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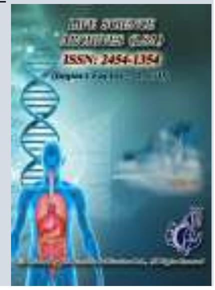

Research Article

PERFORMANCE OF FERTILITY IN RAT MALE BY USING FENUGREEK DRUG (NANOPARTICLES AND EXTRACT) (PERCENT VIABILITY OF SPERM, SPERM ABNORMALITY, SPERM MOTILITY AND SEMEN CONCENTRATION)

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Abstract

The study was carried out at the Animal's house, College of Veterinary Medicine, Babelon for the period between October 2019 to January 2020. A total of 40 adult male Albino rats were used in the current study, at the age of 2 months with body weight 195±15g was divided randomly into four equal groups (10 rats for each group) and treated as follows for 8 weeks. *First group Control group:* In this group, animals were left without any treatment like Negative control. *Second group:* Animals in this group were treated with a daily dose (100 mg/kg) Chitosan given orally by stomach tube. *Third group*: Animals in this group were treated with a daily dose (100 mg/kg) of extract fenugreek given orally by stomach tube. *Fourth group:* animals in this group were treated with a daily dose (100 mg/kg) of nanoparticles fenugreek given orally by stomach tube. Seminal fluid was collected to estimate the Individual motility, Mass motility, Sperm count, Sperm abnormalities and Sperm vitality test. The semen evaluation in the current study appears that dead sperm in the control group and third group showed no significant value between them while a reduction was more prominent in the second group chitosan group so that augmentation between adjuvant of nanoparticles to support a positive reduction in Fourth group that fenugreek nanoparticle. The live sperm, second group, and Fourth group showed a significant elevation when compared with third group and the control groups. The immotile and motile sperm Fourth group showed a significant elevation in sperm movement level when compared with either group. In another kind of semen evaluation, the sperm abnormalities were slightly elevated in rats that received an extract of *Trigonella foenum-graecum* and negative control group, although reduced were more prominent in second group that received chitosan and Fourth group that received *Trigonella foenum-graecum* nanoparticles to be recorded. In addition to semen concentration, there is a significant increase in all groups but more increase in Fourth group that received nanoparticles of *Trigonella foenum-graecum* when compared with a control group which indicate the improvement of nearly all parameters of sperm quality.

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Key words: Antioxidant, Fenugreek drug, Sperm, Rat and Nanoparticles.

1. Introduction

PNPs allow for the alteration of surface characteristics for drug site targeting, as well as improved overall stability, safety, and efficiency for local and systemic drug delivery. Their nanoparticles promote effective permeation *via* membrane barriers enabling boost bioavailability (Mittal *et al*., 2019). Chitosan (CS) is a polymer made from the deacetylation of chitin, a naturally occurring and abundant polysaccharide found in marine crustaceans. When employed to manufacture NPs for drug delivery applications, CS appears to have a number of benefits, including biocompatibility, biodegradability, and low immunogenicity. Its mucoadhesive characteristics and attractiveness for mucosal medication administration are also due to its high positive charge density (Fathalla *et al*., 2016). The cross-linking approach is discovered to be a straightforward way to make CS NPs.

The failure to achieve pregnancy after 12 months or more of frequent unprotected sexual intercourse is characterized as infertility, which is a reproductive system defect (Gurunath *et al*., 2011). Male infertility deficiencies account for around half of all infertility issues; there are a variety of conditions that might affect male fertility. Some male infertility issues are dissected by anatomical reasons such as varicocele, ejaculatory problems, or ductal blockages (Olayemi, 2010). Furthermore, sperm parameter abnormalities can cause male infertility; it has been estimated that sperm production disorders cause 35 – 80 % of male infertility. Other factors that drive male infertility are radiation, infection, cigarette smoking, heavy metals, reactive oxygen species, sperm antibodies, scrotal temperature, hormonal factors, and some therapeutic compounds. Asthenospermia, azoospermia, teratozoospermia, and other sperm abnormalities can develop from all of these conditions (Punab *et al*., 2017).

