



Effect of 6-paradol Nanoparticle on Some Testis and Epididymis Parameters in Diabetic Rats Induced by Streptozotocin

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ARTICLE INFORMATION

Article history:

Received: 27 April 2021

Revised: 05 June 2021

Accepted: 10 June 2021

Published: 24 September 2021

Keywords:

6-paradol,

Semen,

Streptozotocin.

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ABSTRACT

Objective: This study to investigate the role of 6-paradol nanoparticle in the treatment the of the changes occurs in the testis and epididymis and some semen parameters in male rats which induced by streptozotocin.

Methods: Different concentrations 20 mg and 40 mg/kg of 6-paradol nanoparticle used as treatment through 60 days in some physiological and histological criteria in seven groups diabetic rats *Rattus norvegicus* induced by streptozotocin and the parameters that studied were concentration of sperms, motility, percentage of sperms abnormalities, percentage of sperms viability. number of the sperm cell, Sertoli and ledge, diameters and thickness of seminiferous tubules.

Results: the results showed a significant decrease in Concentration of sperms, motility, Percentage of sperms viability, number of the sperm cell, Sertoli and leydig, diameters and thickness of seminiferous tubules in male rats (G3) 60 mg which was given streptozotocin to induce diabetes and showed a significant increase in percentage of sperms abnormalities, while in (G4, G5) 20 mg, 40 mg which was treated by 6-paradol nanoparticle showed a significant increase in Concentration of sperms, motility, Percentage of sperms viability, number of sperm cell, Sertoli and ledge, diameters and thickness of seminiferous tubules and showed a significant decrease in the percentage of sperms abnormalities.

Conclusion: We can consider the 6-paradol is a very good substance because of the efficiency in the treatment of the testis and epididymis and useful as an antibiotic or antioxidant because it is very safe on the body and also tiny size in the nanometres even easily enters to the cell and gives good result.

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CITATION: Abdullah JA, Al-Mayali HK, Al Naeely AJH. "Effect of 6-paradol Nanoparticle on Some Testis and Epididymis Parameters in Diabetic Rats Induced by Streptozotocin". *Sci. J. Med. Res.* 2021;5(19):78-84.

INTRODUCTION

Medical plants have biological effects in the treatment of the disease because they exhibit antioxidant, anti-inflammatory, anti-diabetic, and anti-tumor effects, respectively.¹ Ginger is considered to be ineffective in dysmenorrhea treatment. Also,

there was not much evidence for its analgesic characteristics because of the limited well-conducted trials.² A 6-Paradol is a bioactive compound with anti-diabetic, antioxidative, and anti-tumor-promoting effects, can also be identified from the grains of ginger (*Z. officinale*), paradise (*Amomum melegueta*)

and herbs. The chemical form of 6-paradol is 1-(4-Hydroxy-3-methoxyphenyl)-3-decanone.³ Experimental types of research in animals, both in-vivo and in-vitro showed that 6-paradol might affect insulin sensitivity, carbohydrate metabolism and lipids. Those researches showed promising results on the diabetic complications in the yes, kidneys, liver and nerves.⁴ Nano-medicine has been defined as one of the main scientific techniques that offering miniature devices and sensing technologies to diagnose the disease accurately and within time. There has been a variety of nanotechnology applications in the drug delivery area and in addition to that, for simplifying oral absorption of protein and peptide nanocarriers are modified by certain ligands.⁵ The NPs are utilized to deliver the proteins and RNA for diagnosing diseases and monitoring disease progression. The pulmonary drug delivery means has been defined as one of the sufficient routes except the nanocarriers.^{6,7}

MATERIAL AND METHODS

Preparation of Nanoparticles 6-paradol with Zinc Oxide by Sol gel for Ion Exchange

The study used the method described by⁸ with some modification in preparing the hybrid NP by adding 50 mL of the 6-paradol compound dropwise to the zinc oxide solution resulting from dissolving 1-g of zinc oxide in 50% ethanol and stir the mixture at room temperature for two hours. Then put the mixture in a shaking incubator at 37°C for 24 hours and placed afterward in an incubator at 40°C for 24 hours, then separating the precipitate by centrifugation at a speed of 5000 rpm for 20 minutes and then washed with the deionized distilled water several times and then dried The precipitate is at 40°C. It was ground with a ceramic mortar and finally stored, as shown in Scheme 1.

