE400 ($p<0.001$).

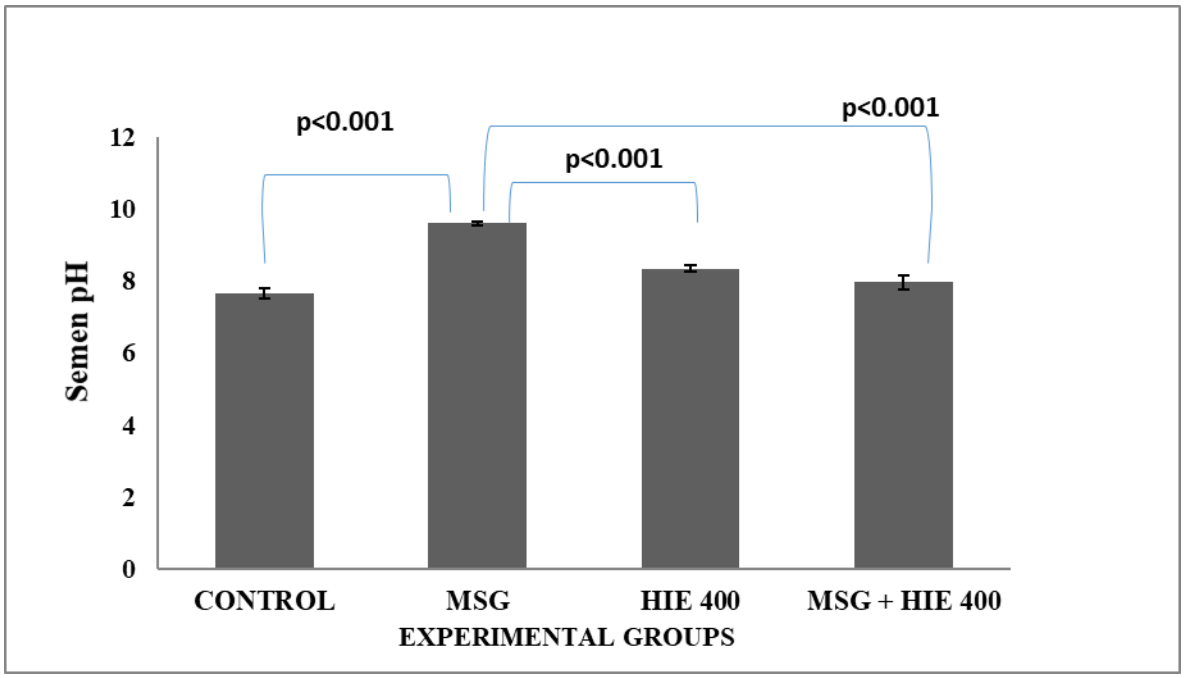

Fig-2: Comparing the pH of the semen from the animals in various experimental groups. MSG versus HIE400 ($p<0.001$), MSG versus Control ($p<0.001$), and MSG versus MSG+HIE400 ($p<0.001$).

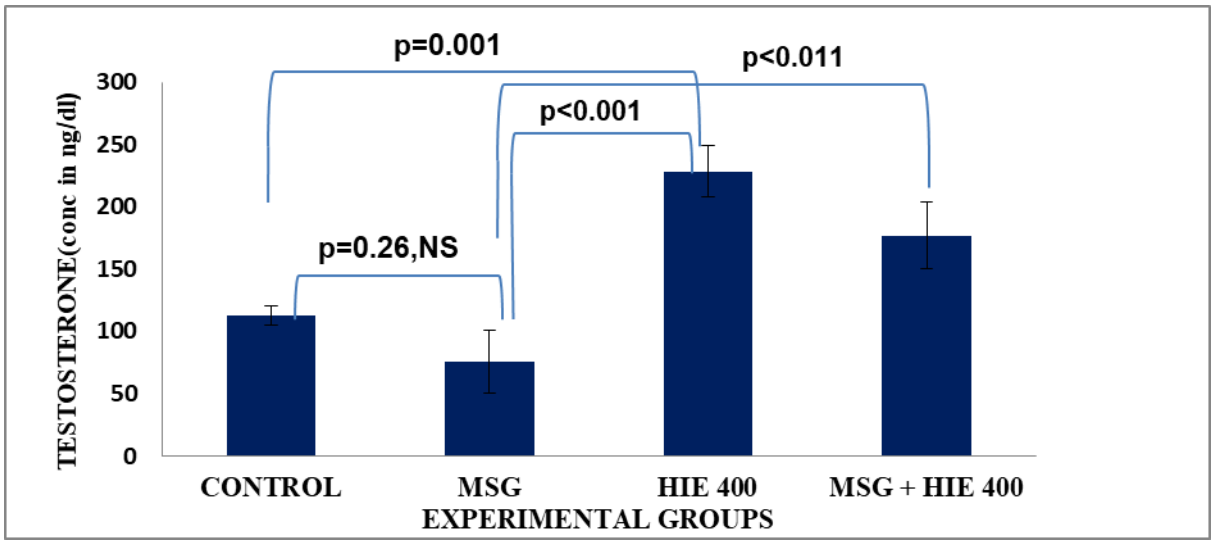

Fig-3: Comparison of Testosterone between the animals of different experimental groups. Control vs MSG (p=0.26, NS), MSG vs HIE400 (p=0.001); MSG vs MSG+HIE400 (p=0.011).

