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Research Article
Study on the Effect of a Mixture of *Hippocampus kuda* Extract and Panax Ginseng on Testosterone Content and Semen Quality in Experimental Rats

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Abstract

Background and Objective: *Hippocampus kuda* and ginseng are commonly known for male sexual function mainly for their potency and libido enhancement effects, however, no study was reported that incorporates these fluids. This study aimed at the extraction of these two ingredients to understand the synergistic effect of increasing testosterone content and semen quality was studied in this article. **Materials and Methods:** After extracting and creating extracts of *Hippocampus kuda* and ginseng, a 1:1 extract was mixed to create a mixture of both ingredients. **Results:** The results obtained from this study confirmed that alcohol extracts from *Hippocampus kuda* and Panax ginseng have a positive effect on testosterone content and semen quality. In particular, the combination of extracts from these two ingredients significantly improves testosterone content and semen quality compared with using extracts separately. **Conclusion:** High-extract mixture extracted by alcohol from *Hippocampus kuda* and Panax ginseng at the rate of 1/1 showed a superior effect in increasing testosterone content and semen quality compared to the extract of each material. A high extract mixture used at a dosage of 240 mg/100 g body weight/day is suitable for increasing the testosterone content, increasing sperm density, increasing the survival rate of spermatozoa and reducing the rate of malformed sperm in real rats experience.

Key words: *Hippocampus kuda*, ginseng, testosterone, sperm, mixture, infertility

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Male infertility with many different causes[1-4] has long been a concern of reproductive scientists. For couples, fertility is considered the main reason to keep happy and maintain the breed. Therefore, for infertile men, there is a risk of unhappiness not only for that person but also for their entire families[5,6]. There have been many studies in the hope of finding solutions to overcome male infertility[7-10]. This research aim is to help overcome infertility so it becomes extremely It is important and urgent now that male infertility is on the rise.

The *Hippocampus kuda* has long been used as a medicine by folklore[8,10]. People use seahorses to treat aches and pains, infections, asthma and physiological weakness. In addition, *Hippocampus kuda* is also known to reduce the size of cancer cells and promote leukocytosis or liquefy of human tumours. When fishermen catch Hippocampus, they preserve products by soaking wine as soon as the seahorse is alive or drying in the sun, then soaking it with wine or gold star powdered to improve health and increase energizing men. Panax ginseng is a medicinal plant that is popularly distributed in Northeast Asia, especially in Korea[1,7]. The research results have shown that ginseng can biosynthesize and accumulate valuable active ingredients such as triterpenes saponins, steroidal saponins, ginsenoside, especially vina-ginsenosides, polysaccharides, flavonoids and some essential oils. For a long time, ginseng has been known as a valuable medicinal herb that plays a role in increasing energy, increasing memory, protecting the body against stress and enhancing men's vitality.

In general, *Hippocampus kuda* and ginseng are commonly known for male sexual function mainly for their potency and libido enhancement effects, however, there are no studies that incorporate these fluids. Extraction of these two ingredients to understand the synergistic effect of increasing testosterone content and semen quality.

This study aimed to extract and create a mixture of *Hippocampus kuda* and ginseng extracts and to use this extract mixture to increase testosterone content and improve semen quality in experimental rats, towards the limit. Infertility for humans.

MATERIALS AND METHODS

Study area: The study was carried out at the Research Center for Anthropology and Mind Development, VNU University of Education, Vietnam from February, 2021 to March, 2022.

Make a mixture of *Hippocampus kuda* extract and Panax ginseng: The *Hippocampus kuda* is washed and crushed, then the ground powder is extracted with 70% ethanol according to the process of Lin *et al.*[8]. The process was summarized as: Seahorse ground powder was soaked in 70% ethanol according to the ratio of seahorse meal/ethanol which was 1/10 (w/v). The mixture of ethanol and seahorse meal was vibrated through ultrasound for 1 hr to disrupt cells and improve extraction efficiency, then the mixture was kept at room temperature for 36 hrs. The final extract was separated from the residue by a filter funnel. The extraction procedure was repeated 3 times[11]. The mixture of alcohol extracts from the extracts was pooled and then the solvent was evaporated by vacuum evaporation to obtain the extract and refrigerated at -200°C before use[12].

