

Effect Of Ribavirin on The Testicles of Adult Rabbit Males and Investigation of The Protective Role of Garlic Plant Extract *Allium Sativum* (Light Microscopic Study)

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Abstract

The aim of this study was to detect the negative effects of Ribavirin on the testicles and to identify the protective role of garlic plant extract, as in this trial used 12 adult male rabbits aged (7-9) months, the animals were randomly distributed to four groups (3 rabbits) in each group, the first group control dosed only distilled water for (30) days, and the second group treatment with ribavirin with , the third group treatment with garlic extract orally dosed with aqueous extract of garlic plant at a dose of (50) mg/kg for body weight per day and for (30) days Fourth group treatment with Ribavirin and garlic extract orally dosed with aqueous extract of garlic plant at a dose of (50) mg/kg for body weight per day with Ribavirin (20) mg/kg by the dosing tube and for (30) days, and then the animals were sacrificed, the results of histological examination of the castrates of male rabbits treated with Ribavirin at a concentration of (20) mg/kg for mature spermatozoa form within the lumen. The study showed a clear improvement and the return of standards to their normal value and improved deterioration in the histological examinations of the castrated male rabbits after treatment Garlic plant extract with Ribavirin and reduce the damage and toxic effects of Ribavirin. The study of the parameters of the sperms showed the appearance of abnormalities in the shapes of the sperms treated with ribavirin compared to the control group, while the group treated with garlic extract at a dose (50) mg/kg of showed the normal shape of the sperms. Sperm shapes improved and returned to the normal level in groups of rabbits treated with garlic extract at a dose of (50) mg/kg of body weight and with ribavirin at a dose (20) mg/kg compared to the ribavirin group.

Keywords

Testes, *Allium sativum*, Ribavirin.

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Introduction

that human exposure to unsafe drugs is a major concern in the field of Public Health and the consequent technological development in various industrial fields to the production of many chemicals such as medicines, pesticides, food additives, cosmetics and other products needed by man. Therefore, it became necessary to study the negative effects of these substances on human health, hence the importance of resorting to the study of the toxic effects of any chemical compound (Gilbert and Knight, 1986). Hepatitis C Virus (HCV) is a global health problem and is considered a causative agent of viral hepatitis. In accordance with the net global health, injured at least 170 million people worldwide are infected with hepatitis C virus (Dixit and Perelson, 2006). Only 15-40% show persistence in the progression of the disease to cirrhosis and hepatocellular carcinoma. At present the combination of Pegylated interferon- α and Ribavirin is considered the basis of the treatment of viral hepatitis (HCV) (Abd El Latif, 2018), in May (2011) the use of two first-generation antivirals Boceprevir and Telaprevir in combination with interferon and Ribavirin in the treatment of viral hepatitis (HCV) was approved, and in December (2013) the second-generation antiviral is Simeprevir in combination with Ribavirin in (HCV) (Yau and Yoshida, 2014). Ribavirin is an antiviral that belongs to a group of similar bases and is a synthetic nucleoside specifically purine (guanosine) (Crotty et al, 2002). It was synthesized in 1970 and in 1972 approved in the treatment of respiratory syncytial virus (RSV) (Sidwell et al, 1972). US regulatory authorities also approved in 1998 the combination of ribavirin (oral) and Interferon alfa (intravenous) in the treatment of hepatitis C virus (HCV) (Tam et al, 1972). Ribavirin has also been used against Congo hemorrhagic fever virus (Graci and Cameron, 2006). Ribavirin belongs to the group of similar bases, as the Purine analogue enters the DNA molecule and inhibits the replication process of DNA and RNA and DNA replication of viruses, inhibiting their reproduction within the host (Howe et al, 2008). Ribavirin has many side effects, including Hematologic, Liver toxicity and embryotoxicity (Narayana et al, 2005), and other studies have proved its toxicity on the male reproductive system through experiments in mice. It was found that Ribavirin after entering the reproductive system and reaching the germ cells, where it acts on the death of germ cells, inhibits IMDH activity, which leads to reduced cell growth and may cause chromosomal damage (Moustafa et al, 2012). Growing interest in the use of medicinal plants as a treatment for various health disorders in the world due to their pharmacological and nutritional importance, *Allium stivum* is one of the oldest plants used in ancient folk medicine (Lestari and Rifai, 2018). Garlic is a flowering plant belonging to the lily family (Liliaceae) spread in Asia, Africa, the Middle East and parts of Latin America (Kamenetsky et al, 2005), garlic contains important compounds including amino acids such as Glycine, Cystine, Arginine, vitamins such as vitamin B1, vitamin B2, vitamin C and mineral elements. It also contains allicin which gives garlic the characteristic smell (Shaikh et al, 2016). Garlic contains important active compounds such as tannins, soaps, carotenoids, flavinoids and clicosides (Shaikh et al, 2016). The use of garlic is reported to be effective in restoring testicular function after experimental testicular insufficiency in mice, although no effect on impotence has been observed (Shang et al, 2019).

