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Ameliorative property of *Hemidesmus indicus* on Mono-Sodium Glutamate Induced deterioration in sperm quality, testosterone level and testicular weight in Male Albino Wistar Rats.

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ABSTRACT

Male infertility is a growing public health concern. Male infertility can be treated surgically or by interventions, yet both are expensive. Herbal therapies are a popular choice for treating infertility due to the lack of adverse effects and inexpensive. *Hemidesmus indicus* (HI) is a medicinal plant that is used in traditional medicine to treat infertility by improving the quality of semen , Thus, the current investigation was conducted to assess the reproductive effect of *Hemidesmus indicus* (HI) extract on Monosodium Glutamate (MSG) triggered alterations in spermiogram and testosterone levels. The ethanolic extract of *Hemidesmus indicus* was given orally (400mg/Kg body weight) on Monosodium glutamate (4gm/kg body weight) induced sub fertile rats for 30 days. The effect of the extract on sperm count, motility, viability, morphology, weight of the testis, pH of the semen, and testosterone levels was assessed and matched with the control and Monosodium glutamate (MSG) given group. *Hemidesmus indicus* extract effectively improved the sperm count ($p<0.001$), sperm motility ($p<0.001$), and sperm viability ($p<0.001$), which was significantly decreased in the MSG group comparing to the control group. The MSG group had significantly more abnormal sperm than the control group ($p<0.001$). The anomaly was significantly lower in the HIE400 group ($p=0.007$) and MSG+HIE400 group ($p=0.001$) compared to the MSG group. Although there was no significant difference in the testosterone level in the MSG group, it significantly increased in the HIE400 group ($p=0.001$) and the MSG+HIE400 group ($p=0.011$) in comparison to the MSG treated group. Consequently, the current investigation showed that monosodium-mediated decline in sperm parameters and testosterone level was ameliorated by the administration of HIE, which enhanced sperm count, motility, viability, and morphology. This proved that HIE plant root extract has the ability to promote fertility and protect the reproductive system from damage triggered by monosodium glutamate.

KEYWORDS: *Hemidesmus indicus* Extract, Monosodium Glutamate, Spermiogram, Sperm Count, Sperm Motility, Sperm Morphology, Sperm Viability, Testosterone

INTRODUCTION:

The inability of a sexually competent male to impregnate with a fertile female is known as male infertility (Pandruvada et al., 2021). It is responsible for 40–50% of infertility in humans. 7% of all males experience it (Hirsh, A., 2003). Semen quality is utilized as surrogate measure of male fecundity since deficiencies in the semen are frequently the cause of male infertility. A more recent development is the development of sophisticated sperm analyses that look at intracellular sperm components (Turner et al.,2020).

Infertility is a common issue, As per the WHO estimates 60–80 million couples worldwide currently suffer from infertility (Calverton, 2004) In India , estimates on infertility differs significantly among Indian states.,In at least 40 % infertility cases, male factor is a significant contributing cause(Sadock BJ et al).

Male infertility, with its psychosocial and clinical implications, poses a significant challenge to the physician and society. There has been considerable worry in recent years regarding the decrease in the quality of semen (Fisher JR and Hammarberg K., 2012). Lifestyle factors such as excessive alcohol intake and obesity, smoking can adversely affect fertility. In addition, exposure to surroundings pollutants and toxins can be directly harmful to gametes (eggs and sperm), resulting in reduced numbers and poor quality (Gore AC *et al*., 2015; Segal TR *et al*., 2019).

Monosodium glutamate (MSG), the sodium salt of glutamic acid, consists of 78% of glutamic acid, 22% of sodium, and water. MSG is used as a flavor enhancer throughout the world (Ataseven N *et al*., 2016). It is extensively used as a food additive in the food processing industry, restaurants, and households. It has been found in a variety of packaged or canned foods (Bojanić V *et al*., 2009).However, There is a possibility of MSG abuse due to its abundance in many food ingredients, most of which are not labelled (Egbuonu AC *et al*., 2009). MSG stimulates the orosensory receptors and improving the deliciousness of meals; therefore, MSG has positive impact on appetite, leading to weight gain (Rogers PJ etal., 1990).Previously various studies were done highlighting the toxic effect of MSG in different animal's tissues. It was reported that high doses of MSG in animals damage the hypothalamic neurons and alter the neural control of reproductive hormone secretion through the hypothalamic–pituitary–adrenal axis (Seo HJ *et al*., 2014). Furthermore, MSG causes damage to the rat liver, kidney (Ortiz GG et al., 2006; El-Meghawry *et al*., 2013), and