2. Materials and Methods

The goal of this research was to find out. According to Saleh *et al*. (2018) techniques of Gas Chromatography–Mass Spectrometry (GC-MS). Five gram of powdered extract was steeped overnight in 15 ml ethanol and filtered with Sodium sulphate through ashless filter paper (2 g). By adding nitrogen to the solution, the extract may be concentrated to 1ml. Both polar and non-polar phytocomponents were present in the extract. GC-MS analysis was performed on 2 liters of Nigella sativa ethanol extract. The components were separated using Helium as the carrier gas at a constant flow rate of 1 ml/min on a fused silica column BR-5MS (5 percent Diphenyl/95 percent Dimethyl poly siloxane), 30 m 0.25 mm ID 0.25 m 2l df on the Clarus 436 GC Bruker utilized in the study. The sample extract was fed into the instrument and identified using the software MS work station 8 and the Turbo Gold Mass Detector (Perkin Elmer). The oven was kept at a temperature of 110 °C for 2 minutes during the 36th minute of the GC extraction procedure. The temperature of the injector was set at 250 °C (Mass Analyser). The various characteristics of the Clarus 436 MS Bruker's functioning were likewise standardized (inlet line temperature: 200 °C). The mass spectra were collected at 70 eV with a 0.5 s scan interval with fragments ranging from 45 to 450 Da. The MS detection took 36 minutes to complete.

3. Result

The T1 group had a substantial increase in live sperm level (74.8 0.49 A) (p0.05) as compared to the control group (65.2 3.25 B, according to the findings of this study. Furthermore, the T2 group had a significant rise in live sperm level (69.22.48 B) (p0.05) when compared to the control group, but the T3 group had a greater increase in live sperm level (771.16 A) (p0.05) when compared to the control group. According to these findings, the T3 group, which received fenugreek nanoparticles, had more notable data on viable sperm levels than the other research groups (T1, T2, and control groups).

The percentage of immotile sperm was substantially higher in group control, T2, when compared to TFG nanoparticles. However, a significant drop in immotile sperm was detected when animals were given nanoparticles to the recorded mean value of (5.4 0.16 B) (p0.05). The results in this study showed that there was significant decrease in immotile sperm level (6 ±0.44 AB) ($p<0.05$) of T1 group when compared with control group (6.9±0.97 A). However, there was slight reduction in percentage of immotile sperm (6.45± 0.77 A) ($p<0.05$) of TFG extract. The results in this study reveal that there was significant elevation in sperm movement level in animal that received 100 mg of chitosan to reach to mean value (61.3±0.91) ($p<0.05$) when compared with negative control group (56.1±2,31 B). In addition, there was slight increase in sperm movement level to 59.7±1.8 B ($p<0.05$) in rats that received TFG extract only when compared with control group. On the other aspect, there is clear prominent increase in sperm movement to level (74±1.2) ($p<0.05$) in rats received orally nanoparticles at dose 100 mg /kg B.W. According to these results, T3 group that received nanoparticles was more effective on sperm quality.

The findings of this investigation revealed that there was no significant difference between animal study groups, despite a lower proportion in T1 that got chitosan and T4 that received TFG nanoparticles to a mean value of (10.8 0.37, 11.5 0.81). Furthermore, sperm abnormalities were somewhat higher in rats given TFG extract compared to the negative control group, although there was no statistically significant difference in the mean value (13.73.30 versus 15.44.6).

When the T1 group that received chitosan empty nanoparticles was compared to the negative control group (22.20.58), the findings indicated a substantial increase in semen concentration (37.60.74) (p0.05). However, there was a significant increase in semen concentration (53.80.80) (p0.05) in rats that received TFG extract only when compared to control group, while there was a clear improvement increase in semen concentration (89.42.46) (p0.05) in rats that received TFG nanoparticles when compared to control group that was given indicate in improvement of nearly all parameters.