Its toxic effects on the male reproductive system and other antiviral drugs is not well studied. The objectives of the study came to know the effect of the drug and the protective role of garlic extract.

Materials and methods:

Collection of plants used in the study:

Obtained from the local market, garlic cloves are exposed to fresh air away from sunlight, dried, grinded and then kept in sealed plastic containers at room temperature until the preparation of the extract begins.

Preparation of aqueous extract of garlic plant:

Garlic the dry cloves of the garlic plant were crushed and turned into a fine powder using an electric grinder from AL-Araby – Take 50 grams of ground dry plant and put in a glass beaker capacity of 1000 ml and add to it 500 ml distilled water after which the material was mixed by the hyter device with magnetic motor hot plate with magnetic stirrer for 20 minutes at a temperature

of 40 M and then leave the mixture for 24 hours on the shaker 40 ° C to obtain the raw aqueous extract (Ilyas et al, 2011) I repeated the process several times to obtain a sufficient amount of the extract .

Animals Used in Study:

In this study, 12 domestic male rabbit animals, purchased from the local market and aged between 9-7 months and weighing (1500-1250 gram), were placed in special rabbit cages made of aluminum with dimensions (46×70×100 cm). - Floor furnished bar wood has been taken into account by the care of the cleanliness of the cages and sterilized with Toggle sawdust every two days, left the animals for a week to adapt to the new conditions were examined animals' laboratory by a veterinarian and make sure that they are free and integrity of fungal diseases and parasitic.

Experience Design:

The of animal were distributed distributed homogeneous of weight to 4 groups of at least 3 animals per group, were treated animals daily for 30-day:

- 1- Group 1 control treatment with distilled water.
- 2- Group 2 treatment with Ribavirin (RBV) 20 mg/kg twice daily by oral tube feeding.
- 3- Group 3 treatment with garlic extract 50 mg/kg once daily by oral tube feeding.
- 4- Group 4 treatment with garlic plant extract and Ribavirin (20 mg/kg) + (50 mg/kg) once daily by oral tube feeding+ Ribavirin at 20 mg/kg twice daily by oral tube feeding.

Sperm collection

The abdomen of the animal was opened using a dissection kit to obtain the testicles that the epididymis is attached to. Then the epididymis was separated from the testicles and placed in a glass petri dish, and using a sharp scalpel, the epididymis was cut into parts and a normal saline was added to them according to the method of (Alchalabi et al, 2019).

Microscopic Examination:

At the determined end date for each group, all rabbits were sacrificed from each group after giving deep anesthesia by ether inhalation. A mid line incision was done. The testis were excised and washed with normal saline and fixed in the fixative 10% formal saline containing 100 ml of formalin, 8.5 grams of sodium chloride and 900 ml of water contained in glass bottles with glass stoppers which had been properly labeled before handling. Tissue processing was done. Histological sections of 3-5 Mm thickness were taken and stained with Hematoxylin and Eosin stain. Slides were observed under the light microscope magnifications (El-Demerdash et al, 2005).

Results

Study of Histological:

Results of control group1 G1:

Light microscope examination:

Examination of HandE-dyed histological sections of the control group rabbits explained the normal shape of the seminiferous tubules, regularity of their forms and differentiation of the stages of spermatogenesis and mature sperms within the seminiferous tubule (Figure 1).