Animals

Forty, 8-week old adult Wister albino male rats that weigh 230 g ± 10g, have been obtained from the animal facility of Pharmacy college in Kerbala University. The male rats have been housed in rooms with controlled temperatures (25°C) with a constant level of humidity (between 40 and 70%) and 12 hours dark/light cycles before the utilization in the experimental settings. All of the animals have been treated based on the Lab Animal Care Principles. Animal Ethical Committee has admitted the experimentation protocols based on guides for care and uses of the lab animals that have been prepared by Animal house in the Pharmacy college in Kerbala University. All the Rats have been given water and a standard diet for 60 days.

Streptozotocin Induced Diabetes

Diabetes has been induced with one intraperitoneal (i. p.) STZ injection in 0.1 M citrate buffer (pH4) at a 60 mg/kg dosage of the bodyweight.⁹ The concentration of the blood glucose and the bodyweight changes have been regularly observed.

Dose of Streptozotocin

Dose of streptozotocin was prepared according to the method of⁹ at a concentration 60 mg/kg of body weight through dissolving the required concentration on the average weight of the animal.

Dose of ZnO

Dose of zinc oxide were prepared according to the method of¹⁰ at concentration 20mg /kg of body weight through dissolving the required concentration on the average weight of the animal.

Dose of 6-paradol Nanoparticle

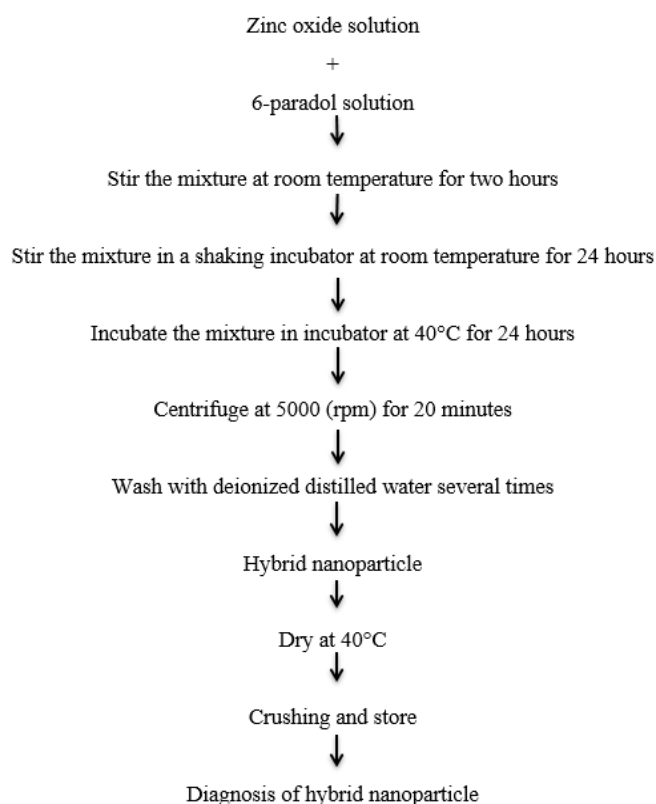
Dose of 6-paradol NP were prepared according to the method of¹¹ at concentration 20 mg, 40 mg/kg of body weight through dissolving the required concentration on the average weight of the animal.

This is why Wister male rats have been divided into five groups that comprise eight animals in every one of the groups as: Group 1 (G1): Control groups have only been administrated DW daily for 60 days.

Group 2 (G2): control group administrated 20 mg/kg/day zinc oxide.

Group 3 (G3): induced diabetic control (60 mg/kg, single i.p. injection of the streptozotocin).

Group 4 (G4): the induced diabetic group was given 20 mg/kg daily of the Nano 6-paradol.



Scheme 1: Preparation of Nanoparticles 6-paradol with Zinc Oxide by Sol-gel for ion exchange

Group5 (G5): the induced diabetic group has been given 40mg/kg daily Nano 6-paradol.

Surgical Process

On 60th day (at the treatment period's end) rats have been sacrificed, following the mixing of the ketamine solution with the solution of the Xylazine (2 mL of 20.0 mg/kg) has been taken by i.p. as an anesthetic, and lower transverse abdominal's incision has opened peritoneal cavity. The two testis and the epididymis have been removed right away from the experimental and control groups. Testis and epididymis fixed in formalin solution until prepare of histological test, testes weight and the epididymis for every one of the group members has been stated.