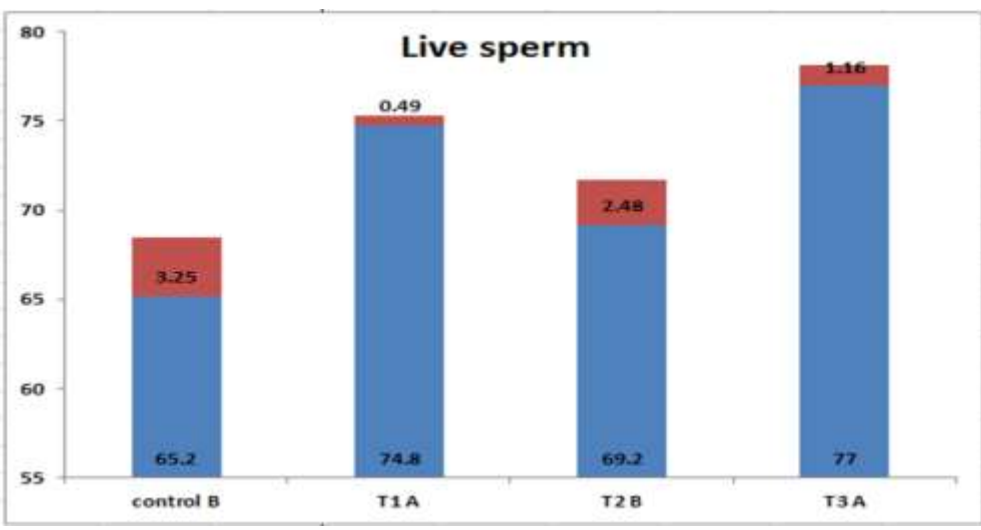

Figure- 1: Effect of Fenugreek (nanoparticles and extract) on viability of Sperm percentage

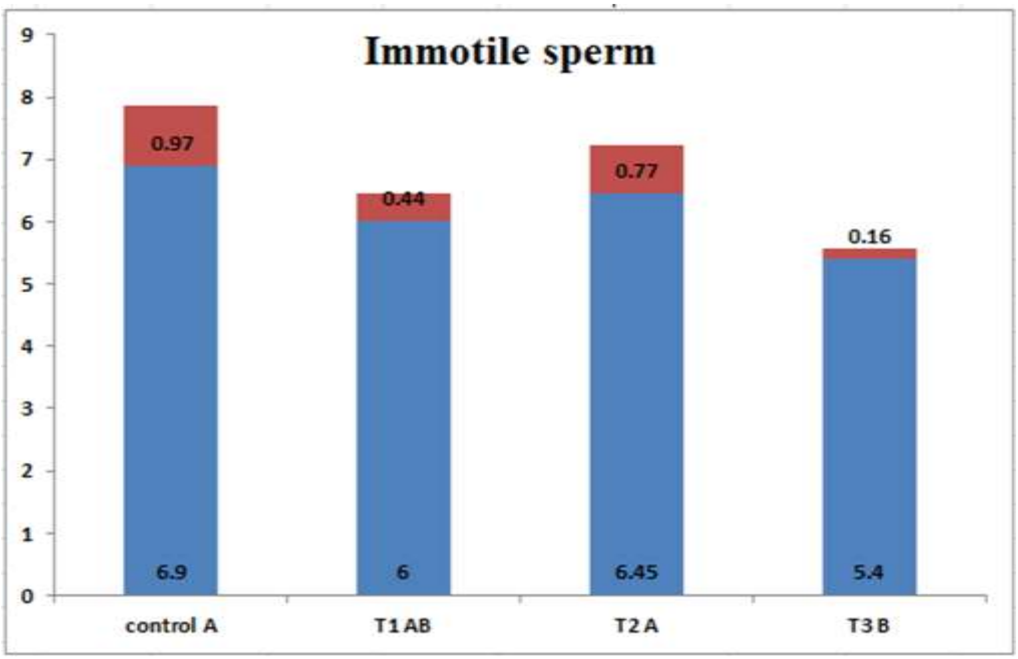

Figure - 2: Effect of Fenugreek (nanoparticles and extract) on the percentage of Sperm immotility

