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Evaluation of the Potential Protective Role of Galangin Associated with Gold Nanoparticles in the Histological and Functional Structure of Testes of Adult Male Albino Mice Administrated with Carbon Tetrachloride

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Abstract

Galangin (Gal) is a natural flavonoid sourced from the roots of the plant *Albina Kalanga*. It possesses a variety of pharmacological activities, such as antioxidant, anticancer, and anti-inflammatory. The current study aims to enhance the efficacy of galangin through the use of gold nanoparticles as a drug delivery system against CCl_4-induced toxicity in the testicular tissue of male albino mice. Forty-two albino mice were divided into seven groups (6 mice/group), treatment with carbon tetrachloride solution for two weeks by intraperitoneal injection, (1 ml/kg) once a week for all groups except the control group, after which a group was injected with gold nanoparticles and two groups were injected with galangin in two concentrations, and two groups were injected with AuNPs + Gal conjugation solution with two concentrations. The animals' bodies were weighed and blood samples were obtained for testosterone hormone analysis and testicles for the purpose of weighing and histological study at the end of the experiments. The study showed that the testicular tissue of mice treated with CCl_4 had a different pathology compared to the control group, the testosterone hormone levels in the CCl_4 group were significantly higher than those of the control group. Results from the AuNPs+Gal group showed a significant reduction in the effects of CCl_4 toxicity on mice testicles, with testosterone levels are returning to normal and histological testicle structures improving, the ratio of testicle weights to animal body weight in the group injected with AuNPs was significantly higher than that of the control and CCl_4 groups. In conclusion, the results revealed that galangin combined with gold nanoparticles could effectively reduced the histological and functional tissue damages caused by CCl_4, presenting a promising natural solution that needs to be further developed.

Keywords: albino mice, CCl_4, Galangin, Gold nanoparticles, testicular tissue.

Introduction

Carbon tetrachloride (CCl_4) is a clear liquid that evaporates very easily and is a colorless volatile liquid with high toxicity. CCl_4 does not easily burn and the name of this chlorinated hydrocarbon is

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tetrachloromethane, according to the International System of Pure and Applied Chemistry (IUPAC) [1]. Carbon tetrachloride is a manufactured chemical whose main use has been in the production of chlorofluorocarbons although it has been also used as a cleaning agent or pesticide. Nowadays, due to its toxic effects its production is restricted. The main routes of exposure for the general population are inhalation of contaminated air and ingestion of contaminated drinking water [2]. Most CCl_4 is eliminated from the body unchanged in its composition, but some may be changed into other chemicals before it is removed from the body (e g, chloroform, hexachloroethane, and carbon dioxide). Chloroform and hexachloroethane may themselves cause harmful effects[3]. Exposure to high concentrations can affect the liver, central nervous system, kidneys, lungs and buildup of waste products in the blood. Carbon tetrachloride is classified as possibly carcinogenic to humans[2]. Exposure to CCl_4 also causes free radical formation in many other organs, including the kidneys, testicles, lungs, and blood. Therefore it is necessary to Monitoring, careful and finds safe solutions and effective for the side effects those products by CCl_4 in environment by environmental specialists and on humans by considering the pathophysiology of side effects, clinical features and treatment of CCl_4-induced toxicity[4]. Flavonoids are a group of natural substances with diverse phenolic structures that are found in fruits, vegetables, grains, roots, stems, and flowers. These natural products have several well-known for their health-promoting effects and many nutraceutical, pharmaceutical and medicinal applications. This is due to their antioxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme functions. They are also known to be potent inhibitors for diverse enzymes, such as xanthine oxidase (XO), cyclo-oxygenase (COX), lipoxygenase and phosphoinositide 3-kinase[5-6]. Galangin (Gal, 3-5-7trihydroxyflavone) is a phytochemical that can be found in the propolis bees and also in the roots of the *Albina kalanga* plant. The flavonoid 3,5,7-trihydroxyflavone (Galangin; Gal) has been reported to have a variety of biological activities, including antitumor, antimutagenic, antioxidative, bactericidal, and antifibrotic effects[7,8]. Nanotechnology has gained wide popularity in the field of applications because the nanoparticles (NPs) have sizes ranging between 1-100 nm and a large surface area relative to their sizes, which increases their ability to interact with target tissues and cells [9,10]. The choice of ‒drug delivery means to the target tissues and cells is no less important than the selection of the therapeutic material itself. Recently, nanoparticles have been used as carriers that help in the delivery of drugs, due to their unique characteristics such as their extremely small sizes and large surface area in relation to the size, as the nanoparticles have the ability to Penetration within biological barriers and membranes easily for hard-to-reach parts [11,12]. The drug delivery systems of nanocomposites provide a targeted delivery system for the ideal dose and reduce the side effects that may result from the delivery system. In addition, the nanoparticles solve problems related to solubility and bioavailability, as these vectors can protect the therapeutic substance from the dangerous internal environment that may cause its degradation in the body [13]. Due to they have particular qualities such as chemical resistance, enzymatic stability, and low cytotoxicity, gold nanoparticles (AuNPs) stand out among other nanoparticles (NPs) as a potential delivery vehicle for several drugs. Moreover, AuNPs have a reputation for being biocompatible and simple to couple with other biologically active compounds such as proteins and Antibodies (Abs) [14]. Also, because of their unique optical characteristics, they bind to active groups including amine and thiol more quickly, which increases the likelihood of changing the cell surface, the less-toxic and non-immunogenic nature of gold nanoparticles and the high permeability and retention effect provide additional advantages by