The Panax ginseng was crushed and extracted with 70% ethanol according to the process of de Souza *et al.*[13]. The process was summarized as follows: Ginseng powder was soaked in 70% ethanol according to the ratio of ginseng powder/ethanol is 1/8 (w/v). The mixture of ethanol and seahorse meal was vibrated through ultrasound for 1 hr to disrupt cells and improve extraction efficiency, then the mixture was kept at 400 in a thermostatic bath (ASTM D323, Switzerland) for 72 hrs. The final extract was separated from the residue by a filter funnel. The extraction procedure was repeated 3 times. The mixture of alcohol extracts from the extracts was pooled and then the solvent was evaporated by vacuum evaporation to obtain the extract and refrigerated at -200°C before use.

Mixture of extracts of *Hippocampus kuda* and ginseng: After extracting and creating extracts of *Hippocampus kuda* and ginseng, a mixture of extracts of 1:1 extract to create a mixture of extracts of 2 ingredients.

Experimental layout method on mouse: Adult male rats include 36 rats from Switzerland. They are divided into 6 groups, each group consists of 6 animals, of equal age and weight, including control lot, lot of *Hippocampus kuda* extract, lot of Panax ginseng, batch drink extract Mixture 1, batch drink extract Mixture 2 and batch drink extract Mixture 3. Before the experiment, all rats were given blood and semen samples to check the consistency of testosterone content and semen quality.

Hippocampus kuda extract and ginseng extract were dissolved with water and taken orally into the mouse body. The oral dosage was 240 mg/100 g body weight. Mixture 1 was given orally at a dose of 120 mg/100 g body weight,

Mixture 2 was given orally at a dosage of 240 mg/100 g body weight, Mixture 3 was administered orally at a dose of 360 mg/100 g of body weight. The high dose extract used in this study was referenced by Hwang *et al.*[1]. When publishing results on the role of seahorses and ginseng in antioxidant and protective properties of testis tissue and health enhancement for experimental rats. The extract and the mixture were dissolved with 0.3 mL of distilled water and used in a syringe with a rubber tube to pass into the digestive tract through the mouth. The control group did the same thing but only with distilled water. Every day, the rats drink at 9 am, after 15 min, feed and drink water as usual. Extraction was used for 3 consecutive weeks.

Biochemical sampling and analysis methods: The blood sampling of rats was done 24 hrs after fasting and after anaesthesia, it can be considered a painless method for rats. Blood samples were taken with a syringe in the caudate vein. The blood samples were analyzed for the concentration of testosterone in the blood plasma using the Active Testosterone RIA DSL-4000 kit, from Diagnostic System Laboratories Inc., USA, at the Research Center for Anthropology and Mind Development, VNU University of Education.

Rat sperm samples were collected according to the method of Hammodi [2]. Sperm samples were diluted according to the method of Turk *et al.*[12] and counted and proportional determined by the Neubauer counting chamber. LABART, Darmstadt, Germany), at the Research Center for Reproductive Health-Family Planning, Hanoi University of Education.

Statistical analysis: The collected data were organized, tabulated and statistically analyzed using SPSS12, software statistical computer package version 22 (SPSS Inc, USA). Data were presented as frequencies and percentages, Chi-square (χ^2) or Fischer exact test, when appropriate, was used as a test of significance. For interpretation of results of tests of significance, significance was adopted at $p<0.05$.

RESULTS

Testosterone levels in the blood of lab rats: Testosterone levels of rats at the beginning of the experiment and the end of the experiment were presented in Table 1. The results in Table 1 showed that the testosterone levels at the start of the experiment were similar in all groups. This proves that the rats used for the experiment had the same testosterone levels. After 3 weeks of *Hippocampus kuda* extract and Panax ginseng extract, testosterone levels differed significantly in the experimental groups and between the experimental and control groups (Table 1).