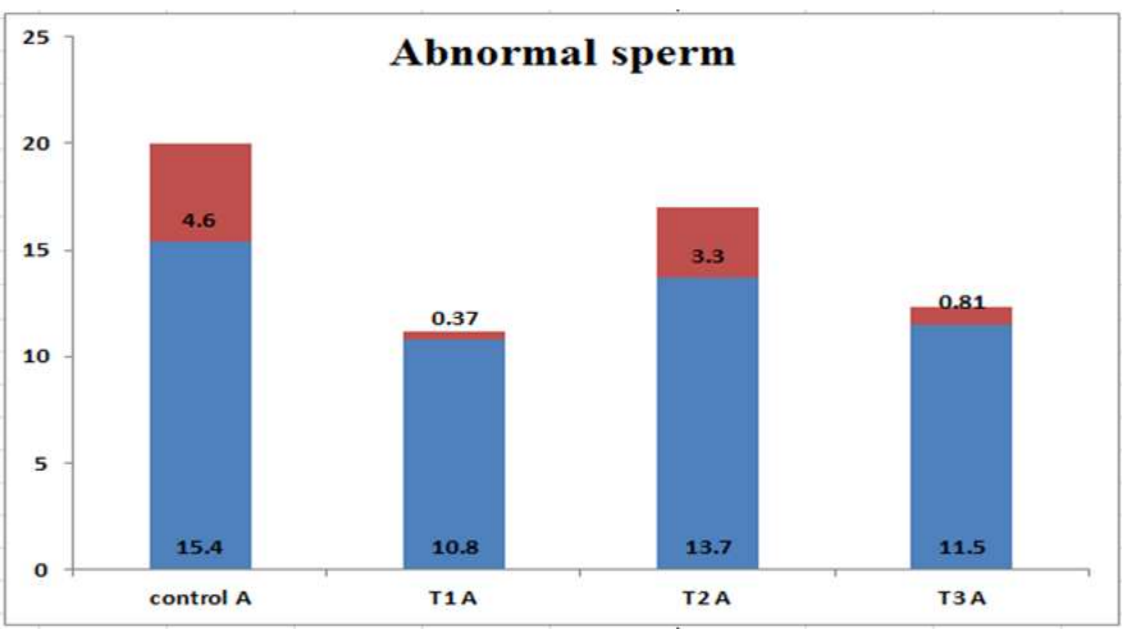

Figure - 3: Effect of Fenugreek (nanoparticles and extract) on the percentage of Abnormal sperm

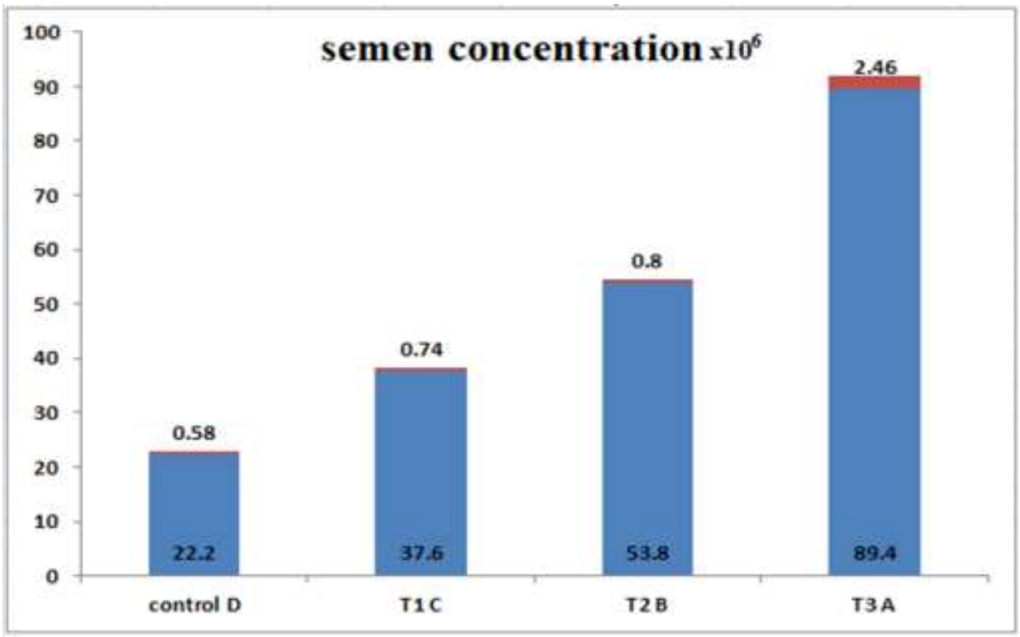

Figure - 4: Effect of fenugreek (nanoparticles and extract) on Semen concentration

4. Discussion

The results of this study were consistent with previous studies on the role of Fenugreek seeds in improving reproductive and physiological performance in male rats, and they demonstrated favorable significant effects in terms of semen quality. As a result, the purpose of this study was to see if fenugreek nanoparticles loaded with chitosan might improve the quality of semen and testis histology in male rats. However, there is a link to the veterinary application of the mentioned articles. Rahman (2010) investigated the role of Fenugreek seeds supplementation to the ration of aged broiler breeder males in activating reproductive performance and improving semen quality in order to extend their productive age. Al-Obaidy (2014), on the other hand, employed fenugreek seeds to improve the productive and physiological performance of elderly laying breeders.