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facilitating penetration and accumulation of drugs at injury sites [15]. Despite unraveling key mechanisms and players in physiological and pathological tissue repair, these findings have not yet led to a substantial improvement in patient care. When considering therapeutic strategies to restore diseased or damaged tissues, it is crucial to realize that most tissue pathologies are due to a combination of underlying systemic disease with regional/anatomical factors that cause tissue stress, an ulcerative lesion, and/or scar formation [16]. Ike another body system, the reproductive system is influenced by a variety of factors that may have negative effects. For that, many studies have looked for alternatives, such as plants, to reduce this factor's effect due to their low cost and potential for daily consumption. Also tissue repair is now understood as a dynamic process that plays a primary role in the extent and resolution of injury. The development of methods to monitor and stimulate tissue repair may significantly improve the prognosis for patients exposed to toxic substances [17]. Histological disorders can affect the function of the organ in it and the health of the individual in general, therefore, it is necessary to find safe and effective ways to overcome tissue damage that may be caused by various factors, including polluting chemicals in the environment [18]. Accordingly, this study concentrated on the Galangin drive by gold nanoparticles as a protective factor and its capacity to mitigate the toxic consequences of CCl_4 exposure.

Materials and Methods

Animals and Experimental Design

obtained Forty-two male albino mice weight 25±5 g, aged 8-10 weeks from Alrazi center/ Where the appropriate environment was provided for them and used in the study after reaching the appropriate weight for the experiment [19] The mice were placed in special cages and distributed appropriately and at temperatures suitable for the animal's temperature of 23 ± 5 ° C, and were in an environment of light and darkness depicted equally during the day, and the mice received a suitable diet for chow and suitable drinking water [20]. care review committee and performed After acclimatization for one week, mice were divided into seven groups N = 6 as follows:

G I : Control.
G II: give 1 ml/kg CCl_4 once a week for 14 days.
G III : give AuNPs (4 ml/kg) for 14 day after treatment CCl_4 once week
G IV: give 10 mg/kg Gal for 14 days and CCl_4 once a week.
G V: give 20 mg/kg Gal for 14 days and CCl_4 once a week.
G VI: give conjugation AuNPs+Gal 10 mg/kg for 14 days and CCl_4 once a week.
G VI1: give conjugation AuNPs+Gal 20 mg/kg for 14 days and CCl_4 once a week.