Rats who received *Hippocampus kuda* extract and Panax ginseng both had increased testosterone levels compared to the control, although the difference was not statistically significant. However, when rats were given a mixture of *Hippocampus kuda* extract and Panax ginseng, the testosterone content was significantly higher than the control and the difference was statistically significant ($p<0.05$). These results showed that the *Hippocampus kuda* alcohol extract and Panax ginseng had the effect of increasing the testosterone levels of the lab rats and the blend of these two ingredients was higher than every single ingredient in increasing testosterone levels in rats.

Sperm density of lab rat: The sperm density of the experimental rat was shown in Table 2. The results showed that the sperm density of rats before the experiment was not different, on average was 1.5 million sperm/mL. This means that the rat used in the experiment had a uniform sperm density across the lots. At the end of the experiment, there was a clear difference in sperm density between groups. The control group had a sperm density of 1.79 million mL^{-1}, while the group drinking *Hippocampus kuda* extract of Panax ginseng at a dose of 240 mg/100 g body weight/day all had a higher sperm density, reaching times. 1.99 and 1.91 million mL^{-1}, respectively.

Meanwhile, when using a mixture of extracts of these two ingredients, the sperm density of the experimental rats increased significantly, reaching 2.28, 2.62 and 2.71 in Mixture 1 plots, respectively: Mixture 2 and Mixture 3 (Table 2).

Survival rate of lab rats' sperm: The amount of surviving sperm in the semen plays a very important role in fertilization. Results of the sperm survival rate of rats in the experiment were shown in Table 3. The results showed that, at the beginning of the experiment, the rat had a homogeneous sperm survival rate, reaching about 70%. After 3 weeks of drinking *Hippocampus kuda* extract and ginseng, the survival rate of rats' spermatozoa in the batches changed. Specifically, the control group achieved the lowest results and the survival rate of spermatozoa in the batches given single extract or high extract mixture tended to increase and the highest increase in groups Mixture 2 and 3, although the difference between lots was not statistically significant.

Ratio of malformed spermatozoa in laboratory rats: The percentage of malformed spermatozoa in the experimental rat was shown in Table 4. Results showed that the rate of

Table 1: Test rats' blood testosterone levels (ng mL^{-1}) in different groups

	Experimental plots					
Parameters	Control	*Hippocampus kuda*	Panax ginseng	Mixture 1	Mixture 2	Mixture 3
Before experiment	2.46±0.11	2.42±0.16	2.53±0.21	2.44±0.17	2.50±0.15	2.59±0.16
After experiment	2.59±0.16[a]	3.08±0.15[ab]	2.97±0.16[ab]	3.42±0.19[b]	3.98±0.22[c]	3.97±0.22[c]

Data are expressed as Mean±Standard error and values in the same row with different letters differ significantly ($p<0.05$)

Table 2: Rat sperm density (million mL^{-1}) in different groups

	Experimental plots					
Parameters	Control	*Hippocampus kuda*	Panax ginseng	Mixture 1	Mixture 2	Mixture 3
Before experiment	1.53±0.11	1.49±0.13	1.51±0.15	1.51±0.10	1.51±0.13	1.52±0.07
After experiment	1.79±0.15	1.99±0.14	1.91±0.12	2.28±0.16	2.62±0.12	2.71±0.21

Data are expressed as Mean±Standard error differ significantly ($p<0.05$)

Table 3: Ratio of viable spermatozoa (%) in different groups

	Experimental plots					
Parameters	Control	*Hippocampus kuda*	Panax ginseng	Mixture 1	Mixture 2	Mixture 3
Before experiment	68.4±4.4	70.1±4.6	69.8±3.9	68.6±4.1	69.0±5.1	70.7±3.7
After experiment	70.5±4.6	74.2±4.8	75.8±3.7	77.2±3.9	79.4±4.5	78.6±4.2