4. Discussion

The present study investigated the potential of Hemidesmus indicus in enhancing spermiogram including sperm count, motility and morphology an viability paramters in male Wistar rats. MSG, a commonly used food enhancer, has been implicated in disrupting the growth and function of the male reproductive system, as evidenced in both human and animal studies (Moore, 2003).The present study also conclude the implications of MSG on sperm paramters of rat testis.

Administration of MSG was associated with a significant decline in sperm count, motility, and viability, consistent with prior research indicating MSG's adverse effects on testicular function (Nayanatara et al., 2008; Ekaluo et al., 2013). In contrast, treatment with Hemidesmus indicus extract, particularly in combination with MSG, significantly improved sperm parameters, indicating its potential in ameliorating MSG-induced testicular toxicity. Previous studies have suggested that the phytochemical constituents of Hemidesmus indicus, such as alkaloids, steroidal lactones, and flavonoids, may influence spermatogenesis and enhance fertility (Devi BR et al., 2014).

MSG has been associated with the generation of oxygen-free radicals and oxidative stress in various animal tissues, further exacerbating reproductive dysfunction (Onyema et al., 2012; Kumar & Bhandari, 2013). In contrast, Hemidesmus indicus root extract has been shown to possess antioxidant properties, potentially mitigating oxidative damage induced by MSG (Mary NK et al., 2007). The present study also showed the influence of MSG on Testis weight, which has decreased significantly compared to HI and MSG+HI Group,which might be due to the atrophy of testicular tissues following MSG administration

The current study found a decrease in testosterone levels in the MSG-treated group. In contrast, the hormone level in the HIE group and MSG+HIE group increased dramatically when compared to the MSG group and control, demonstrating clearly that Hemidesmus indicus has altered the hypothalamic pituitary-gonadal axis. Additionally, MSG's neurotoxic effects on the hypothalamic-pituitary axis and central nervous system negatively impact

reproductive parameters (Samuels, 1999). However, administration of Hemidesmus indicus extract appears to counteract these effects, possibly through its antioxidant potential and modulation of hormonal balance as in the group HI and group HI+MSG.

Furthermore, MSG ingestion led to an elevation in semen pH, which could negatively affect spermatogenesis and sperm quality. In contrast, Hemidesmus indicus extract maintained normal semen pH levels, highlighting its protective effects on reproductive function.

5. Conclusion

Hemidesmus indicus root ethanolic extract demonstrates fertility-boosting properties and protects against MSG-induced reproductive impairment. The extract improves testicular weight, sperm parameters, and maintains optimal semen pH and hormonal balance, suggesting its potential as a therapeutic agent for reproductive health issues.

6. References

1. Abd-Ella, E. M., Mohamed, A., & Mohamed, A. (2016). Attenuation of monosodium glutamate-induced hepatic and testicular toxicity in albino rats by *Annona muricata* Linn. (*Annonaceae*) leaf extract. *J. Pharm. Biol. Sci.*, *11*, 61-69.

2. Alalwani, A. D. (2014). Monosodium glutamate induced testicular lesions in rats (histological study). *Middle East Fertility Society Journal*, *19*(4), 274-280. https://doi.org/10.1016/j.mefs.2013.09.003

3. Anzila, I., Marhendra, A. P. W., & Rahayu, S. (2019). The effect of monosodium L-glutamate (MSG) treatment for short and long terms to the semen quality of adult male rats. *Journal of Experimental Life Sciences*, *9*(2), 116-121. https://doi.org/10.21776/ub.jels.2019.009.02.09

4. Ataseven, N., Yüzbaşıoğlu, D., Keskin, A. Ç., & Ünal, F. (2016). Genotoxicity of monosodium glutamate. *Food and Chemical Toxicology*, *91*, 8-18. https://doi.org/10.1016/j.fct.2016.02.021

5. Bojanić, V., Bojanić, Z., Najman, S., Savić, T., Jakovljević, V., Najman, S., & Jancić, S. (2009). Diltiazem prevention of toxic effects of monosodium glutamate on ovaries in rats. *General Physiology and Biophysics*, *28* Spec No, 149-154.