RESULTS

1- Concentration of Sperms (million/mL)

Table 1 demonstrated there was a significant rise at ($p \geq 0.05$) for sperms concentration to group treated by 6-paradol nanoparticle (G4, G5) when compared with a control group (G1) and another animal groups. In addition, there was a significant decrease at ($P \geq 0.05$) for sperms concentration in animal group (G3) compared with a control group and other groups. While there was no significant difference at ($p \leq 0.05$) in sperms concentration in animal groups treated by (G2) when compared with the control group and other groups.

2- Percentage of Sperms Motility (%)

In this study, **Table 1** demonstrated do not show any change at ($p \leq 0.05$) in sperms motility between animal groups that treated with (G2) and control group (G1). Furthermore, there was a significant decrease ($P \geq 0.05$) in sperm motility in an animal group (G3) when compared with control group and treated groups with 6-paradol (G4, G5) and (G2). As well as, there were a significant increase at ($p \geq 0.05$) for sperm motility

in animal group which treated with 6-paradol nanoparticle (G4, G5) when compared with (G1).

3- Percentage of Sperms Abnormalities (%)

Table 1 showed a considerable rise at $p \geq 0.05$ of abnormal sperm ratio for an animal group (G3) when compared with a control group and treated groups by 6-paradol (G4, G5, and G2) respectively. In addition, there was a reduction significantly at $p \geq 0.05$ of abnormal sperm ratio for animal group, which 6-paradol nanoparticle treated (G4, G5) when compared with (G1).

4- Percentage of Sperms Viability (%)

In this study, **Table 1** demonstrated a significant reduction at $p \geq 0.05$ to the percentage for sperm viability in an animal group (G3) when compared with control group (G1) and other groups, respectively. In addition, there was a considerable rise at $p \geq 0.05$ to a percentage for sperm viability in the animal group treated with 6-paradol nanoparticle (G4, G5) when compared with (G1, G2) respectively.

5- Number of Leydig's Cells

In this study, **Table 2** demonstrated a considerable reduction at $p \geq 0.05$, represented by the declining number of Leydig's cell in animal group (G3) compared with a control group (G1) and other treated groups. Besides, there was a considerable rise at ($p \geq 0.05$) in number for Leydig's cell of the animal group treated with 6-paradol nanoparticles (G4, G5) compared with the control group and another treated group. but there was showed no significant difference ($p \leq 0.05$) in number for Leydig's cell between (G1) and (G2).

6- Number of Sertoli Cells

In this study, **Table 2** demonstrated a considerable reduction at $p \geq 0.05$, represented by a declining number for Sertoli cells in an animal group (G3) when compared with a control group (G1) and other groups. Furthermore, there was a considerable

Table 1: Effect of 6-paradol on semen parameters in rats induced diabetes by STZ

Sperms Groups	Sperms con (million/ml)	Sperms motility %	Sperms abnormality %	Sperms Viability %
G1	86.46 ± 0.75 d	73.54 ± 3.40 d	10.30 ± 11.30 b	81.64 ± 0.97 d
G2	85.78 ± 1.72 d	73.41 ± 4.10 d	10.43 ± 0.98 b	80.55 ± 1.20 d
G3	62.26 ± 1.86 e	43.36 ± 2.91 e	14.65 ± 0.73 a	53.05 ± 2.14 e
G4	91.73 ± 1.40 a	88.64 ± 3.38 a	7.79 ± 1.01 d	89.57 ± 1.27 a
G5	89.82 ± 0.34 b	85.75 ± 2.52 a	7.84 ± 1.17 d	86.68 ± 1.37 b
LSD	1.59	3.32	0.87	1.2

Numbers = Mean ± SE.

Different letters = Significantly difference ($P \geq 0.05$).

G1= Control group injected by only DW per day during 60 day.

G2= Control group injected by 20 mg/kg/day zinc oxide

G3= induced diabetic group (injected by 60 mg/kg STZ, in intra peritoneal)

G4= induced diabetic group by (STZ) injected by 20 mg/kg/day Nano 6-paradol.

G5= induced diabetic group by (STZ) injected by 40 mg/kg/day Nano 6-paradol.