cerebellum (Hashem HE *et al*., 2012). Further, MSG has a toxic effect on rats' testis, possibly contributing to male infertility (Alalwani AD, 2013; Abd-Ella EM *et al*., 2016). Oligozoospermia and abnormal sperm morphology were observed in male Wistar rats given MSG dose-dependent (Onakewhor J *et al*., 1998). Oxidative stress occurs when there is an imbalance between reactive oxygen species (ROS) production and its removal in cells. MSG can induce oxidative damage in various organs by producing ROS, elevated lipid peroxidation, and change in the level of antioxidant enzymes (Hemalatha *et al*., 2003).
Medicinal plants, since times immemorial have been used in virtually all cultures as a source of medicine. Fertility control with plant preparations has been reported in ancient literature on indigenous medical systems, but only limited trials have been made to prove theseassertions (Sofowora *et al*., 2013). Traditional Indian medicine, with its evolution through centuries, has always fascinated practitioners and researchers for its potential applicability in treatment on a scientifically established research background.
Hemidesmus indicus R.Br. (Asclepiadaceae family) commonly known as "Indian sarsaparilla "is a widely scattered medicinal plant in India. The Root of the plant has been used as a traditional medicine mentioned in Ayrveda, siddha and Unani medicine,it is used as tonic and 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. Pharmacologically, it is claimed to possess, antioxidant (Ravishankara MN et al.,2002), renoprotective (Kotnis MS et al 2004), antinociceptive (Verma PR et al,. 2005) and hepatoprotective activity(Prabakan M *et al*,. 2000).It mainly consists of essential oils and phytosterols, e.g. hemidesterol, hemidesmol, and saponins. Though *Hemidesmus indicus* has many proven pharmacological actions, its fertility- enhancing effect has not been reported. Therefore, the present study was undertaken to evaluate the fertility effect of *Hemidesmus indicus* Extract (HIE) on Monosodium glutamate (MSG) induced changes in spermiogram and testosterone levels.

Material and Methods

Chemicals

The chemical used was Monosodium Glutamate, purchased from SF Traders, UP, India (Purity 99% NT). A stock solution was prepared by dissolving 100gm MSG crystals in 100 ml of distilled water. The dose schedule was so adjusted that the amount of MSG administration per animal was as per their respective weights.

Preparation of plant extract

Hemidesmus indicus was collected from a local herbal supplier (Power Lab), Marthandam, Tamilnadu. Botanist Dr. Ajith Kumar, Department of Botany, Government College, Trivandrum, did identification and authentication. The Roots were cleaned, shade-dried and

ground. The ethanolic extract was prepared using the soxhlet apparatus for 72 hours (Ingle KP et al 2017). The semi-solid materials were formed by removing the solvent from the plant extracts in a vacuum rotary evaporator under reduced pressure. The ethanolic extract (yield 9.8 %) was dark brown in color and sticky in nature. The extract was refrigerated until it used.

Experimental animals

Adult male albino Wistar rats weighing approximately 200 grams obtained from the central animal house, KMCH College of Pharmacy, were used. The rats were housed in polypropylene cages with paddy husk bedding under the laboratory conditions of (30±2) temperature and (50±4) humidity to acclimate for two weeks. In each cage, three rats were housed to avoid any stress due to overcrowding. The rats were fed with Laboratory chow and water ad-libitum (laboratory pellets; Lipton India Ltd.). All the experimental procedures followed the strict guidelines of the Institutional Animal Ethical Committee. 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, and 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 were treated everyday morning at 10.30 AM. MSG was administered (4gm/Kg body weight) before the daily HIE administration for 30 days. Animals were sacrificed 24hr after the last treatment by euthanasia. The laparotomy was done to expose the reproductive system after the last day of treatment .Testis were carefully removed and epididymis were detached from testis ,cleaned of accessory structures and weighed.

