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## Effect of Hibiscus Rosa nanoparticles on sperm parameters of male albino rats

**Samer Habib Kareem**

College of Science, University of Babylon, Iraq

\*Corresponding author email:

[samir.kareem.scihigh177@student.uobabylon.edu.iq](mailto:samir.kareem.scihigh177@student.uobabylon.edu.iq)

**Faris Naji A. Alhady**

College of Science, University of Babylon, Iraq

**Adnan Mansour**

College of Veterinary Medicine, Al-Qasim Green University, Iraq

**Abstract**--Background; Herbal medicine is the world's oldest type of healthcare. Hibiscus rosa flower and leave extracts are used as natural remedies to treat various diseases, and as a reversible antifertility for males. Aim and objectives; this study aimed to investigate the effectiveness of nanoparticles to improve the impact of Hibiscus rosa flower extract as an anti-fertility substance. Animals and methods; twenty male albino rats were divided into five groups: G1 (-ve control): treated with normal saline (0.2 ml per day), G2 (+ve control): treated with nanoparticles (300mg empty liposome per day), G3: treated with flower extract nanoparticles (100mg/kg per day), G4: treated with flower extract nanoparticles (200mg/kg per day), and last group G5: treated with flower extract nanoparticles (300mg/kg per day). Sperm function tests (sperm concentration, sperm motility percent, and sperm viability percent) were estimated after the treatment in the five groups after 55 days of daily treatment. Besides, the diagnosis of compounds in the extracts of palmitic acid “ethyl ester”, ascorbic acid, 9-octadecenoic acid “methyl ester”, methyl stearate, oleic acid, 9,12-octadecadienoic acid, eicosane, heptadecane, 1-(1,5-dimethyl hexyl, fumaric acid, butanediol, stigmasterol, and beta-sitosterol, was performed using gas chromatography-mass (GC/MS) method. Results; the results showed that increasing the dose leads to a significant decrease in sperm concentration and sperm viability percent, also sperm motility percent was significantly decreased compared with the control group. Conclusion; many of the components derived from medicinal plants are used directly or indirectly for therapeutic applications, as the results of our current

study showed that dosing with extract of *Hibiscus rosa* flowers loaded nanoparticles for 55 days leads to beneficial results.

**Keywords**---hibiscus, extract, nanoparticles, sperm, chromatography.

## Introduction

Natural plant products are widely used nowadays due to the increased burden of diseases. Many plant extracts can be used as contraceptives by inhibiting the fertility of males and females [1, 2]. Various medicinal plants possess properties that reduce male fertility either by curbing the spermatogenesis process or reducing sperm viability or affecting their motility [3]. One of these plants is the extract of *Hibiscus rosa-sinensis* L. flowers which has inhibitory properties on fertility [4]. The phytochemical findings indicate that the flowers of *H. rosa-sinensis* comprised phenolic compounds, tannins, flavonoids, alkaloids, and anthraquinones [5, 6]. In addition to glycosides, protein, reducing sugars, essential oils, and steroids, played a role in the plant's medicinal functionality [7, 8]. It has several beneficial properties, including antihypertensive [9]. Anti-inflammatory [10]. And analgesic [11]. Besides, this plant has hypolipidemic and anti-diabetic effects [12–14]. Anti-parasitic effect [15]. Wounds healing [16]. The cardiac protective effect and reduce blood pressure and is useful for the treatment of hypercholesterolemia [17].

Nanoparticles (NPS) have the potential to enter cells and unite with them. Their DNA molecules are extremely tough to cope with, because of their immense complexity, small sizes, and specific physical and chemical characteristics that distinguish them from bigger materials composed of the same elements [18]. Various sorts of NPs have diverse characteristics and specific applications, due to the nature of the parent material and other considerations [19]. Despite recent advancements, some nanostructures might have toxic effects on cells, especially germ cells, and must be optimized with the right ingredients [20]. Furthermore, the germ cells could impair fertilization and fetal development [21]. Since fertility and effective reproduction are critical to the survival of a species, the present study aimed to investigate the effectiveness of nanoparticles to improve the impact of *Hibiscus rosa* flower extract as an anti-fertility substance.