Chemicals

Tetracholroareic acid ($HAuCl_4.3H_2O$), Gal, and dimethyl sulfoxide (DMSO) were purcushed from and were provided by Sigma Chemical Co. (St. Louis, MO, USA). Testosterone, kit from LineaR Chemicals, S.L(Spain),

AuNPs preparation and Gal

AuNPs were prepared according to the citrate sodium reduction Turkevich standard method and Gal was conjugation on the synthesized AuNPs.

Blood samples collection

Blood collection two weeks after the experiment, in which blood samples were drawn from the heart immediately after local anesthesia. Blood samples were left for 30 minutes for coagulation, centrifugation at 4,000 rpm for 20 minutes to isolate blood serum, maintaining blood at a freezing point of 20 C° they were used to identify hormones testosterone in the blood [21].

Histopathological examination

The animals were completely anesthetized and sacrificed After drawing blood and performing serum separation by Centrifuge, the testicles were removed from the associated tissues, washed, the testicles with

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sodium chloride solution, dried in filter paper, weighed, and kept in a 10% formalin for 48 hours for histological studies, The testicular organs under study were preserved in a 70% ethanol solution and then the method of preparing the textile slides was used by burying with paraffin wax, cutting and dyeing with hematoxylin and eosin (H&E) [22].

Statistical analysis

Statistics SPSS program (version 23.0) was used for statistical analysis of all data using the ANOVA test, and LSD was used to measure the significant differences, and the value was accepted at P≤ 0.05, and the data was exposed as mean ±SD [23].

Results

Preparation AuNPs and Gal conjugation

AuNPs were chemically prepared by the method of reducing gold salt $HAuCl_4.3H2O$ by sodium tricitrate, where the color of the solution was changed from yellow to dark red to indicate the formation of AuNPs after a while Gal was added to form the conjugation solution (Data not shown).

Testes weight and index:

The experimental groups did not record any significant difference ($p > 0.05$) in the weight of the testicle after treatment for two weeks with different solutions, compared to the other groups in the experiment after CCl_4 treatment (1 ml / kg B.W./once / week for two weeks). The results also recorded a significant increase ($P \leq 0.05$) between the ratio of average testicular weights to body weight in the group of animals injected with AuNPs + Gal 10 mg/kg solution which recorded 0.0033 g, compared to the control group ratio which recorded 0.0027 g and compared to the ratio of the CCl_4 group, which recorded 0.0026 g. Table 1.

The study recorded a significant increase (P≤ 0.01) between the ratio of average testicular weights to body weight in the group of animals injected with AuNPs solution, which recorded an average testicular weight ratio of 0.0035 g, compared to the control group ratio which recorded 0.0027 g. and compared to the ratio of the CCl_4 group, which recorded 0.0026 g. As well as compared to the ratio of the Gal 20 mg group which recorded 0.0028 grams Table 1.

Table 1. Shows Testis Weight gain after all group mice treatment 14 days.

Groups	Testis Weight (gm) (Mean ± S.E.)	Testis/Wb (gm) (Mean ± S.E.)
Control	0.078 ± 0.002	0.0027 ± 0.0001
CCl_4	0.076 ± 0.0044	0.0026 ± 0.0002
Gal 10 mg	0.081 ± 0.0044	0.0029 ± 0.0001
Gal 20 mg	0.074 ± 0.0076	0.0028 ± 0.0002
AuNPs	0.076 ± 0.0030	0.0035 ± 0.0002 [abc]
AuNPs+ Gal 10 mg	0.083 ± 0.0029	0.0033 ± 0.0001 [ab]
AuNPs +Gal 20 mg	0.070 ± 0.0017	0.0030 ± 0.0003

significan(P≤0.05)
a significant vs control group
b significant vs CCl_4
c significant vs Gal 20 mg