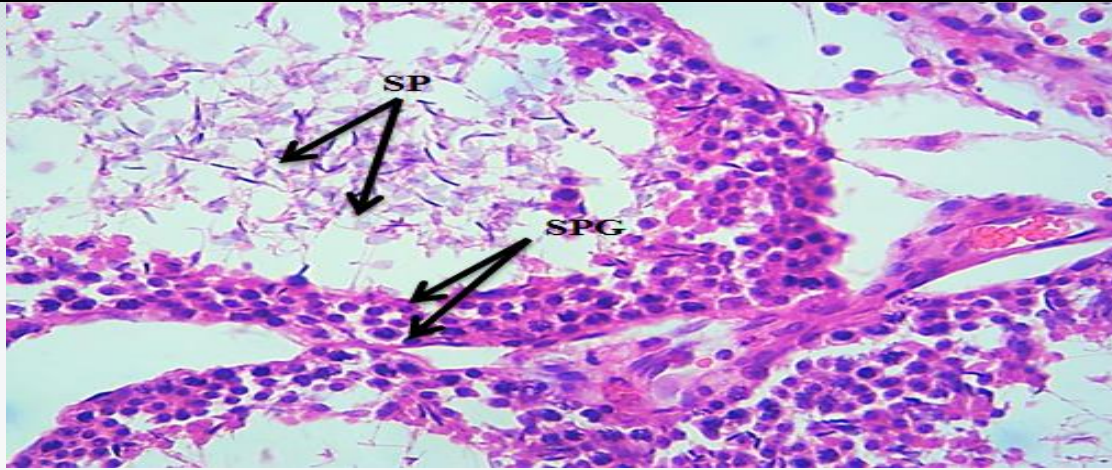


Figure (1) The control group testicle clip shows the stages of spermatogenesis (SPG) and mature sperm within the seminiferous tubule (SP) .H and E 400X,

Results of the experimental group 2 G2:

Histological examination of the testes of the rabbit group treated with RBV shows that stages the sperm formation are incomplete and that the mature sperm is not seminiferous tubule lumen (Figure 2).

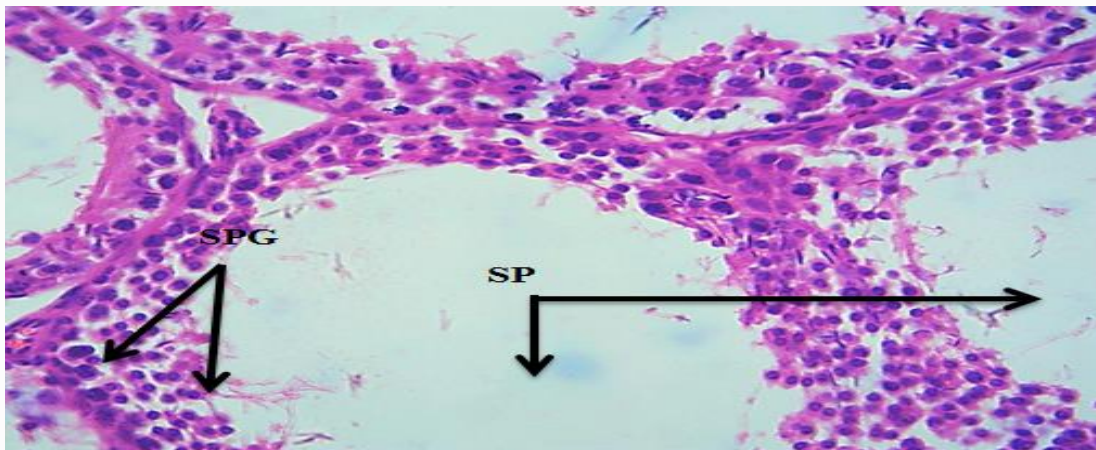


Figure (2) The segment of the testes of the group treated with Ribavirin shows that the spermatogenesis (SPG) and that the mature sperm is not seminiferous tubule lumen (SP).H and E 400X

Results of the experimental group3 G3:

Histological examination of the testes rabbit of this group shows formation stages of spermatogenesis and mature spermatozoa within the seminiferous tubule lumen is naturally (Figure 3).