The current study showed that semen quality rats received Fenugreek was less accurate when compared with nanoparticles loaded chitosan as well as chitosan received rats that may be due to chitosan has potent antioxidant activity as well as immune stimulator when compared with extract. In mice, long-term oral administration of fenugreek at higher doses resulted in reduced fertility, lower motility, sperm count, and an increase in the proportion of abnormal sperms, according to a number of studies. This could be due to a return to difficulty overcoming metabolism with the accumulation of free radicals.

Previous research of Al-Majed *et al*. (2006) have shown that glutathione deficiency causes spermatotoxicity and aberrant sperm morphologies. In addition, an endocrinological research in male mice's plasma revealed a rise in estradiole and a decrease in testosterone, both of which are known to influence fertility. The current study was conducted on a modest dosage hydro alcoholic extract of Fenugreek that enhances the semen parameters (volume, consistency, mass, and individual motility of spermatozoa, sperm concentration, percentages of living, dead, and defective spermatozoa) in Awassi rams (Etches, 1996). The influence on FSH and LH hormone concentrations that was proven by fenugreek seeds treatments might explain the clear improvement in semen concentration and quality (Taha, 2008).

Animals exposed to CCl4 and treated with CNPs exhibited considerable increases in antioxidant enzymes, reduced oxidative stress indicators, tumor markers, and significant improvement and regeneration of hepatocytes, according to a number of studies (Al-Baqami and Hamza, 2021).The present study showed that nanoparticles of fenugreek and empty chitosan showed a clear improvement of spermatogenesis quality, hormonal and concentration that is due to small size, and increase uptake by cell because modulated by TPGS that inhibit glycoprotein Pg that permit entry of active compound into intracellular and augmentation of endogenous antioxidants. These findings are consistent with those of Wen et al., who found that CNPs with a mean diameter of 83.66 nm protect cells from H2O2-induced cell damage by restoring the activity of endogenous antioxidants (SOD, GPx, and CAT) and increasing their gene expression. Despite the fact that no scientific research has been published to substantiate the involvement of CNPs in spermatogenesis modulation. These authors demonstrated that, in addition to normalizing oxidative stress markers, which is attributed to chitosan's antioxidant properties, with stabilizing the cell membrane, as well as the counteraction of free radicals due to its antioxidant nature and its ability to inhibit lipid accumulation due to its antilipidemic property, chitosan can also inhibit lipid accumulation by its antilipidemic property (Anraku *et al*., 2010). Oxidative stress is caused by Reactive Oxygen Species (ROS) such as H2O2, hydroxyl radicals, and superoxides, and it is at the root of a variety of diseases including cancer (Manda *et al*., 2009), reproductive damage (Zhang *et al*., 2010), rheumatoid arthritis, and inflammation (Filippin *et al*., 2008). Chitin, like vitamin C, has antioxidant properties and can be employed as a component in the development of functional foods to prevent age-related and diet-related disorders (Park and Kim, 2010; Kerch, 2015). In our research, we discovered that chitosan nanoparticles may stimulate efficient plant defense responses against oxidative stress in the open field, with higher efficacy than natural chitosan. The approach for making CNPS-loaded fenugreek allowed for the creation of homogenous, practically spherical NPs with a low polydispersity index and dimensions (range 30–200 nm) small enough to pass through stomatal pores and potentially unload their content into mesophyll intercellular spaces. Chitosan is also employed in the pharmaceutical industry, as well as for wound healing and tissue regeneration. Chitosan has been used as an excipient to prevent the active substance from being released from the nanocapsule. Chitosan with a high molecular weight is more viscous, and it's utilized to provide pharmaceuticals a longer shelf life, increase therapeutic efficiency, and lessen adverse effects (Kofuji *et al*., 2005). Because of its good film forming and muco adhesive characteristics, chitosan has also been reported for usage as a coating material in drug delivery applications, resulting in controlled drug release. Oral treatment of chitosan improved the phagocytic function of mice peritoneal macrophages, raised the index of immunological organs such as the thymus and spleen, activated lymphocytes to generate IL-2 cytokines, and boosted the activity of natural killer cells, according to research. By stimulating macrophages, chitosan and extract with TPGS increased the release of additional cytokines, resulting in a cascade response. Fenugreek enhances the body's immunity and acts as an antioxidant, according to several studies, and is known as an immunological booster (Gupta *et al*., 2021).