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Testosterone concentration

The study recorded a significant increase ($P \leq 0.05$) in the percentage of Testosterone in the group injected with CCl_4 solution, where 2.011(ng/dL) was recorded compared to all other experimental groups, where animals of the control group recorded 0.200(ng/dL), the Gal group of 10 mg recorded 0.453(ng/dL), the Gal group of 20 mg recorded 0.253(ng/dL), the AuNPs group recorded 0.466(ng/dL), the conjugation group AuNPs+Gal 10 mg recorded 0.238(ng/dL), and the conjugation group AuNPs+Gal of 20 mg recorded 0.257(ng/dL). While the rest of the groups did not record any significant difference among them Table 2.

Table 2. Shows changes in Testosterone hormone concentration in serum blood of all group mice treatment 14 days.

Groups	Testosterone (n g/dL) (Mean ± S.E.)
Control	0.200 ± 0.061
CCl_4	2.011 ± 0.33 [a]
Gal 10 mg	0.453 ± 0.009 [b]
Gal 20 mg	0.253 ± 0.024 [b]
AuNPs	0.466 ± 0.017 [b]
AuNPs +Gal 10 mg	0.238 ± 0.011 [b]
AuNPs +Gal 20 mg	0.257 ± 0.026 [b]

a significant vs control group
b significant vs CCl_4

Histopathological study:

No pathological histological changes were recorded in the Mice testicles in the control group where, the seminiferous tubules, sperm cell stages, and interstitial tissues showed standard histological parameters. Conversely, the testicles of mice in the group injected with CCl_4 revealed congestion of the blood vessels of the testicle and interstitial capillaries. Medium and multifocal numbers of seminiferous tubules showed remarkable lysis in epithelial lining cells described by large, pale, separate, swollen vacuoles, usually exchanging the cytoplasm and sometimes displacing the edge of the cell nucleus, a thick basement membrane warped and lined with one or two sheets of dissolved germ cells. These variations were frequently attended by reduced spermatogenesis and the lack of sperm in the lumen of the decaying tubules.

Galangin contains many biological activities such as antioxidant, antibiotic, anti-inflammatory, antifungal and anti-cancer. The testicles of mice in the group treated with the conjugation AuNPs+Gal solution displayed the normal histological form of the seminiferous tubules, mid-congestion of the blood vessels in the testicle, and moderately lively spermatogenesis (Fig.1).