6. Calverton, Maryland, USA: ORC Macro and the World Health Organization; 2004. World Health Organization. Infecundity, Infertility, and Childlessness in Developing Countries. DHS Comparative Reports No 9.

7. Chatterjee R, Bhattacharya B. A.(1995).note on the isolation of sitosterol from Hemidesmus indicus. Journal of Indian Chemical Society.;32:85.

8. Devi BR, Mohan C, Manjula P, Kumar BK, Naresh B, Devi BP.(2014)Phytochemical and micropropagation studies in Hemidesmus indicus (L.) R. BR. The Journal of Indian Botanical Society.;93(1and2):76-81.

9. Egbuonu, A. C., Obidoa, O., Ezeokonkwo, C. A., Ezeanyika, L. U., & Ejikeme, P. M. (2009). Hepatotoxic effects of low dose oral administration of monosodium glutamate in male albino rats. *African Journal of Biotechnology*, *8*, 3031-3035.

10. Ekaluo, U. B., Ikpeme, E. V., Ibiang, Y. B., & Amaechina, O. S. (2013). Attenuating role of vitamin C on sperm toxicity induced by monosodium glutamate in albino rats. *Pakistan Journal of Biological Sciences*. https://doi.org/10.3923/pjbs

11. El-El-Meghawry El-Kenawy, A., Osman, H. E., & Daghestani, M. H. (2013). The effect of vitamin C administration on monosodium glutamate induced liver injury.

An experimental study. *Experimental and Toxicologic Pathology*, *65*(5), 513-521. https://doi.org/10.1016/j.etp.2012.02.007

12. Fisher, J. R., & Hammarberg, K. (2012, January). Psychological and social aspects of infertility in men: An overview of the evidence and implications for psychologically informed clinical care and future research. *Asian Journal of Andrology*, *14*(1), 121-129. https://doi.org/10.1038/aja.2011.72

13. Gore, A. C., Chappell, V. A., Fenton, S. E., Flaws, J. A., Nadal, A., Prins, G. S., Toppari, J., & Zoeller, R. T. (2015). EDC-2: The Endocrine Society's second scientific statement on endocrine-disrupting chemicals. *Endocrine Reviews*, *36*(6), E1-E150. https://doi.org/10.1210/er.2015-1010

14. Gowda, K. M., Moodithaya, S. S., Kedilaya, V. R., Nayanatara, A. K., & Suchetha Kumari, N. (2021). A Nutraceutical approach to enhance Reproductive longevity and Ovarian health using *Curcuma longa* in Wistar rats. *Research Journal of Pharmacy and Technology*, *14*(10), 5385-5390.

15. Hashem, H. E., El-Din Safwat, M. D., & Algaidi, S. (2012). The effect of monosodium glutamate on the cerebellar cortex of male albino rats and the protective role of vitamin C (histological and immunohistochemical study). *Journal of Molecular Histology*, *43*(2), 179-186. https://doi.org/10.1007/s10735-011-9380-0

16. Hemalatha S, Wahi AK, Singh PN, Chansouria JPN. Anticonvulsant and free radical scavenging activity of Hybanthus enneaspermus: A preliminary screening. Ind J

Traditional Knowledge 2003; 2(4): 383-388. http://nopr.niscpr.res.in/handle/123456789/25972

17. Hermanussen, M., & Tresguerres, J. A. (2003). Does high glutamate intake cause obesity? *Journal of Pediatric Endocrinology and Metabolism*, *16*(7), 965-968. https://doi.org/10.1515/jpem.2003.16.7.965

18. Hirsh, A. (2003). Male subfertility. *BMJ*, *327*(7416), 669-672. https://doi.org/10.1136/bmj.327.7416.669

19. Ingle KP, Deshmukh AG, Padole DA, Dudhare MS, Moharil MP, Khelurkar VC.(2007) Phytochemicals: Extraction methods, identification, and detection of bioactive compounds from plant extracts. *J Pharmacogn Phytochem.*;6:32–6.

20. Kotnis MS, Patel P, Menon SN, Sane RT.() Renoprotective effect of *Hemidesmus indicus*, a herbal drug used in gentamicin-induced renal toxicity. *Nephrology (Carlton)* ;9:142–52.

21. Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: A review of literature. J Hum Reprod Sci. 2015 Oct-Dec;8(4):191-6. doi: 10.4103/0974-1208.170370. PMID: 26752853; PMCID: PMC4691969.