Data are expressed as Mean±Standard error with $p<0.05$

Table 4: Ratio of malformed spermatozoa in lab rats (%) in different groups

	Experimental plots					
Parameters	Control	*Hippocampus kuda*	Panax ginseng	Mixture 1	Mixture 2	Mixture 3
Before experiment	11.3±1.1	12.0±0.7	11.8±1.2	13.1±1.2	12.3±1.2	12.9±0.9
After experiment	13.5±1.5[a]	11.1±1.3[ab]	10.9±1.3[ab]	10.3±0.9[ab]	6.9±0.9[b]	6.6±0.8[b]

Data were expressed as Mean±Standard error with different letters differing significantly ($p<0.05$)

malformed sperm in rats at the beginning of the experiment did not differ between groups, at an average of 12%. However, at the end of 3 weeks of drinking *Hippocampus kuda* extract and ginseng, the percentage of malformed sperm in semen was significantly reduced.

DISCUSSION

Table 1 shows that, a mixture of extracts of 1/1 from seahorse extract and Panax ginseng extract at a dose of 240 mg/100 g body weight/day was consistent with the increase in testosterone levels in the blood of experimental rats.

Testosterone is important in sperm production and the maintenance of sex drive. When testosterone levels are depleted, mature sperm differentiation is affected. Many studies have shown that testosterone levels play an important role in maintaining health, sexual desire and sexual characteristics in men and maintaining appearance and masculinity in animals[7]. The extract in this study increased the concentration of testosterone in the blood, clearly a positive effect in improving the semen quality of lab rats.

In Table 2, although the difference compared with the control group was not statistically significant. This result showed that seahorse extract and Panax ginseng extract both tended to increase the sperm density of lab rats.

Table 3 shows the difference in sperm density of using high extract mixture Mixture 1 compared with control and high extract mixture Mixture 2 or Mixture 3 compared with *Hippocampus kuda* extract or ginseng was statistically significant ($p<0.05$). Thus, the combination of 2 types of extract from *Hippocampus kuda* extract and ginseng at the rate of 1/1 has a superior effect in increasing the sperm count of lab rats and the dose using 240 mg/100 g body. weight/day is suitable for increasing sperm count. In this experiment, the mixture of *Hippocampus kuda* extract and ginseng extract significantly increased sperm count. The results of this study are similar to those of Kopalli *et al.*[10].

Table 4 shows that the control group had the highest rate of malformed sperm, with 13.5%. In a batch of drinking seahorse extract or ginseng alone, the rate of malformed sperm tended to decrease, respectively 11.1 and 10.8%. In particular, when giving the rat a mixture of extracts of these two ingredients, the rate of malformed sperm decreased to

6.9 and 6.6% in Mixtures 2 and 3. This result proves that combining these two extracts and using a dosage of 240 mg/100 g of body weight/day has helped reduce the rate of malformations of sperm in the sperm of lab rats. The change in the percentage of malformed spermatozoa that decreased when given the extracts to mice was also found in previous studies, which shows that this is a highly feasible research direction[7,8,10].

General, the high-extract mixture extracted by alcohol from *Hippocampus kuda* and Panax ginseng showed a superior effect in increasing testosterone content and semen quality.

The results in this study showed superior efficacy of the mixture of *Hippocampus kuda* and Panax ginseng compared with other previous studies[9,12,13]. Ginseng extract combined with seahorse has been shown to improve sperm and semen quality[7,8]. However, the ratio between these two ingredients is different, so the effectiveness of improving the quality and quantity of sperm is also different[7,8,10,13]. The results of this study have implications for the application of the product to testing on rats, the next step will be to conduct tests on humans to improve the quality of men's sperm. The limitation of the study is that it has not been tested on other animals to confirm the product's effectiveness.