Table 2: Effect of 6-paradol on germ cells in rats induced diabetes by STZ

Sperms Groups	Number of Leydig's cells	Number of Sertoli cells	Number of Spermatogonia	Number of Spermatocyte	Number of Spermatid
G1	21.52 ± 1.04 d	20.70 ± 1.25 d	66.26 ± 0.66 e	96.22 ± 0.52 d	126.42 ± 1.04 d
G2	21.03 ± 0.7 d	20.46 ± 0.62 d	65.16 ± 0.92 f	95.68 ± 0.72 d	125.13 ± 0.81 e
G3	13.09 ± 1.63 e	12.27 ± 1.25 e	40.69 ± 0.53 g	51.12 ± 0.63 e	58.98 ± 2.03 g
G4	26.05 ± 0.28 a	25.87 ± 0.77 a	73.75 ± 0.65 a	117.09 ± 3.74 a	131.85 ± 0.41 a
G5	24.89 ± 0.68 b	24.70 ± 0.91 b	71.93 ± 0.97 b	109.54 ± 4.42 b	130.09 ± 0.61 b
LSD	0.82	0.8	0.78	2.36	0.89

Numbers = Mean ± SE. Numbers = Mean ± SE.

Different letters = Significantly difference ($P \geq 0.05$).

G1= Control group injected by only DW per day during 60 day.

G2= Control group injected by 20 mg/kg/day zinc oxide

G3= induced diabetic group (injected by 60 mg/kg STZ, in intra peritoneal)

G4= induced diabetic group by (STZ) injected by 20 mg/kg/day Nano 6-paradol.

G5= induced diabetic group by (STZ) injected by 40 mg/kg/day Nano 6-paradol.

Table 3: Effect of 6-paradol on the seminiferous tubules in rats induced diabetes by STZ

Seminiferoustubules Groups	Thickness of Seminiferous tubules (micrometer)	Diameter of Seminiferous tubules (micrometer)
G1	26.61 ± 0.48 f	5.6 ± 0.25 c
G2	29.15 ± 6.82 c	5.2 ± 0.32 d
G3	18.12 ± 0.48 g	3.16 ± 0.14 e
G4	32.12 ± 0.81 a	6.46 ± 0.08 a
G5	30.65 ± 0.37 b	6.12 ± 0.12 b
LSD	0.54	0.22

Numbers = Mean ± SE. Numbers = Mean ± SE.

Different letters = Significantly difference ($P \geq 0.05$).

G1= Control group injected by only DW per day during 60 day.

G2= Control group injected by 20 mg/kg/day zinc oxide

G3= induced diabetic group (injected by 60 mg/kg STZ, in intra peritoneal)

G4= induced diabetic group by (STZ) injected by 20 mg/kg/day Nano 6-paradol.

G5= induced diabetic group by (STZ) injected by 40 mg/kg/day Nano 6-paradol.

rise at $p \geq 0.05$ in number for Sertoli cell of the animal group treated with 6-paradol nanoparticles (G4, G5) compared with the control group. While there were showed do not showing any change $p \leq 0.05$, in number for Sertoli cell between the control group (G1) and the animal group treated with Zinc oxide (G2).

7- Number of Spermatogonia

In this study, **Table 2** demonstrated considerable reduction at $p \geq 0.05$, represented by a decrease in the number of spermatogonia cell in animal group (G3) compared with a control group (G1) and another group. In addition, there were a considerable rise $p \geq 0.05$, in number for Spermatogonia cell in

rats which treated with 6-paradol nanoparticles (G4, G5) when compared with a control group (G1) and (G2), and noticed a significant reduction at $p \geq 0.05$, a number for Spermatogonia cell between (G1) and (G2).

8- Number of Spermatocyte

In our study, **Table 2** demonstrated a considerable reduction at $p \geq (0.05)$, represented by a decrease in the number of spermatocytes in the animal group (G3) when put to comparison with the (G1) and another group. Besides, there was a considerable rise at $p \geq (0.05)$, represented by an increase in the number of spermatocytes in the animal group, which was treated with 6-paradol nanoparticles (G4, G5) when compared with the control group (G1) and other groups.

9-Number of Spermatid

In this study, **Table 2** demonstrated a considerable reduction at $p \geq (0.05)$ represented by a decrease in the number of Spermatid in the animal group (G3) in comparison to the control group (G1) and other groups. In addition, there were a considerable rise of $p \geq 0.05$, represented by increase in the number of Spermatid in the animal group which was treated with 6-paradol nanoparticles (G4, G5) when compared with control group (G1) and other groups.

10- Thickness of Seminiferous Tubules (micrometer)

Table 3 demonstrated a significant reduction at $p \geq 0.05$, revealed by a decrease of the thickness of testes and epididymis seminiferous tubules for an animal group (G3) when put to the comparison control group (G1) and other groups. In addition, clarified a considerable rise at $p \geq 0.05$, represented by an increase in the thickness of seminiferous tubules of testes and epididymis in the animal group treated with 6-paradol nanoparticles (G4, G5) when compared with control group (G1) and other groups.