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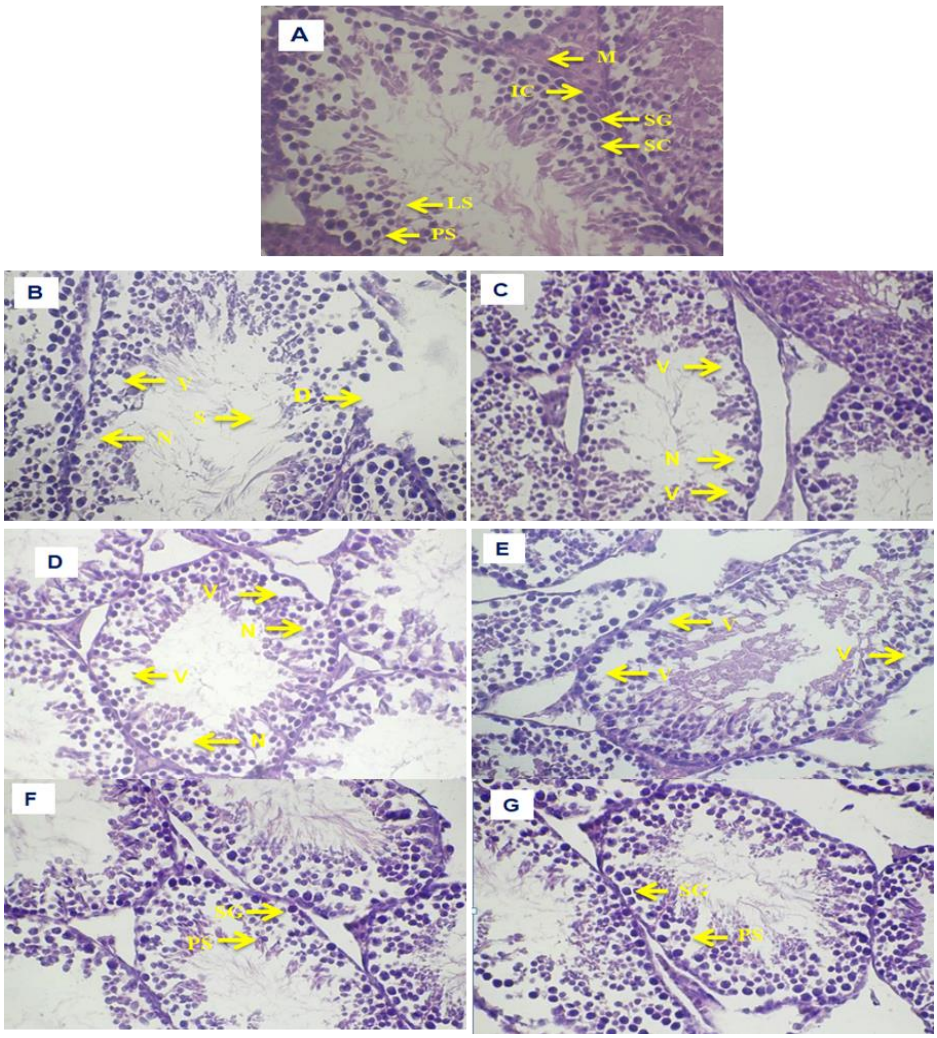

Figure 1. Histological pathological results of castration seminiferous tubule of mice (A): Transverse segment of the control group shows a normal histological appearance of Sertoli cells (SC), spermatogonia (SG), primary spermatocytes(PS), late spermatids(LS), mediastinum(M), interstitial cells(IC) 400 x. H&E, (B): Testicles of mice in the CCl_4 group show damage to the basement membrane (D), vacuolation cytoplasm of spermatozoa (V) necrosis of seminiferous tubule (N) accompanied by a decrease in sperm and loss of sperm in the cavity (L). (C, D)Cross-sectional of the two groups treated with Galangin 10.20 mg/kg respectively showing some cytoplasm vacuoles (V) and necrosis of seminiferous tubule cells (N) (E): Transverse section in the testicular tissue of the group treated with gold nanoparticles showing cytoplasmic vacuoles of sperm-forming cells (F, G) group (conjugation) showed normal histological parameters of spermatoposites with moderately active spermatogenesis).

Discussion

The current study showed that there were no significant differences in the weights of the testicles between the group injected with CCl_4 compared to the other study groups and the reason may be the low of use of concentration and dose represented by 1 ml/kg of CCl_4 and treatment once a week for 14 days. While the previous studies were represented by a significant decrease in the weights of the testicles and the ratio of testicular weights to body weight

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compared to the control group due to the increased concentration and daily doses given to the experimental animals, While a significant increase was found ($P \leq 0.05$) in the ratio of testicle weights to animal body weights in the group injected with gold nanoparticles compared to the control group and the carbon tetrachloride group, a significant difference was found in the conjugate group AuNPs+Gal of 10 mg/kg compared to the control group and the group injected with carbon tetrachloride, and according to the following studies.

The results of the study by [24] showed a decrease in the weights of rat testicles after treatment with carbon tetrachloride, where they were injected intraperitoneally with a single dose of CCl_4 dissolved in coconut oil at a concentration of 3 mg/kg body weight for 15 days, and histological examination revealed the occurrence of testicular atrophy, Increased pituitary weight, changes in the tubules that carry sperm [24]. Another study reported that testicular weight and testicular weight/body weight ratio in the CCl_4 treatment group were significantly lower than the control group due to the daily dose of up to four weeks at a concentration of 3 ml/kg body weight [25].