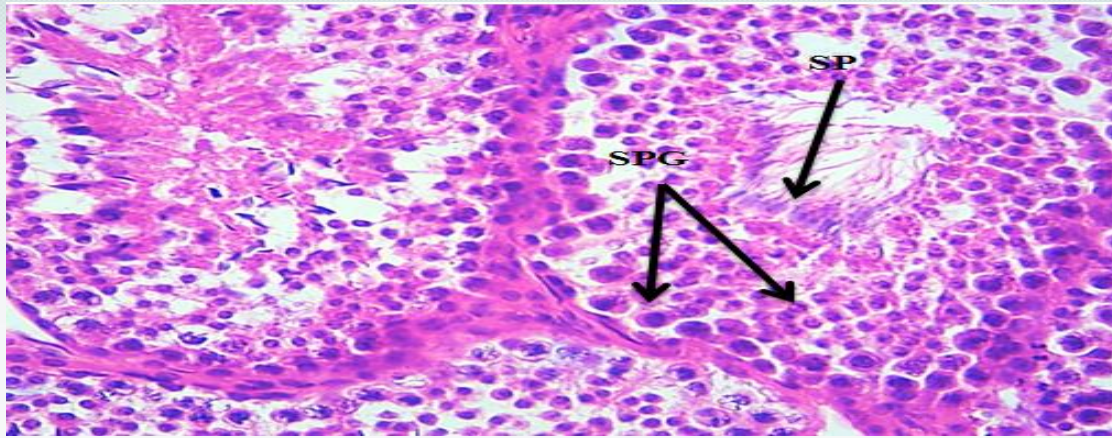


Figure (3) The group testicle clip group with garlic plant extract shows the stages of spermatogenesis (SPG) and mature spermatozoa within the seminiferous tubule lumen is naturally .H and E 400X,

Results of the experimental group 4 G4:

The treatment of rabbits with Ribavirin and garlic extract resulted showed tissue changes in the testicle (Figure 4), which illustrates the stages of spermatogenesis and maturing of sperm within the seminiferous tubule lumen is naturally.

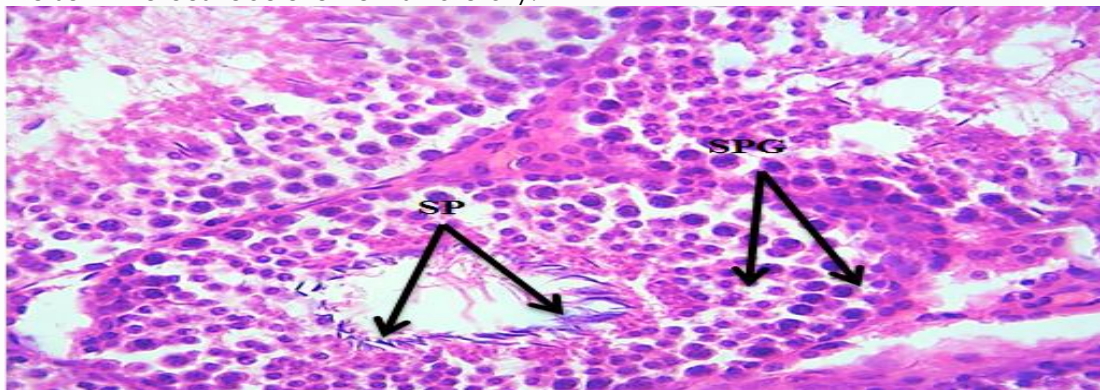


Figure (4) Clip testicles Group treatment with Ribavirin and garlic plant extract shows the stages of spermatogenesis (SPG) and maturing of sperm within the seminiferous tubule lumen is naturally .H and E 400X,.

Study of Sperms Parameters:

From the microscopic examination of the sperm of rabbits in the control group, as well as the dose of garlic extract 50 mg/kg of body weight, the normal shape of the sperm in terms of head, midsection and tail, pictures (Figure 5), (Figure 6) were observed. While it is noted through the microscopic examination of the sperm of rabbits treated with RBV, the appearance of abnormalities clearly, represented by the presence of two-headed sperm, in addition to the observation of the sperm in which a deformation appeared in the median pieces represented by holes and a large bending or even separation from the head of the sperm. Large sperms with coiled tails, Figure (7), (8), (9), (10). Microscopic examination of rabbits' sperm treated with RBV and garlic extract showed, The normal shape of the sperm and it's similar to the shape of the sperms in the control group, Figure (11).



Figure (5) rabbit's sperm in control group shows the normal shapes of the sperm. 400x



Figure (6) rabbits Sperm of group treated with garlic extract shows the normal shapes of sperm .400X.