## Materials and Method

The flowers of *Hibiscus rosa-sinensis* plant were collected from the nurseries of Babylon and Karbala governorates in the period between October to November 2021, it was washed and left to dry in the air at room temperature for three weeks and ground in an electric mill and kept in a tube until use.

### Preparation of crude extract to *Hibiscus rosa-sinensis* L.

Five grams of fine powder of *H.rosa* flowers was extracted with 100 ml of ethanol (70%) and 100 ml of acetone in a round bottom flask with a magnetic stirrer for 10 hours at room temperature. The flower extract was then filtrated by Whatman filter paper then the extract was placed in the oven at 40 °C until removed all

solvent. After grinding it, sodium azide is added to prevent bacterial growth. The extract is then kept in the refrigerator until used.

### **Preparation Nanoparticles** **Phytosome preparation**

The liposomes were prepared with the dried thin lipid film technique as described previously [22]. The lipid phase components include L- $\alpha$  phosphatidylcholine 0.25 g and cholesterol 0.25 g was dissolved in 15 ml of mixed organic solvents chloroform: methanol (2:1) mixed in the 25 ml glass test tube, Vortexed for 30 minutes, 1500 rpm. The mixture was kept warm in a water bath at 40 °C for 15 minutes and transferred to a round bottom flask connected with a vacuumed rotary evaporator machine at; 80 rpm, 40 °C attached to a vacuum pump. The round-bottom flask was immersed in a thermostatic water bath at 40 °C for 2 hours to affirm the dryness of the thin lipid film deposited on the inner walls of the round-bottom glass flask. The empty liposome was formed and suspended by a phosphate buffer.

### **Phytosome Loaded with *Hibiscus rosa-sinensis* L.**

The 400 mg of prepared extract was added to the 800 g of empty liposome and vortexed at 1500 rpm for 30 minutes and then phytosome solution was sonicated for 5 minutes using a probe sonicator (Hielscher sonicator, ultrasonic processor up 100 H [100 W, 30 kHz, 0.8 cycles, 80% amplitude]), and Tween 80 was added until final concentration 5% while stirring the solution and it was further sonicated for 5 minutes rejoined with the previous set of rotary evaporator under 80 rpm, 80 °C for 30 min.

### **Laboratory animals**

The study involved 20 male albino rats divided into five groups (4 animals for each group). All the animals were treated for 55 days and after that were anaesthetized and dissected and epididymis sperm was taken from the right epididymis for the sperm function test. The groups of this study as the following: G1 (-ve control): treated with normal saline (0.2 ml per day), G2 (+ve control): treated with nanoparticles (300mg empty liposome per day), G3: treated with flower extract nanoparticles (100mg/kg per day), G4: treated with flower extract nanoparticles (200mg/kg per day), and last group G5: treated with flower extract nanoparticles (300mg/kg per day).

### **GC-MS analysis**

Agilent 5975C Series GC/MS was used in the current study, and the analysis uses a compact silica column packed with DB-WAX (100% dimethyl polysiloxane, 30 m x 0.25 mm ID x 2.5  $\mu$ m). The oven temperature was set at 60 °C for 10 min, then raised at 20 °C/min to 230 °C and held at 250 °C for 10 min. The carrier gas, helium, was set to a linear velocity of 30 cm/sec.

## Statistical analysis

The SPSS was used to infer the significance and analysis of variance (ANOVA), and the least significant coefficient (LSD) was used to compare the results, the Correlation Coefficient test in addition to the standard statistical methods used to determine the mean and standard deviation (SD) [23].