Treatment with CCl_4 in the group of mice at dose and concentration (1 ml/kg once a week for two weeks). to higher serum testosterone compared to the control group, The two conjugation groups attributed the normal levels of testosterone to testosterone after treatment with CCl_4, while the two free Gal groups were close to the ratios of the two control groups, while the free AuNPs group was the highest among the rest of the groups after the CCl_4 group.

The endocrine system is responsible for the production of steroid hormones in various tissues such as the ovaries, testicles, and adrenal glands, including luteinizing hormone LH, testosterone and follicle-stimulating hormone FSH are the chief controllers of spermatogenesis [26]. The low level of androgenic enzyme activity in the testicle led to a decrease in the level of testosterone in the blood and testicle [27]. The sperm totals in the testicle are reduced through inhibition of testosterone making during pressure situations due to excessive stages of corticosterone hormones and adrenocorticotropic [28].

Cellular functions are maintained by maintaining a balance between (ROS) and antioxidants [29]. ROS are shaped either through cellular metabolism or exposure to certain chemicals. In the present study, we used CCl_4 as a model for chemical exposure and intraperitoneal injection of experimental mice. Cell damage caused by ROS can be prevented or mitigated by antioxidants [30].

In this study, a decrease in the ratio of testicular weight to body weight, and higher testosterone concentration in rats using CCl_4 compared to the control group were observed due to the toxic effect of CCl_4. Which generates free radicals leading to the formation of ROS that affect the germ line of the testicle and the level of testosterone. Definite by histopathological results of the testicle to the deterioration of testicular tissue and epithelium lining a little seminiferous tubules escorted by compact spermatogenesis and the lack of sperm. The defensive role of conjugation against the harmful effect of CCl_4 in the testicle tissue and natural testosterone return can be explained by the antioxidant capabilities of Galangin and the conductivity of gold nanoparticles.

Previous results showed that conjugation AuNPs+Gal therapy alleviated CCl_4-induced imbalance in the testicles' oxidation- antioxidant arrangement due to its antioxidant possessions and successfully reduced the intracellular concentration of testosterone. These results are consistent with other studies [31].

The results of the current study showed histological abnormalities in the testicles using CCl_4 compared to the control group due to the toxic effect CCl_4. This generates free radicals and leads to the formation of reactive oxygen species that affect the testicular germ line and testosterone level. Reduce CCl_4 complex, impaired sperm morphological integrity and reduced testicular dysplasiatic indicators. CCl_4 significantly reduced the number of Sertoli cells, spermatogonia male germ cells, spermatocytes and sperm compared to the control group. The pathological histological results of the testicle confirm the degeneration of testicular tissue and the epithelium lining a few

seminal tubules accompanied by decreased spermatogenesis and the absence of sperm. The defensive role of the AuNPs+Gal conjugation solution against the harmful effect of CCl_4 in testicular tissue and the return of natural testosterone can be explained by the antioxidant abilities of the Galangin and the conductivity of gold nanoparticles. Cellular functions are maintained by maintaining a balance between reactive oxygen species (ROS) and antioxidants[32,33].

The current study results indicated that the conjugation solution AuNPs+ Gal can effectively protect sperm cells from damage caused by oxidative stress and apoptosis and regulate testosterone secretion.

In mice treated with conjugation solution AuNPs+Gal, germ cell density and sperm count increased. In this group, the integrity of the tubular wall was preserved, and the cavity space was filled with normal sperm. The height of the seminal epithelium was increased compared to the CCl_4 group. Sperm mothers of type A and B are observed at the junction on the wall of the seminal tubules next to the well-arranged Sertoli cells. In contrast, these arrangements were lost in the CCl_4 group as well. Due to the antioxidant properties of Galangin and gold nanoparticles, all of the listed indicators improved and well-preserved the structure and function of the germ epithelium and sperm strains against the destructive effects of a Carbon tetrachloride.

