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Effect of grape seed and flaxseed oils on kidney and testicular tissue in male rabbits exposed to tramadol-induced oxidative stress

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ABSTRACT

The study aimed to determine how tramadol affects oxidative stress and how flaxseed Oil (FSO) and grape seed oil (GSO) can enhance the biological functions of testicular and renal tissues in male rabbits exposed to tramadol-induced oxidative stress. The experiment was divided into four groups, each comprising five rabbits, with rabbits randomly assigned to groups. Findings: Tramadol significantly increased malondialdehyde (MDA) levels and decreased glutathione (GSH) levels ($P<0.05$). On the other hand, GSH levels significantly increased ($P<0.05$), and MDA levels decreased ($P<0.05$) in groups treated with (FSO) and (GSO). Histological examination of G1 showed stages of spermatogenesis; G2, the stages of sperm formation, are incomplete, with an observed proliferation of sperm-forming cells without the formation of mature sperm. Group G3 shows the stages of sperm formation and mature sperm in their natural state. Group G4 shows the stages of sperm formation and mature sperm in their natural state. In male rabbit kidney tissue, in group (G1), the renal corpuscle, the area surrounding the corpuscle (C), the proximal convoluted tubule, and the distal convoluted tubule are all evident. Group G2 shows swelling of the renal glomerulus and loss of the periglomerular space, with hemorrhage into the renal tissue (H) and enlargement of the distal convoluted tubule and proximal convoluted tubule cells. Group G3, by contrast, displays the renal glomerulus, the surrounding area, and the proximal and distal convoluted tubules in their typical configurations. The renal glomerulus, the area around the glomerulus, and the proximal convoluted tubule and distal convoluted tubule in their typical configuration. They are also visible in group G4.

Keywords: Glutathione, Malondialdehyde, Oxidative Stress, Free Radicals, injuries

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تأثير زيوت بذور العنب وبذور الكتان على أنسجة الكلى والخصية في الذكور الأرانب المعرضة للإجهاد التأكسدي الناجم عن الترامادول

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الملخص

هدفت الدراسة إلى معرفة تأثير عقار الترامادول على الإجهاد التأكسدي وكيف يمكن لزيت بذور الكتان وزيت بذور العنب تعزيز الخصائص البيولوجية لأنسجة الخصية والكلى لدى ذكور الأرانب المتأثرة بالإجهاد التأكسدي الناتج عن عقار الترامادول. وقد تم تصميم التجربة في 4 مجموعات، تتكون كل منها من 5 أرانب، موزعة عشوائيًا. وقد أظهرت النتائج أن عقار الترامادول رفع مستويات مالوندیالدهید (MDA) بشكل ملحوظ وخفض مستويات الجلوتاثيون (GSH) بشكل ملحوظ ($P < 0.05$). من ناحية أخرى، زادت مستويات GSH بشكل ملحوظ ($P < 0.05$) وانخفضت مستويات MDA ($P < 0.05$) في المجموعات المعالجة بـ (FSO) و (GSO). أظهر الفحص النسيجي G1 مراحل تكوين الحيوانات المنوية G2، ومراحل تكوين الحيوانات المنوية غير مكتملة، وهناك ملاحظة لانفجار الخلايا المكونة للحيوانات المنوية دون تكوين الحيوانات المنوية الناضجة. في الصورة (ج)، تُظهر المجموعة G3 مراحل تكوين الحيوانات المنوية وملاحظة الحيوانات المنوية الناضجة في شكلها الطبيعي. في الصورة (د) تظهر المجموعة G4 مراحل تكوين الحيوانات المنوية وملاحظة الحيوانات المنوية الناضجة في شكلها الطبيعي. أما بالنسبة لأنسجة الكلى لدى ذكور الأرانب، فقد تبين في المجموعة (G1) أن الكبيبة الكلوية (ج) والأنبوب الملتوي القريب والأنبوب الملتوي البعيد كلها واضحة. أما بالنسبة للصورة (ب) للمجموعة G2، فهي تظهر تورم الكبيبة الكلوية واختفاء المساحة المحيطة بالكبيبة، وتتضمن نزيفًا في أنسجة الكلى (ح) وتضخم خلايا الأنبوب الملتوي البعيد والأنبوب الملتوي القريب. من ناحية أخرى، تعرض المجموعة G3 الكبيبة الكلوية (المنطقة المحيطة بالكبيبة) والأنابيب الملتوية القريبة والبعيدة في تكويناتها النموذجية. تظهر أيضًا الكبيبة الكلوية والمنطقة المحيطة بالكبيبة والأنبوب الملتوي القريب والأنبوب الملتوي البعيد في تكوينها النموذجي في صورة المجموعة G4.