Sperm count and Morphology

On one side, the cauda epididymal duct was exposed and incised. The sperm oozing from the incision was soon drawn into a capillary tube up to 0.05µL level. It was diluted with phosphate buffer saline. The diluted semen was used for the spermatological analysis after being thoroughly mixed. Sperm count was performed using neubauer's counting chamber according to the standard procedure. One drop of eosin yellow was added to one part of diluted semen and smear was prepared using clean glass slide and dried. Morphological defects were analyzed after visualizing under microscope (40X or oil immersion). At least 200 spermatozoa were examined from different field of each slide for morphological evaluation. The number of spermatozoa with abnormal morphology were counted and recorded in percentage.

Sperm viability:

Viability was determined using eosin nigrosine staining (Nayanatara, A.K. *et al*., 2008). One drop of eosin stain Y and one drop of Nigrosine are added to an Eppendorf tube to assess sperm vitality. One drop of semen is added and mixed with a Pasteur pipette. A drop of the mixture was transferred to a microscope slide, covered with a coverslip, and at least 200 spermatozoa were identified, with stained spermatozoa, being regarded as dead and unstained spermatozoa as living.

Viability = (number of live sperm/total of sperm) ×100%

Semen pH:

A sterile pin was used to puncture the epididymis immediately following dissection. The pin's semen was rubbed against pH paper (4.0–10.0). The color changes coincide with pHand are read from the paper.

Estimation of sex hormones:

At the end of the treatment, the rats were weighed, and blood samples were withdrawn by retro-orbital puncture into sampling tubes. Sera were separated by centrifugation of blood samples at 3500 rpm for 15 min. Serum testosterone level was estimated using the electrochemiluminescence immunoassay "ECLIA" and performed according to the manufacturer's instructions in the "cobas e immunoassay analyzers" kit.

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

RESULTS:

The body weight of experimental animals was recorded using digital balance before and after the experiment to measure the changes. The body weight increased proportionally with time in the control, MSG, HIE400, and MSG+HIE400 groups. The body weight increased in the MSG group non-significantly compared to the control group. Animals treated with HIE also showed no significant decrease in body weight compared to control. In contrast, animals treated with MSG+HIE400 showed a significant decline (0.021) in body weight compared to the MSG-only treatment group (Fig-1).

Testicular weight

Changes in the weight of the testis were recorded and expressed in Fig-2. The weight of the testes relatively decreased compared to the control, whereas weight significantly increased in the HIE400 group ($p<0.001$) and the MSG+HIE400 group ($p<0.001$) compared to the MSG

group.

Semen pH

Monosodium Glutamate administration caused significant variation in the semen pH (Fig-3). The pH of the semen increased significantly in MSG treated group ($p<0.001$) compared to the control, whereas semen pH in HIE400 and MSG+HIE400 remained near the control value. The pH was significantly lower in MSG+HIE400 than in the MSG Group ($p<0.001$).

Spermiogram

The sperm count, sperm motility, sperm viability, and sperm abnormality of the control and experimental groups were recorded and represented in table-1. The sperm count significantly declined in the MSG group compared to the control ($p<0.001$). In contrast, the count was significantly increased in the HIE400 group ($p<0.001$) and MSG+HIE400 group ($p<0.001$) respectively compared to the MSG group. The sperm motility significantly declined in the MSG group compared to the control ($p<0.001$). In contrast, the motility was significantly increased in the HIE400 group ($p=0.020$) and MS+HIE400 group ($p=0.002$) compared to the MSG group, respectively.

The sperm viability significantly declined in the MSG group compared to the control ($p<0.001$). In contrast, the viability was significantly increased in the HIE400 group ($p=0.017$) and MSG+HIE400 group ($p<0.001$) compared to the MSG group, respectively. The sperm abnormality significantly increased in the MSG group compared to the control ($p<0.001$). In contrast, the abnormality was significantly decreased in the HIE400 group ($p=0.007$) and MS+HIE400 group ($p=0.001$) compared to the MSG group, respectively

Testosterone concentration

The testosterone level (Fig-4) did not show significant variation in the MSG group compared to the control ($p=0.26$). In contrast, it showed a significant increase in the HIE400 group ($p=0.001$) and MSG+HIE400 ($p=0.011$) when compared to MSG treated group.