## Results

The results pertaining to GC-MS analysis led to the identification of a number of compounds from the GC fractionations of the ethanolic extract of Hibiscus flowers. The results of the present study were tabulated in (Figure 1). The results revealed that the presence of many active compounds such as Palmitic acid, ethyl ester Ascorbic acid, 9-Octadecenoic acid, methyl ester, Methyl stearate Oleic acid 9,12-Octadecadienoic acid, Eicosane, Heptadecane, 1-(1,5-dimethyl hexyl, Fumaric acid, Butanediol, Stigmasterol, and Beta-Sitosterol. The results of the sperm function test for the animals treated with nanoparticles (phytosome) loaded with the extract of *H. rosa sinensis* flower. The result showed a significant decrease ( $p < 0.05$ ) of epididymal sperm concentration in three groups treated with nanoparticles loaded with *H. rosa* flower extract (100 mg/kg/day, 200 mg/kg/day, 300 mg/kg/day) of body weight compared to negative control group and group that received empty liposome, the G5 that received 300mg of *H. rosa sinensis* loaded with phytosome recorded continuously a significant decline ( $p < 0.05$ ) in comparison to G3 and G4 (Figure 2).

The present study showed that progressive sperm motility percent in G3 and G5 were significantly decreased ( $p < 0.05$ ) compared to G4, and control groups, also G4 recorded a significant decrease ( $p < 0.05$ ) compared to control -ve and control +ve (Figure. 3). On the other aspect current data showed that non-progressive motility percent were significantly decreased ( $p < 0.05$ ) in rats that received *H.rosa* loaded liposome with graded dose (100,200 and 300 mg/kg) compared to negative and positive control groups, also it was noticed that the more decreasing in non-progressive sperm motility in G3 compared with G4 and G5. While there is no significant difference ( $p > 0.05$ ) between negative and positive control groups (Figure 4). The current study reported that empty liposome has no influence on the motility of sperm and recorded data mimic with negative. Findings of the current study revealed that immotile sperm value between treated groups of *H. rosa* loaded with liposome showed a significant elevation ( $p < 0.05$ ) comparison with negative and empty liposome (Figure 5). In addition, the animals which were received *H. rosa* nanoparticles at a dose of 300 mg/kg showed clear reduction in the percentages of sperm viability compared with low doses (100, 200 mg/kg) that recorded a mean value that was no different from the negative and positive control group (Figure 6).

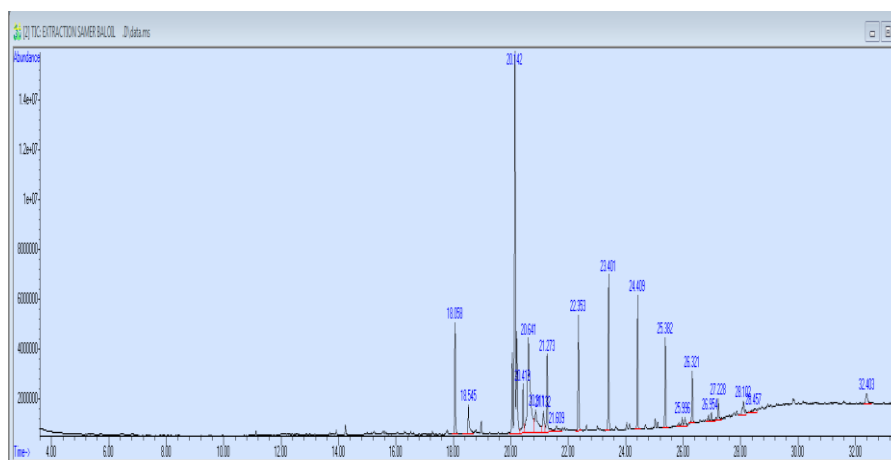


Figure 1. GC-MS analysis and fractionations of the ethanolic extract of *Hibiscus rosa* flowers.

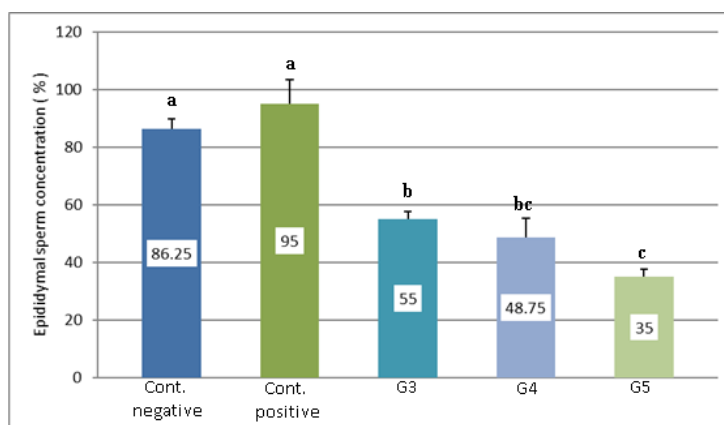


Figure 2. Epididymal sperm concentration in rats treated with *Hibiscus rosa* nanoparticles