CONCLUSION

High-extract mixture extracted by alcohol from *Hippocampus kuda* and Panax ginseng at the rate of 1/1 showed a superior effect in increasing testosterone content and semen quality compared to the extract of each material. A high extract mixture used at a dosage of 240 mg/100 g body weight/day was suitable for increasing the testosterone content, increasing sperm density, increasing the survival rate of spermatozoa and reducing the rate of malformed sperm in real rats experience. The results of the study have important implications for the application of the product in improving the quality and quantity of sperm and overcoming infertility in men.

SIGNIFICANCE STATEMENT

This study explores the effect of a high-extract mixture extracted by alcohol from *Hippocampus kuda* and Panax ginseng. This extract effect increases testosterone content and semen quality compared to the extract of each material. This study will help researchers uncover important issues related to semen quality and quantity to overcome male infertility.

REFERENCES

1. Hwang, H., J. Kim, J. Park, H. Yun and W.K. Cheon *et al.*, 2014. Red ginseng treatment for two weeks promotes fat metabolism during exercise in mice. Nutrients, 6: 1874-1885.
2. Hammodi, A.S., 2012. Effect of mobile phone on male fertility in rats. Mesopotamia J. Agric., 40: 1-8.
3. Bilia, A.R. and M.C. Bergonzi, 2020. The G115 standardized ginseng extract: An example for safety, efficacy, and quality of an herbal medicine. J. Ginseng Res., 44: 179-193.
4. Reay, J.L., D.O. Kennedy and A.B. Scholey, 2006. Effects of *Panax ginseng*, consumed with and without glucose, on blood glucose levels and cognitive performance during sustained 'mentally demanding' tasks. J. Psychopharmacol., 20: 771-781.
5. Sengupta, P., S. Dutta, I.R. Karkada and S.V. Chinni, 2022. Endocrinopathies and male infertility. Life, Vol. 12. 10.3390/life12010010.
6. Su, Y. and Y. Xu, 2015. Study on the extraction and purification of glycoprotein from the yellow seahorse, *Hippocampus kuda* Bleeker. Food Sci. Nutr., 3: 302-312.
7. Hwang, S.Y., W.J. Kim, J.J. Wee, J.S. Choi and S.K. Kim, 2004. *Panax ginseng* improves survival and sperm quality in guinea pigs exposed to 2,3,7,8-tetrachlorodibenzo- p-dioxin. BJU Int., 94: 663-668.
8. Lin, Q., J. Lin, J. Lu and B. Li, 2008. Biochemical composition of six seahorse species, *Hippocampus* sp., from the Chinese coast. J. World Aquacult. Soc., 39: 225-234.
9. Ryu, B.M., Z.J. Qian and S.K. Kim, 2010. Purification of a peptide from seahorse, that inhibits TPA-induced MMP, iNOS and COX-2 expression through MAPK and NF-κB activation, and induces human osteoblastic and chondrocytic differentiation. Chem. Biol. Interact., 184: 413-422.
10. Kopalli, S.R., K.M. Cha, S.Y. Hwang, M.S. Jeong and S.K. Kim, 2019. Korean red ginseng (*Panax ginseng* Meyer) with enriched Rg3 ameliorates chronic intermittent heat stress-induced testicular damage in rats *via* multifunctional approach. J. Ginseng Res., 43: 135-142.
11. Tambi, M.I.B.M. and M.K. Imran, 2010. *Eurycoma longifolia* Jack in managing idiopathic male infertility. Asian J. Androl., 12: 376-380.
12. Türk, G., A. Atessahin, M. Sönmez, A. Yüce and A.O. Çeribasi, 2007. Lycopene protects against cyclosporine A-induced testicular toxicity in rats. Theriogenology, 67: 778-785.
13. de Souza, L.R., A.L. Jenkins, E. Jovanovski, D. Rahelić and V. Vuksan, 2015. Ethanol extraction preparation of American ginseng (*Panax quinquefolius* L) and Korean red ginseng (*Panax ginseng* C.A. Meyer): Differential effects on postprandial insulinemia in healthy individuals. J. Ethnopharmacol., 159: 55-61.