Table-1: Spermiogram of control and treated experimental animals. N=6 in each group

	CONTROL	MSG	HIE 400	MSG + HIE 400
Sperm count ($x10^6$ /mL)	62.67 ±0.65	41.71 ±0.54 * p= 0.018	76.29 ±1.42 # p<0.001	69.1 ±4.39 ¶p<0.001
Motility (%)	78.84 ±0.32	61.44 ±0.75 * p=0.020	68.68 ±0.42 #p=0.004	72.38 ±3.39 ¶p=0.035
Viability (%)	84.15 ±0.86	65.2 ±1.23 * p=0.01	72.98±1.25 #p=0.001	79.86 ±3.11 ¶p=0.041
Abnormalities (%)	22 ±1.15	37.67 ±0.88 * p=0.01	29.8 ±0.49 #p=0.002	25.92 ±2.69 ¶p=0.029

Note:control vs MSG, # MSG vs HIE400, ¶ MSG vs MSG + HIE400

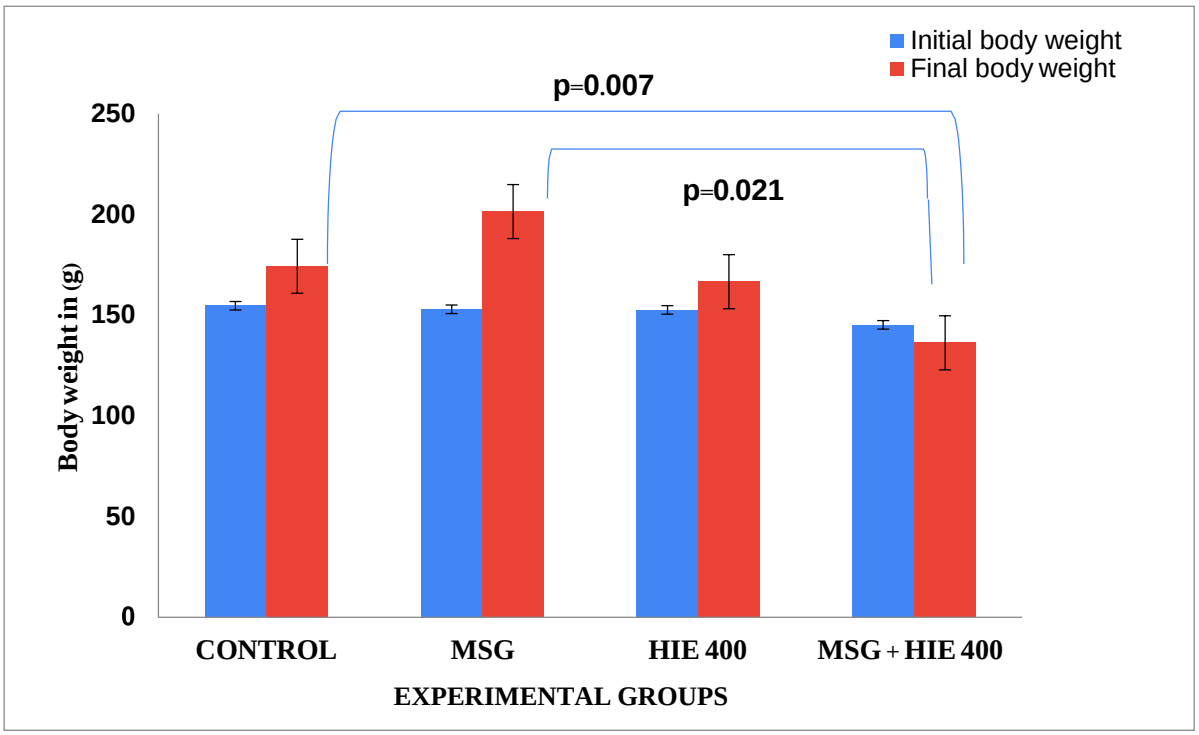

Fig-1: Comparison of final body weight between the animals of different experimental groups. Control vs MS+HIE400 (p=0.021): MSG vs MSG+HIE400 (p=0.021).