Conclusions

The results of the current study showed the effectiveness of galangin loaded on gold nanoparticles in reducing the toxicity of CCl_4 and reducing the histological damage it causes in the testicular tissues of albino mice, by improving the level of testosterone hormone and replacing the damaged tissues with new tissues in the testis, as the galangin may prevent the process of programmed death for cells because it is an effective antioxidant. Gold nanoparticles also helped in the delivery of galangin to the target tissues, which led to better results. Thus, it can be used as a promising method to deliver various therapeutic materials. However, further investigation to study the underlying mechanisms involved in *in vitro* and *in vivo* are essential.

Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Authors sign on ethical consideration's approval
- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad.

Aurthor's Contribution

B. S. A. and H. S. Al. conceptualized the study and contributed to methodology. Both of authors were responsible for data curtain. H. S. Al. supervised the study. All authors have read and agreed to the published version of the manuscript.

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تقييم الدور الوقائي المحتمل للكالانجين المقترن بجسيمات الذهب النانوية في التركيب النسيجي والوظيفي في خصى ذكور الفئران البيض البالغة المعاملة برباعي كلوريد الكربون

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الخلاصة

الكالانجين(Gal) Galangin هو فلافونويد طبيعي من مصادره جذور نبات *Albina Kalanga* . يمتلك العديد من الفعاليات الدوائية مثل مضاد للأكسدة والسرطان والالتهابات. تهدف الدراسة الحالية إلى تعزيز فعالية الكالانجين من خلال استخدام جسيمات الذهب النانوية AuNPs كنظام لتوصيل الدواء ضد السمية التي يسببها رباعي كلوريد الكاربون في أنسجة الخصية لذكور الفئران البيض. تم تقسيم اثنين وأربعين من الفئران البيض إلى سبع مجموعات (6 فئران / مجموعة)، كانت المعاملة بمحلول رباعي كلوريد الكاربون لمدة أسبوعين بالحقن داخل الصفاق , (1 مل / كغم) مرة واحدة في الأسبوع لكل المجاميع ماعدا مجموعة السيطرة، بعدها حقنت مجموعة بجسيمات الذهب النانوية ومجموعتين تم حقنها بالكالانجين بتركيزين ، وتم حقن مجموعتين بمحلول الاقتران AuNPs + Gal بتركيزين, .تم وزن أجسام الحيوانات وأخذ عينات دم لتحليل هرمون التستوستيرون والخصيتين لغرض الوزن والدراسة النسيجية في نهاية التجارب. أظهرت الدراسة أن أنسجة الخصية للفئران المعاملة برباعي كلوريد الكاربون كان لها أمراض مختلفة مقارنة بمجموعة السيطرة ، وكانت مستويات هرمون التستوستيرون في مجموعة CCl_4أعلى بكثير من تلك الموجودة في مجموعة السيطرة. أظهرت النتائج من مجموعة الاقتران AuNPs + Gal انخفاضا كبيرا في آثار سمية CCl_4 على خصيتي الفئران ، مع عودة مستويات هرمون التستوستيرون إلى طبيعتها وتحسين تركيب الخصية النسيجية. كانت نسبة أوزان الخصية إلى وزن جسم الحيوان في المجموعة المحقونة ب AuNPs أعلى بكثير من تلك الخاصة بمجموعة السيطرة ، .في الختام ، كشفت النتائج أن الكالانجين عند اقترانه مع جزيئات الذهب النانوية يمكن أن يقلل بشكل فعال من الامراض النسيجية والوظيفية التي يسببها CCl_4 ، مما يقدم حلا طبيعيا واعدا يحتاج إلى مزيد من التطوير.

الكلمات المفتاحية: الفئران البيض، رباعي كلوريد الكاربون، كالانجين، جسيمات الذهب النانوية، أنسجة الخصية.