Figure (7) rabbits sperm of group treated with ribavirin shows abnormalities in the shapes of some sperms, as it is noticed that there are many sperms that suffer from tail wrap (red indicator). 400X

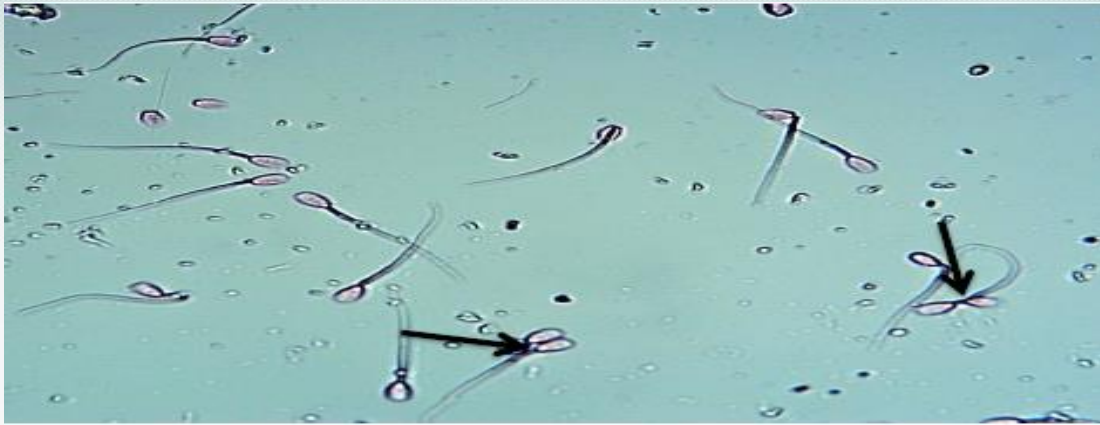


Figure (8) rabbits Sperm of group treated with ribavirin shows abnormalities in the shapes of some sperm, where it is noted that there are two-headed sperm (black indicator) .400X.



Figure (9) rabbits Sperm of group treated with ribavirin , Showed that there are holes in the midsection (blue pointer) 400X.



Figure (10) rabbits Sperm of group treated with ribavirin showed some sperms with two tails (black indicator) and other sperms suffer from tail warp (red indicator) .400X.

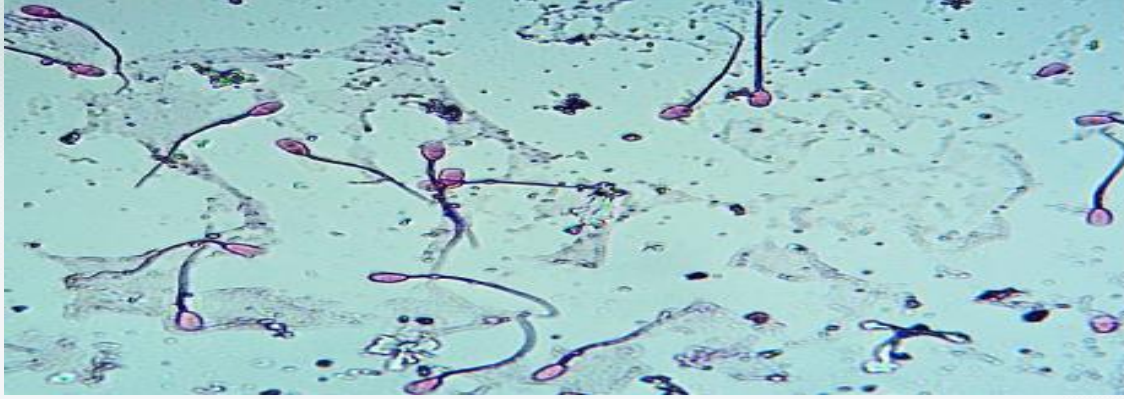


Figure (11) rabbits Sperm of group treated with ribavirin and garlic extract shows the normal shapes of sperm .400X.

Discussion:

Ribavirin is an antiviral drug that belongs to the group of nucleoside analogues as the Purine analogue enters the DNA molecule and inhibits the duplication process of DNA and inhibits RNA and DNA replication of viruses, which lead to inhibiting their reproduction within the host (Howe et al, 2008). Ribavirin has many side effects, including Hematologic and Teratogenicity, and other studies have demonstrated its toxicity to the male reproductive system through experiments (Crotty et al, 2002). The histological study of the testicles of adult male rabbits treated with RBV at a dose of 20 mg/kg indicated the incomplete stages of spermatogenesis and the lack of mature sperm within the seminal tubule, as it shows the epithelial cells of the epididymal wall and the absence of mature sperm within the lumen of epididymis, these results agreed with (Hay Autifi et al, 2017) which conformed the effect of RBV at dose (20, 100, 200)mg/kg for different periods in the tissues and cells of the testes, regularity of sperm-forming epithelial cells in the tubules and there necrosis with lack of sperm and the irregular diameters of Leydig cells in the tubule cavity, may be due to thickening of the seminiferous tubule walls caused by the collagen fibers secreted from Sertoli cells, thus causing sperm weakness and with this increase a lot of changes appear within the testicle, especially in Sertoli cells as it directly affects the differentiation and development of germ cells (El-Demerdash et al, 2008). The result was consistent with the study conducted by (Batool et al, 2008), which showed histopathological changes in the testicles of rats after treating with different doses of RBV, where there was a decrease in the maturation and differentiation of sperm cells and an increase in the amount of intercellular tissue in the testicles of rat, with end of spermatogenesis a necrosis of seminiferous tubule with decrease of primary and secondary spermatocyte and presence of reduction in the number of spermatozoa and presence of inflammatory cells and absence or loss of spermatozoa in the seminiferous lumen, The results also agreed with (Narayana et al, 2005), who observed narrowing of the epididymal ducts and decay in the static Cilia with decreased numbers of mature spermatozoa probably There is an inverse relationship between the ratio of glutathione in the cells and the form of spermatozoa, since treatment with ribavirin depletes the amount of glutathione in the sperm cells and thus enhances the susceptibility to poisoning and inhibition of sperm, and that changes in lysozyme activity and glutathione levels together play a significant role in sperm malformations and therefore that prolonged intake of ribavirin by humans has serious effects on fertility (Ibrahim et al, 2020; Farias et al, 2012).

The treatment with aqueous garlic extract at a dose of (50) mg / kg body weight led to the natural shape of the testicular tissue. While the treatment with RBV at a dose of (20) mg/kg and aqueous garlic extract at a dose of (50) mg / kg of body weight led to the return of normal standards of testicular and epididymis tissue because the aqueous garlic extract has a significant role in reducing the toxicity of ribavirin. These positive effects of garlic may be due to its active role in activating the enzymatic and non-enzymatic antioxidant defense system within the body, as well as containing natural antioxidant nutrients such as vitamin C and selenium (Almasry et al, 2017). Vitamin C is an antioxidant because it is a source of energy and activates enzymes and metabolic processes as it activates the enzyme Adenyl Cyclase and inhibits the enzyme Phosphodiesterase Isoenzymes, which leads to increased levels of cyclic adenosine monophosphate cAMP and metabolic processes in tissues need this increase and leads to cell

growth and differentiation (Kemper et al, 2000). Some research has indicated that Selenium has positive effects on tissue lesions in chemical poisoning cases (Asadpour et al, 2013). the antioxidant effectiveness of garlic is due to the fact that garlic extract contains phytochemicals that prevent tissue breakdown resulting from oxidation, these include water-soluble compounds, fat-soluble compounds, flavinoids, and Alixin (Obidike et al, 2012), where garlic has shown antioxidant efficacy through its free radical action enhanced by antioxidant enzymes such as glutathione peroxidase, catalase, and increased glutathione in the cell, in addition to its sulfur compounds interfere with different steps of lipid peroxidation and since garlic extract inhibits lipid peroxidation, it protects the epithelial cells (Abdel-wareth et al, 2019). While treatment with garlic extract has a role in reducing deformities of sperm forms compared to the group treated with RBV because garlic contains selenium and vitamin C, which contribute as antioxidants, garlic has a highly effective role in improving overall sperm count and mobility and reducing dead and abnormal sperm numbers (Amagase et al, 2001). Treatment of male rabbits with ribavirin at a dose of 20 mg/kg leads to a significant change in the parameters of the sperm compared to the control group. The reason for the increase in the number of deformed sperms in the animals treated with ribavirin is due to several changes in the testicular tissue such as destruction in the epithelium cell in vascular and its damage, which leads to the interruption of blood supply and the occurrence of atrophy and degeneration of the testicle (Narayana et al, 2002), or it may be the cause of the disturbance in the hormonal regulation of the process of spermatogenesis due to a decrease in testosterone produced by Leydig cells as a result of The effect of ribavirin (Durazzo et al, 2002). Ribavirin also cause histological changes in the testis, including an increase in the desquamation of the seminal epithelium in the epididymis. It also works on the atrophy of the seminal vesicles and the prostate gland, as these toxic effects lead to a reduction in semen fluid and abnormalities in sperm (Pecou et al, 2009). ribavirin transport to the testis through the peritoneum and its reach to the germ cells and their accumulation in the clumps of germ cells, where ribavirin stimulates changes in the shape of the sperm through point mutations in the germ cells, which leads to the appearance of abnormalities in the shape of the sperm (Hofer et al, 2010), or the reason may be that ribavirin leads to the generation of free radicals such as free oxygen radical (-O), and hydrogen peroxide (H₂O₂), and types of active oxygen that cause damage to various biomolecules present inside the cell (DNA, lipids and proteins), as well as a change in the cell membranes of sperm, which affects the vitality of kindness and its parameters (De Santis et al, 2003).