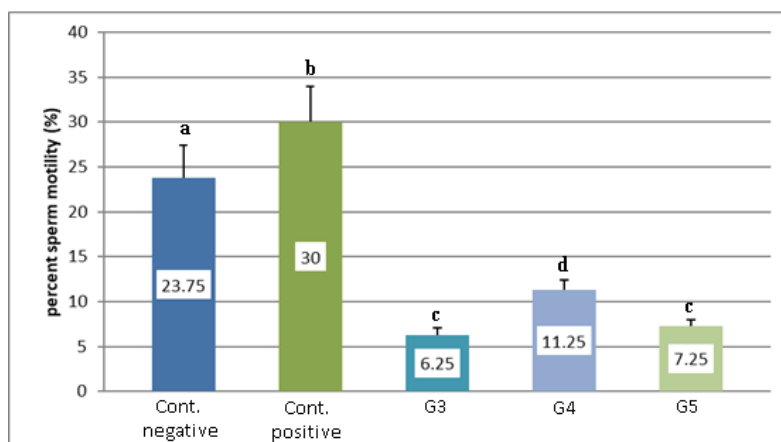


Figure 3. Epididymal progressive sperm motility percent in rats treated with *Hibiscus rosa* nanoparticles

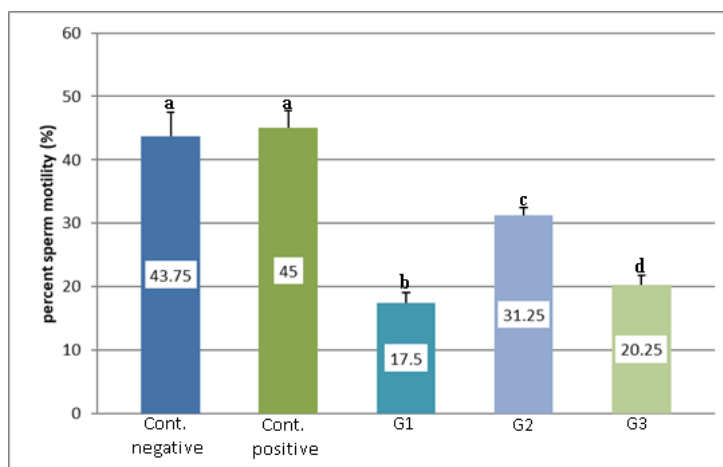


Figure 4. Epididymal nonprogressive sperm motility percent in rats treated with *Hibiscus rosa* nanoparticles

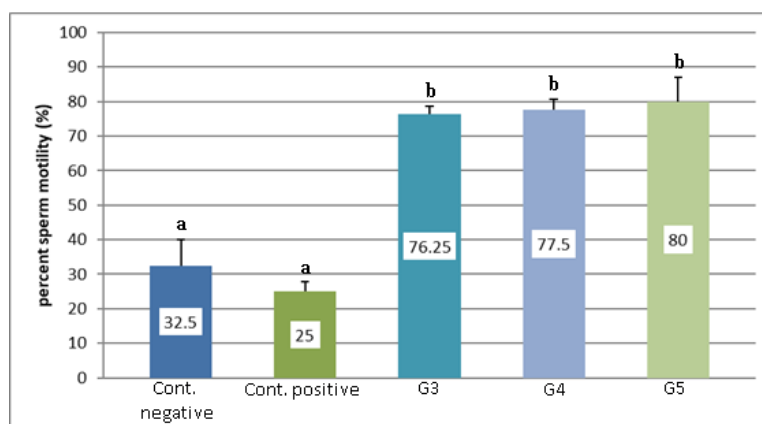


Figure 5. Epididymal immotile sperm motility percent in rats treated with *Hibiscus rosa* nanoparticles