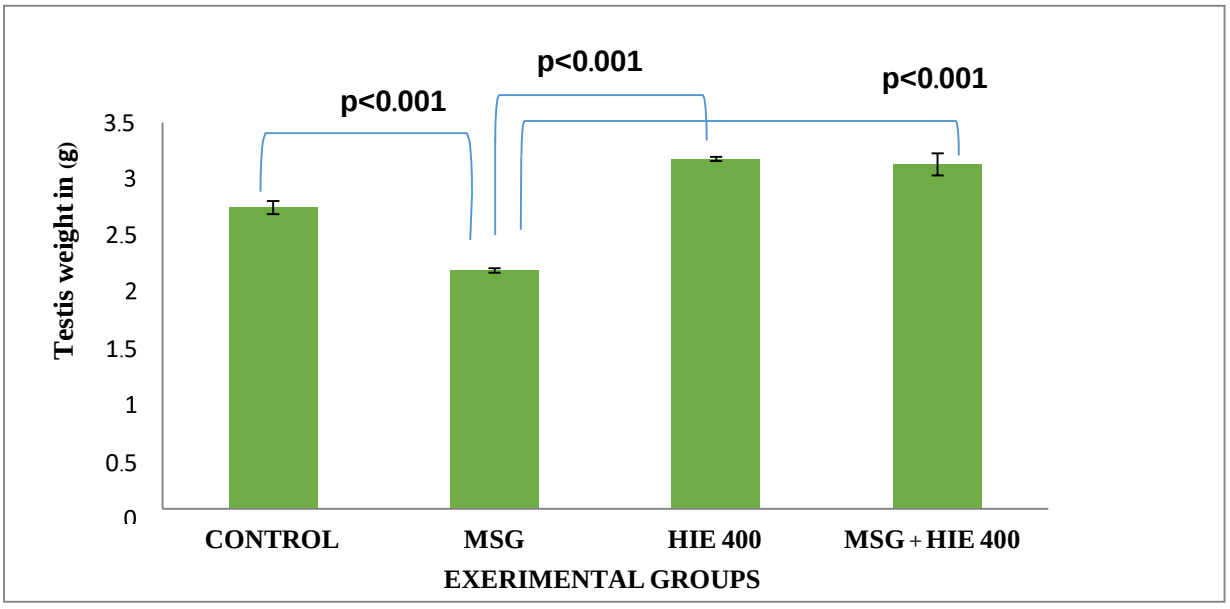

Fig-2: Comparison of testes weight between the animals of different experimental groups. Control VS MSG (p<0.001); MSG vs HIE400 (p=0.005); MSG vs MSG+HIE400 (p<0.001).