11- Seminiferous Tubules Diameter (micrometer)

In this study, Table 3 revealed a significant reduction at ($P \geq 0.05$) represented by a decrease in the diameter of seminiferous tubules of testes and epididymis in an animal group (G3) in comparison to the control group (G1) and other groups. Also demonstrated a considerable rise $p \geq 0.05$, represented by an increase in diameter for seminiferous tubules for testes and epididymis in the animal group treated with 6-paradol nanoparticles (G4, G5) compared with control group (G1) and other group.

DISCUSSION

Caudal epididymis sperm count and motility showing a considerable rise $P \geq (0.05)$ following treatment by 6-paradol for 14- and 28-days in a duration- and dose-dependent way in comparison to controls. There was no change on another parameter sperm related to morphology and viability compared to group control.¹² These results correspond with our results. The identified increases from 6-paradol in sperm work to rats administered might lead to increased favorable sperm to genic activities due to the high testosterone levels. In addition, testosterone is critically involved in developing sperm cells, while derangement majorly led to testicular steroid genic disorder and Leydig's cell dysfunction.¹³ The concentration of sperms in epididymis and testes has been considerably reduced ($p \geq 0.05$) in the rats subjected to treatment with different STZ dose compared with the control animals. There has been a considerable decrease in the sperm concentration in other groups subjected to treatment via high STZ dose, and when put to comparison with each other ($p \geq 0.05$), the results exhibited that their STZ is affecting the sperm parameters. Also, the results exhibited a significant reduction in the motility and viability percentage in the treated group when put to comparison with the control group ($p > 0.05$). At the same time, the abnormality percentage has been significantly increased ($p > 0.05$) in rats subjected to treatment with different STZ doses in comparison to the control group¹⁴ These results correspond with our results. Clinical and experimental¹⁵ evidence showed that diabetes miletus (DM) badly affects sperm and spermatogenesis-related parameters. Many researches utilizing STZ induced diabetes miletus type one (T1DM) animal model showed a daily decrease in motility, sperm count and sperm production^{16,17} morphologies.¹⁸ The result of our study correspond with these results. In accordance with such results; this research showed an increment in testes weight, levels of testosterone, and sperm accumulations in seminiferous tubules' lumen and showed that 6-paradol nanoparticle has androgenic and antioxidant activities in the dosages of 20 and 40 mg/kg and has a significant impact on sperm parameters and spermatogenesis in rats.¹⁹ indicated an increase in the abnormal sperm volume with a reduction in the viability of the sperm in the test group compared with the control-sham rats. In addition, the sperm motility is reduced with time in each one of test groups compared with the control rats. Also, the analyses of the sperm count have exhibited a

reduction in sperm volume. There were various supportive results In this study, such as increased abnormal sperm content with various properties including pyriform (pear-shape) head, elongated head, bent and cytoplasmic droplets, an increase in the infiltration related to immune mononuclear cells/mm² of the interstitial connective tissues, degenerated germinal cells in the STZ exposed groups specifying the main role of imbalanced oxidative stress in creating many disorders. The rats with the STZ induced diabetes have significantly decreased sperm numbers, viability, and motility compared to the control group. In a case of STZ given cinnamon, ginger, and combined cinnamon and ginger, the increase has been noticed in all three parameters and in all three groups of treatment. The comparison of all of the three with the healthy control group has been considerable ($p > 0.05$). The last one of the groups, combining cinnamon and ginger had shown a rapid increase in comparison with the cinnamon and ginger separately.^{20,12} have stated that *Z. Officinale* have positive effects on the reproductive function in the male rats that have been coinciding with other researches on increased sperm counts, testosterone, motility, and reduction in the levels of the malondialdehyde. Ginger was shown earlier to result in the stimulation of spermatogenesis.²¹ It has been shown as well that administering ginger may result in significant increases of the levels of testosterone in the plasma.²² STZ causes damage to testicular tissue due to the drug's toxic effects and a significant reduction in the number of spermatogonia, spermatids, sertoli cells, and Leydig's cells²³ this result corresponds with our results. Treating rats with chemical treatments disrupts sperm production by damaging the cellular components of sperms such as proteins and fats and damaging their membranes, as well as an imbalance of energy metabolism or by affecting DNA synthesis for sperm which affects cell division leading to its death and thus impaired fertility or sterility.²⁴ The changes in the number of cells in this study are due to the effect of STZ in reducing the level of testosterone, which affected the spermatogenesis process, especially the last stages of this process. It is because of the mitosis of spermatogonia and the formation of primary sperms may not require hormonal stimulation may deal with,²³ therefore, animals treating with STZ treatment leads to the death of spermatogonia due to the direct effect of the drug on the enzyme topoisomerase II, which affects the process of DNA synthesis, especially sperm cells,²⁵ these results also correspond with our results. The decrease in the number of spermatogonia in our study may be due to the degeneration of Leydig's cells leading to their increased breakdown and reduced numbers, especially in rat treated with STZ, which causes a decrease in the concentration of testosterone due to the direct effect of free radicals, the testosterone is of high importance in the sperm cells development these results may correspond with.²⁶ The decrease in the percentage of spermatids is also due to the lack of numbers of Sertoli cells as they are affected by the free radicals generated by the STZ drug.²⁷ Or it may be due to the decrease in the concentration of FSH which was indicated by the current