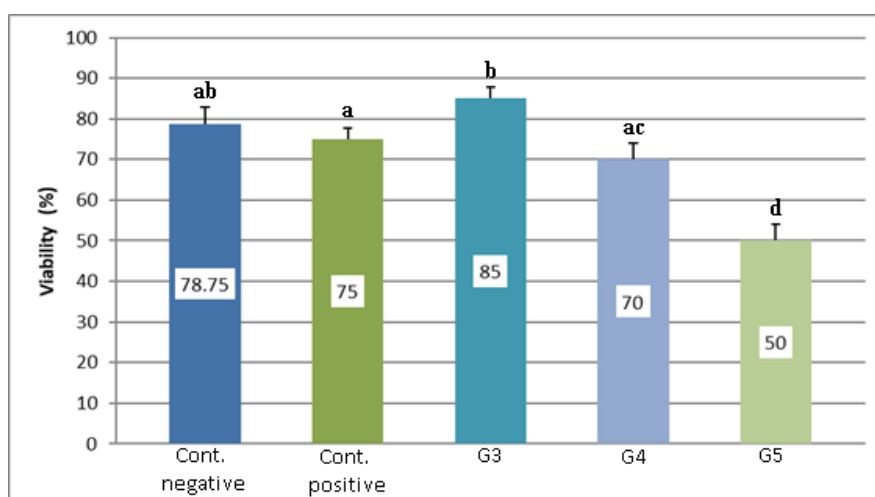


Figure 6. Epididymal sperm viability percent in Rats treated with *H. rosa* nanoparticles

## Discussion

Fertility regulation, which includes contraception and infertility management, is an essential component of reproductive health [24–26]. The effect of anti-androgens on the reproductive system and fertility of male albino rats was described by Al-Hady [24]. *Hibiscus Rosa Sinensis* Linn is flavones, containing, quercetin-3-sophorotrioside, cyaniding-3,5-diglucoside, and other constituents are cyanidin chloride, ascorbic acid, riboflavin, taraxeryl acetate,  $\beta$ -sitosterol, thiamine, hentriacontane, malvalic acids, and cyclic acids sterculic [27]. These compounds were shown to have antioxidant, cancer preventive, pesticide, Hypocholesterolemic, Dermatitigenic, and Anemia genic and Antioxidant [8, 27].

The present study was in concord with modern study confirmed [28]. That GC-MS analysis of Hibiscus flowers (ethanolic extract) showed many compounds as Propanal, (Ethanimidic acid, 1-Propanol, 2-methyl-(1.57%), ethyl ester (31.43%), Hexadecanoic acid, methyl ester (2.99%), (S)-(2.72%), 1,3,5-Triazine-2,4,6-triamine (2.48%), 2,3-dihydroxy (12.58%), Ethylenediamine (6.71%), O-Methylisourea hydrogen sulfate (4.06%), Ethene, ethoxy-(3.63%), N-Formyl- $\beta$ -alanine (2.36%), (Z)6,(Z)9-Pentadecadien-1-ol (1.70%), Butanediol (1.65%), Propanamide, N-ethyl-(10.69%), 7-Formylbicyclo(4.1.0) heptanes (2.80%), 2-Butanamine and Methanecarbothiolic acid (1.08%).

The results of the current study indicated that oral administration of phytosome of *H. rosa-sinensis* at a dose of 200 and 300 mg/kg of body weight for 55 days had a significant negative effect on sperm parameters in the tail of the epididymis, as there was a significant decrease in the average sperm concentration in the epididymis, the percentage of motile sperm, the degree of sperm activity, the percentage of live sperm, and an increase in Percentage of anaplastic sperms in the caudal epididymis as well as a decrease in fertility in treated males compared to the control group. These findings agreed with previous studies, as it was found that oral administration of the aqueous extract of *H. rosa sinensis* flowers to male mice at a dose of 300 and 500 mg/kg of body weight caused a significant decrease in the concentration of sperm in the testes and epididymis and a decrease in the percentage of fertility [8, 29]. Resulted from the dosing of the turpentine extract of the flowers *H. rosa sinensis*. At a dose of 175 mg/kg of body weight, sperm parameters and sex hormones decreased and the percentage of male fertility in egg rats decreased [30].