The effects of chitosan and fenugreek on male reproductive capacity were studied, and it was discovered that they considerably increased male reproductive capacity, boosted testes antioxidant capacity, and stimulated sperm production. Methanol and ethanol both had outstanding yield percentages, with mean values of 25.8 and 25.3, respectively, according to the research (Bhanger *et al*., 2008). Because antioxidant activity might be connected with polyphenolic components, ethanol extract had higher Total Phenolic Content (TPC), Flavonoid content (FC), and chelating activity of organic solvent extracts of fenugreek expressed as gallic acid, quercetin, and Na_2 EDTA equivalent, respectively. Hydroxymethyl furfural, Gingerone,

- Curcumene, Bergamotene, and Gingerol are the primary chemicals in Fengreek. They prevent cell development and fermentation and are utilized as antioxidants. They also help to overcome male and female sterility (Ishtiaq*et al*., 2020, Sunitha *et al*., 2018).

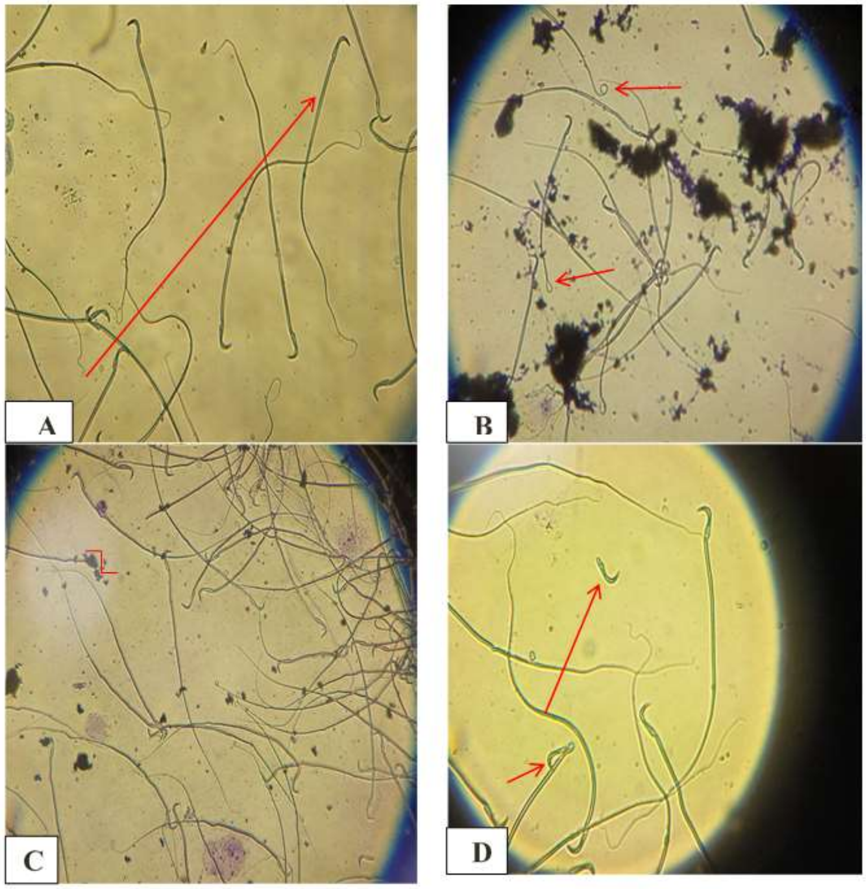

Figure – 5: Normal sperm (A), Coiled tailed (B), Banana head (C), Detached head and coiled head (D)

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