22. Kumar, P., & Bhandari, U. (2013). Protective effect of Trigonella foenum-Graecum Linn. on monosodium glutamate-induced dyslipidemia and oxidative stress in rats. *Indian Journal of Pharmacology*, *45*(2), 136-140. https://doi.org/10.4103/0253-7613.108288

23. Mary NK, Achuthan CR, Babu BH, Padikkala J. (2003).In vitro antioxidant and antithrombotic activity of Hemidesmus indicus (L) R. Br. Journal of Ethnopharmacology. Aug 1;87(2-3):187-91

24. 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.

25. Moore, K. L. (2003). Congenital malformations due to environmental factors. In *Developing humans* (2nd ed) (pp. 173-183). W. B. Saunders Company Ltd.

26. Nakai, M., Miller, M. G., Carnes, K., & Hess, R. A. (2002). Stage-specific effects of the fungicide carbendazim on Sertoli cell microtubules in rat testis. *Tissue and Cell*, *34*(2), 73-80. https://doi.org/10.1016/s0040-8166(02)00006-x

27. Nayantara, A. K., Vinodini, N. A., Damodar, G., Ahmed, B., Ramaswamy, C. R., & Shabarienth, S. (2008). Role of ascorbic acid in monosodium glutamate mediated effect on testicular weight, sperm morphology and sperm count in rat testis. *Journal of Chinese Clinical Medicine*, *3*(1), 1-5.

28. Onakewhor, J., Oforofuo, I., & Singh, S. (1998). Chronic administration of monosodium glutamate induces oligozoospermia and glycogen accumulation in Wistar rat testes. *African Journal of Reproductive Health*, *2*, 190-197.

29. Onyema, O. O., Aisil, C. S., & Ihetuge, A. P. (2012). Monosodium glutamate induces oxidative stress and affects glucose metabolism in the kidney of rats. *International Journal of Biochemistry Research and Review*, *2*(1), 1-11. https://doi.org/10.9734/IJBCRR/2012/827

30. Ortiz, G. G., Bitzer-Quintero, O. K., Zárate, C. B., Rodríguez-Reynoso, S., Larios-Arceo, F., Velázquez-Brizuela, I. E., Pacheco-Moisés, F., & Rosales-Corral, S. A. (2006). Monosodium glutamate-induced damage in liver and kidney: A morphological and biochemical approach. *Biomedicine and Pharmacotherapy*, *60*(2), 86-91. https://doi.org/10.1016/j.biopha.2005.07.012

31. Pandruvada, S., Royfman, R., Shah, T. A., Sindhwani, P., Dupree, J. M., Schon, S., & Avidor-Reiss, T. (2021). Lack of trusted diagnostic tools for undetermined male infertility. *Journal of Assisted Reproduction and Genetics*, *38*(2), 265-276. https://doi.org/10.1007/s10815-020-02037-5

32. Prabakan M, Anandan R, Devaki T. Protective effect of *Hemidesmus indicus* against rifampicin and isoniazide-induced hepatotoxicity in rats. *Fitoterapia*. 2000;71:55–9.

33. Ravishankara MN, Shrivastava N, Padh H, Rajani M..(2002 Evaluation of antioxidant properties of root bark of *Hemidesmus indicus* R. Br. (*Anantmul*) *Phytomedicine*. ;9:153–60.

34. 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. https://doi.org/10.1016/0031-9384(90)90230-2

35. Sadock BJ, Sadock VA. 9th ed. Philadelphia: Lippincott Williams and Wilkins; (2003). Kaplans and Sadocks Symptoms of Psychiatry Behavioral Sciences Clinical Psychiatry; pp. 872–4.

36. 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

37. Segal, T. R., & Giudice, L. C. (2019). Before the beginning: Environmental exposures and reproductive and obstetrical outcomes. *Fertility and Sterility*, *112*(4), 613-621. https://doi.org/10.1016/j.fertnstert.2019.08.001

38. 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 Journal of Physiology and Pharmacology*, *14*(4), 213-221. https://doi.org/10.4196/kjpp.2010.14.4.213

39. Sofowora, A., Ogunbodede, E., & Onayade, A. (2013, August 12). The role and place of medicinal plants in the strategies for disease prevention. *African Journal of Traditional, Complementary, and Alternative Medicines*, *10*(5), 210-229. https://doi.org/10.4314/ajtcam.v10i5.2

40. Turner, K. A., Rambhatla, A., Schon, S., Agarwal, A., Krawetz, S. A., Dupree, J. M., & Avidor-Reiss, T. (2020). Male infertility is a women's health issue-Resaerch and clinical evaluation of male infertility is needed. *Cells*, *9*(4), 990. https://doi.org/10.3390/cells9040990

41. Verma PR, Joharapurkar AA, Chatpalliwar VA, Asnani AJ. Antinociceptive activity of alcoholic extract of *Hemidesmus indicus* R. Br. in mice. *J Ethnopharmacol.* 2005;102:298–301.