Furthermore, [38]. Found that the treatment of male albino rats with the phenolic components of *H. rosa sinensis* flowers at a dosage of 300 mg/kg for 60 days resulted in a significant decrease in testosterone level, substantial drop in testicular and epididymal weights, sperm motility percent, grade activity, epididymal sperms concentration, and live sperm percentage, with a significant elevation in the epididymal sperm abnormality. In a recent study [31]. Showed a significant decrease in the level of Testosterone and Progesterone in treated rats compared with control groups for 30 and 60 days of treatment, and a significant decrease in the expression of the androgen receptors and progesterone receptor in the testicular cells treatment group with phenolic compounds of *H. rosa-sinensis* flowers at a dose of 300 mg/kg/day of body weight for 60 days compared to control groups.

This effect may be attributed to the decrease in the secretion of FSH and LH hormones from the anterior lobe of the pituitary gland, which leads to the inhibition of the production of the testicular lipotropic hormone, which affects the process of sperm development, and then a decrease in the concentration of sperm inside the seminiferous tubules and the fertility of animals [32]. The production of naturally mature sperm is the basis of male fertility [33]. The production of sperm and testicular lipotropic hormone in the testicle is mainly regulated by the FSH and LH hormones, which are released from the pituitary gland, which are the main regulators of the process of spermatogenesis [34].



In treated animals, because of the inhibition of the androgen is responsible for the initiation and completion of the process of spermatogenesis during the normal maturation of the developing or developing sperm [35]. And that initiating the process of spermatogenesis and maintaining it quantitatively and qualitatively naturally requires the presence of adequate levels of hormones so that any decrease associated with severe abnormalities in the sperm and may lead to a state of oligospermia or asthenia. Androgen has an important role in the last stages of spermatogenesis via stimulating the conversion of round to elongated spermatoblasts during the spermatogenesis cycle, in addition to the fact that androgen deficiency impairs the process of sperm liberation [36, 37].

Perhaps the reason for the decrease in the concentration of sperm in the testes and epididymis is the effect of the extract on Sertoli cells responsible for incubating spermatoblasts and their differentiation with the effect of FSH, which stimulates the maturation of sperm in the seminiferous tubules by enhancing the production of androgen-binding protein by Sertoli cells by FSH receptors on the basal lateral membranes, which are important for the initiation of spermatogenesis [38]. However, in another study concerning Insulin-like growth factors (IGFs) showed insignificant differences in the level of IGF1, and IGF2 between the treated and control groups [39]. In this study, it was shown that the effective dose was 100 mg/kg.BW. So that of nanoparticles loaded with hibiscus rosa while ED50 of H.rosa flower extract approximately 300 mg/kg.BW. So that the benefit of nanoparticle using works is decreasing the effective dose of extraction without using nanoparticles.

The anti-fertility activity of the beautiful flower plant may be attributed to the presence of phytoestrogen compounds such as  $\beta$ -Sitosterol, Stigmasterol, Quercetin, Apigenidine, and Kaempferol, which are confirmed in the GC-MS that mimic functions of hormones in the reproductive system in mammals due to the similarity of their structural structure with estrogen [40]. This enables it to bind with estrogen receptors, causing misregulation of the latter and thus affecting the function of the reproductive system, and these compounds have been linked to fertility in mammals by affecting sexual development and reproductive system function [41]. It is now known that some plant estrogens such as genistein have the ability to bind to estrogen receptors  $\alpha$  and  $\beta$  and change the transcription of genes that respond to estrogen [42].

## **Conclusion**

Many of the components derived from medicinal plants are used directly or indirectly for therapeutic applications, as the results of our current study showed that dosing with extract of Hibiscus rosa flowers loaded nanoparticles for 55 days leads to beneficial results.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

### Authors' contributions

All authors had 1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND 2. Drafting the work or revising it critically for important intellectual content; AND 3. Final approval of the version to be published; AND 4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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