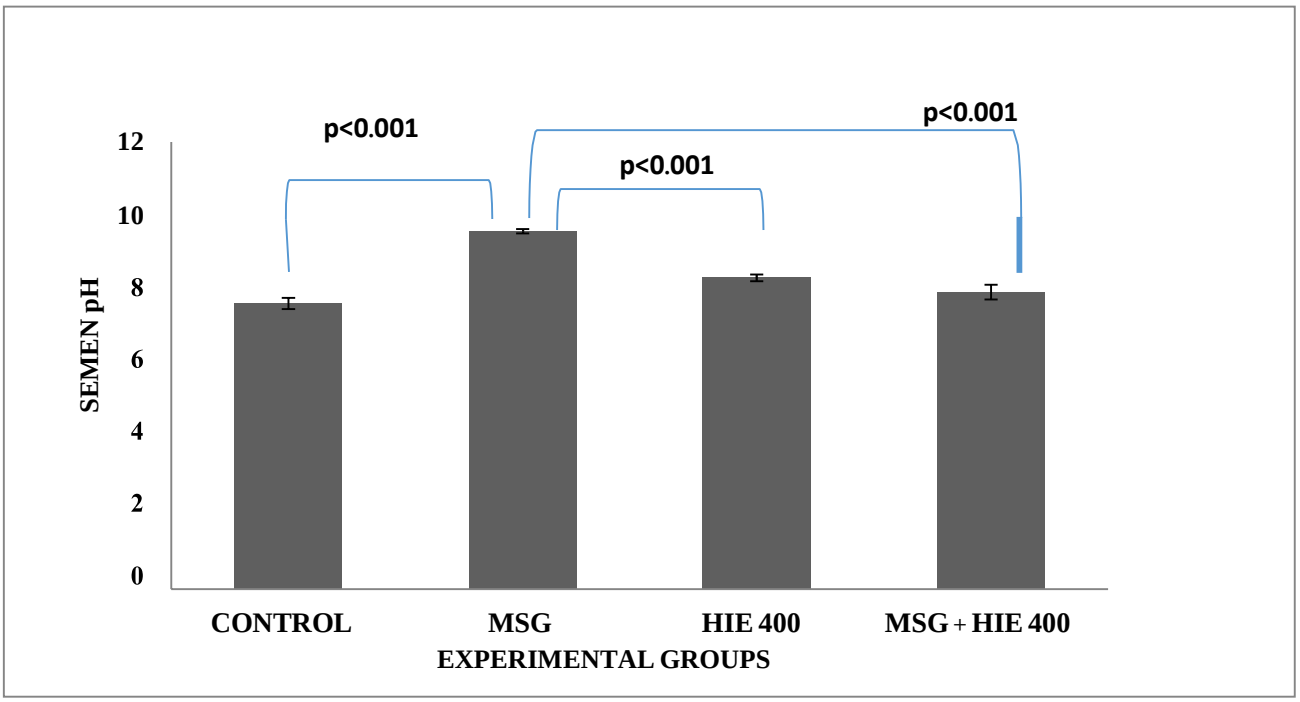

Fig-3: Comparison of semen pH between the animals of different experimental groups. Control vs MSG ($p<0.001$), MSG vs HIE400 ($p<0.001$); MSG vs MSG+HIE400 ($p<0.001$)

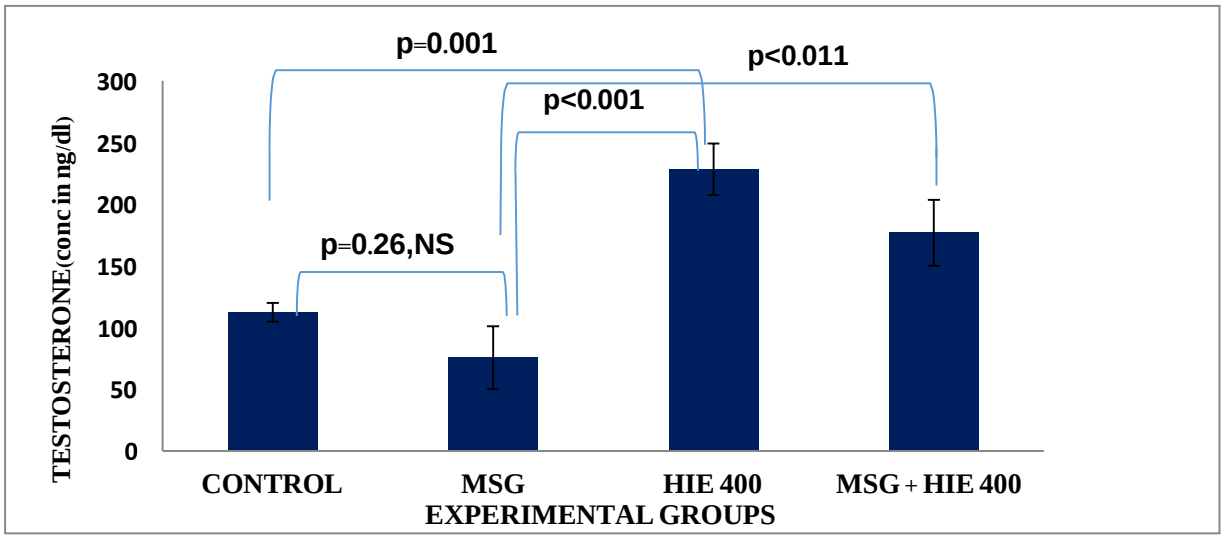

Fig-4: Comparison of Testosterone level between the animals of different experimental groups. Control vs MSG ($p=0.26$, NS), MSG vs HIE400 ($p=0.001$), MSG vs MSG+HIE400 ($p=0.011$).