Ribavirin also causing the abnormal shape of the mid-piece and tail through its effect on the sperm membrane, although the reason for its effect on the sperm membrane is not clear, but the reason for its effect on the membrane may be due to its formation of free radicals, especially the types of active oxygen that work To attack the double carbon bonds that are found within the polyunsaturated fatty acid structures that enter the composition of the outer membranes of the sperm, which results in the breakdown of the outer membrane of the sperm and the transformation of unsaturated fat into lipid peroxide, which results in an increase in the concentration of MDA, which affects the sperm vitality and leads to Appearance of abnormalities in the mid-piece and tail (Roomer et al, 2011), the lack of GSH in the blood serum and seminal plasma leads to damage to the midsection of the sperm, thus distorting its appearance and disrupting its movement (Hansen and Deguchi, 1996). The cause of the deformity in the mid-piece of the sperm can be attributed to the low level of fructose in the semen, as fructose is produced in the seminiferous tubule and its production is under the control of male hormones in the testis, where ribavirin depletes ATP and thus stops the process of glycolysis (Lim et al, 1998). The scientists also agreed that ribavirin is a selective cytotoxic for rapidly dividing cells, and it follows from this that ribavirin or its receptors act as cytotoxins in the testicles, which affects the number of sperms and increases abnormalities (Kochhar et al, 1980). From the microscopic examination of rabbit sperm in group treated with garlic extract 50 mg/kg showed the normal shape of the sperm in terms of head, mid-piece and tail. And rabbits treated with ribavirin and garlic extract showed high improvement in sperm parameters, as garlic extract contains multiple bioactive phenolic compounds that have a role in scavenging reactive oxygen species (ROS) and protecting DNA from damage in addition to its role in Increasing the activities of enzymatic and non-enzymatic antioxidants, and thus garlic extract have a role in the vitality of sperms (Bertrand et al, 2016). Also, garlic extract has an effective role in improving the number and quality of sperms, as it contains vitamin (E) and (C) which is important in sperm health by regulating their maturation It also raises the level of the testosterone hormone, and the reason for this rise is that garlic extract contains a high percentage of vitamin E, which works in maintaining the cells responsible for the production

of testosterone necessary for the formation of sperm and protecting them from oxidative damage (Ghalehkandi, 2011). garlic extract has the ability to raise the concentration of the hormone SSH (Odo et al, 2018), which stimulates the process of spermatogenesis in the testicles, as well as raises the concentration of the hormone ICSH which stimulates the formation and secretion of testosterone from Leydig cells. This leads to an increase in the concentration of testosterone and thus increases the number of sperms. Also, the protective role of garlic extract against ribavirin toxicity to rabbit testicles, as ribavirin toxicity leads to a decrease in the concentration of antioxidants GSH, SOD and CAT in the testicles, and this leads to a decrease in ICSH activity. Which stimulates Landck cells to form and secrete testosterone, and in return, the multiple phenolic compounds found in garlic, especially Allicin , which Reduces the oxidation process, prevents ribavirin toxicity and increases the formation and secretion of testosterone from the testicles, which is essential for initiating and maintaining spermatogenesis (Abdennour and Ouarda, 2011) . (Hewan et al, 2020) the vitamin E in garlic allows free radicals to dehydrogenate antioxidants instead of unsaturated fatty acids and thus break the chain of free radical reactions, leading to a marked decrease in free radical reactions and protection of sperm membranes (Asadpour et al, 2013).

Conclusion

The current study showed that garlic has an antioxidant and sperm- improving effect and protects testicular cells from the toxic effect of ribavirin on the male reproductive system.

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