study, which indicates the oxidative damage that affects the body.²³ indicated that major changes occurred in the cytoplasm of the Sertoli cell, as the cell shrinks with the presence of dense bodies and large fatty droplets in the cytoplasm, which stops the cell's secretory function in rats injected by STZ. Also,²⁶ confirmed a decrease in the transferrin protein produced by Sertoli cells, which has a role in developing and growing sperm cells. The increase in the number of Sertoli cells and Leydig's cells is due to the effect of 6-paradol NPs on gonadotropin in the blood serum, especially LH, which results in increasing FSH and the number of Leydig's cells and then increasing sertoli cells.²⁸ The role of flavonoids especially 6-paradol is to protect spermatogenesis, germ cell proliferation, Sertoli and Leydig's cells against free radical attack.²⁹ The results of our study in groups that were treated with 6-paradol NPs revealed a rise for cells number in comparison with the controls that corresponded with³⁰ and were caused by a high level of antioxidant enzymes like (GSH, SOD and CAT) because of the flavonoids that work to inhibit the oxidative stress caused by the STZ drug. Flavonoids also contribute to spermatogenesis activation and increase the number of spermatocytes by stimulating the secretion of the sex hormones LH, FSH and testosterone.³¹ 6-paradol nanoparticle reduces the oxidative stress caused by the STZ by reducing free radicals, and 6-paradol affects sperm indirectly through its effect on the sexual organs, which stimulates the secretion of testosterone responsible for the growth and development of the spermatocyte, the antioxidant activity of 6-paradol is also very important for the mitosis of spermatogonia which lead to the production of sperm without abnormalities, while the role of the epididymis is to provide the requirements of the sperms to remain normal without damage deal with.³² The 6-paradol nanoparticle group of rats showed an increase in spermatocyte, Leydig's and sertoli cells in comparison to the control group, and this explains the role of flavonoids in scavenging free radicals and protecting cell membranes from oxidative stress with increased antioxidant enzymes, in addition to the role of flavonoids in inhibiting free radicals.³³ These results may correspond with our results, and perhaps the reason of the change in thickness and diameter of seminiferous tubules may be caused by oxidative stress due to STZ-treatment, oxidative stress is the essential mechanism for the destruction of tissue, when the drug accumulates in the testis and epididymis, especially the mitochondria, and causes inhibition of respiratory chain enzymes as a result of the action of radical oxygen species like hydroxyl radicals, Hydrogen peroxide radical and Superoxide radicals which causes oxidative stress. STZ also causes severe depletion of the spermatogenic epithelium in the testis and epididymis and causes apoptosis of normal cells, affecting the spermatogenesis process.³⁴ Which helped improve its composition, as 6-paradol's ability to protect against oxidative stress that causes cell damage with scavenging free radicals, in addition to that 6-paradol possesses other antioxidant mechanisms that contribute to 6-paradol's strength against oxidation by binding to minerals such as iron and zinc, which

inhibits the breakdown of fats, in addition to other biochemical mechanisms such as inhibiting the enzymes Xanthine oxidase and Nitric oxidase synthase, as 6-paradol nanoparticle can spread on the surface of the two-layer lipids as well as the hydrophilic layers in the cell membrane, which facilitates the scavenging of free radicals deal with.³⁵ The groups treated with 6-paradol nanoparticle witnessed an increase in the seminiferous tubules' diameters of epididymis and testis in addition to the epithelial lining cells in comparison to the control group. This increase in the thickness and diameter of seminal tubules is due to the role that flavonoids play in activating antioxidants in tissues with an increase the rate of cell metabolism and the manufacture of proteins and enzymes essential for tissue growth and construction³⁶

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