DISCUSSION

The current study established the fertility-boosting property of *Hemidesmus indicus* in Monosodium Glutamate-induced reproductive abnormality in male Wistar rats. Monosodium glutamate (MSG), one of the popular enhancers used in food products, is known to hamper the growth and function of the male reproductive system and has been demonstrated to be damaging to human testicles as well as those of experimental animals (Moore 2003).

In the first 30 days of the present study, rats given MSG displayed higher body weight than their age-matched control group; this was in accordance with the earlier study (Rogers *et al*., 1990), who demonstrated that oral intake of MSG gave rise to increased body weight in rats. MSG stimulates orosensory receptors, increasing body weight. MSG also showed positive impact on the appetite center, which led to an increase in weight gain due to an increase in food palatability (Hermanussen and Tresguerres, 2003; Hermanussen *et al*.,2006). In the present study, there was a non-significant increase in the body weight in rats treated with MSG for a more extended period (30 days) compared to their age-matched control groups. Some studies showed that MSG administration for a more prolonged period caused reduction in body weight, and changes are only temporary (Mohamed *et al*., 2017). The current study showed that administration of MSG resulted in a significant decline in sperm count and motility, a significant reduction in viability and showed an increase in motility; this is in parallel with the previous study. These findings concur with those of other studies on the impact of MSG on testicles. Nayanatara *et al*., 2008 claimed that treating rats with MSG decreases the sperm count and increases the incidence of abnormality of sperm. According to Ekaluo *et al*., 2013, MSG treatment decreased testis and epididymis weight, sperm count, and increased sperm abnormalities. Even in the present study, which showed significant improvement in the sperm count in the HIE400 and HIE400+MSG group compared to the MSG Treated group, Moreover Motility and Viability of the sperm in the group HIE400 and MSG+HIE400 has increased significantly compared to the MSG Group. This demonstrates that the administration of *Hemidesmus indicus* ethanolic extract has ameliorated the toxic changes caused by the MSG. According to Devi BR *et al*., 2014, the plant contain alkaloids steroidal lactones, and flavonoids that may influence *Hemidesmus indicus* ability to increase fertility by influencing spermatogenesis. Based on previous studies, MSG was linked to

producing oxygen-free radicals and oxidative stress in many experimental animal tissues (Onyema *et al*., 2012; Kumar & Bhandari, 2013). Free radicals significantly contribute too many oxidative processes resulting in cell damage; hence, antioxidants are essential for preventing their formation (KM Gowda *et al*., 2021).

The ability of the *Hemidesmus indicus* root extract to scavenge free radicals was studied (Mary NK, et al.,2007). This ability of the plant to scavenge the free radicals may be the reason for enhancing the sperm parameters on Monosodium glutamate-mediated changes in sperm quality. The present study showed a reduction in the testosterone level in MSG treated group. In contrast, the hormone level in the HIE400 group and MSG+HIE600 group elevated significantly comparing the MSG group and control, indicating clearly that *Hemidesmus indicus* has influenced the Hypothalmo pituitary-gonadal axis.The hypothalamic-pituitary (HP) axis and other parts of the central nervous system (CNS) are affected negatively by MSG's neurotoxic effects (Samuels, 1999). The toxic effect of MSG on various reproductive parameters has been ameliorated by the *Hemidesmus indicus* plant extract, probably by the significant antioxidant potential of the plant as claimed earlier (Mary NK, et al., 2007) that might have probably come to protect the decline in sperm quality and ameliorating testosterone level. The pH of semen raised compared to the control group, and the pH remained normal in HIE600 and HIE400+MSG groups. The optimum pH is necessary for normal spermatogenesis and sperm count and motility; Low pH can affect spermatogenesis and sperm quality negatively; the present study showed that ingestion of MSG led to an elevation in pH, which might be due to the effect of MSG on acidic secretions from accessory glands.

CONCLUSION

Our findings show that *Hemidesmus indicus* plant root ethanolic extract has fertility-boosting capabilities as well as protection against Monosodium Glutamate-mediated impairment to the reproductive function. The extract aids in increasing testosterone levels, raising testicular weight, sperm counts, sperm motility, viability, and maintain normal morphology, and maintaining optimum pH.

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