INTRODUCTION

Tramadol is a medication approved by the U.S. Food and Drug Administration (FDA) to treat moderate to severe pain. Tramadol's propensity for abuse and addiction has led the FDA to classify it as a Schedule IV restricted narcotic. Its application is thus restricted to pain situations. Because it is an opioid, tramadol selectively binds to different opioid receptors in the central nervous system [(1)]. Another study, however, has shown more and more evidence that tramadol is being abused, leading to deliberate overdoses or poisoning [(2)]. Seizures (15%), agitation (10%), respiratory depression (5%), and altered awareness (30%) are all consequences of severe poisoning caused by tramadol [(3)]. In an experimental study, long-term therapeutic doses of tramadol induced only minor histological alterations in the kidneys, confined to tubular cells. Creatinine and blood urea nitrogen levels also did not alter[(4)]. Numerous organ systems, including the respiratory, cardiovascular, gastrointestinal, central neurological, renal, and endocrine systems, can be impacted by tramadol intoxication. It also results in serotonin syndrome and rhabdomyolysis. To evaluate its risks and

advantages in pain management, doctors should be aware of its negative effects, high potential for abuse, and drug interactions. To stop additional poisoning, individuals with a history of overdose may be eligible for alternative therapy[5]. A controllable oxidative stress response is a major cause of many diseases.

In medicinal plants, many polyphenolic compounds, including flavonoids, alkaloids, and other phenolic compounds, exhibit anti-inflammatory properties. Treating illnesses with these herbs is cost-effective and lessens the strain on public health. [6]. Flaxseed oil (*Linum usitatissimum L.*) has become more popular due to its potential health benefits, including protection against diabetes, cancer, heart disease, and inflammation.[7]. Alpha-linolenic acid (ALA), lignans, ferulic acid, p-coumaric acid, and phytosterols are among the beneficial compounds in flaxseed oil that contribute to its therapeutic effects in fatty liver disease and other conditions. Phenolic compounds found in olive oil include oleic acid, hydroxytyrosol, and tocopherols.[8]. High concentrations of bioactive substances, such as lignans, dietary fiber, and alpha-linolenic acid, are found in flaxseeds. Flaxseed ingestion can play a significant role in preventing disease, particularly nutritional diseases. Frequent flaxseed ingestion may reduce the insulin resistance index, fasting glucose, and blood pressure, and improve the lipid profile, as well as the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR). In addition to their anticancer and antioxidant properties, flaxseeds can improve skin health, accelerate wound healing, and significantly reduce symptoms of menopause, constipation, and mental exhaustion. [9]. The grape is one of the most widely consumed fruits worldwide. The leaves and sap of grapevines have long been utilized in traditional European medicine. Grape skins and seeds contain numerous polyphenols, particularly proanthocyanidins, which can be used as functional ingredients to support the body's natural functions and treat a variety of health conditions. They are also a good source of vitamins and fiber. Grape seeds are readily available because they are a byproduct of winemaking, as evidenced by extensive research.[10]. A product made from seed grape pomace is grape seed oil GSO. GSO has a variety of biological activities, including antioxidant, anti-inflammatory, metabolic disease-reducing, and skin health- and wound-healing-promoting effects, due to its high intrinsic levels of fatty acids, sterols, vitamin E, and phenols. This shows great potential for use and is worthwhile. [11]. Grape seeds are among the most valuable components of grape pomace, a by-product of winemaking. Utilising grape seeds as a raw material to produce practical products will help the wine industry grow sustainably and enable the recycling and reuse of grape pomace. The application of (GSO), which is rich in bioactive compounds with a variety of health-promoting properties, may be very advantageous for the food, cosmetic, and pharmaceutical industries.[12].

MATERIALS AND METHODS

Design and Experimental Animals:

In this study, 20 local rabbits were used, aged between 3 and 4 months and weighing 500-750 grams. They grew up in the animal house section of the University of Kirkuk's College of Agriculture. The experimental animals were divided into four groups, each consisting of 5 rabbits, randomly assigned based on similar weights as follows: Group 1 (G1) received a standard diet with drinking water for 21 days. Group 2 (G2) was administered tramadol at a dose of 2.5 ml/kg. [13]. Group 3 (G3) was treated with tramadol (2.5 g/kg) along with 2 ml of grape seed oil by mouth. Group (G4) was treated with tramadol (2.5 g/kg) along with 2 ml of flaxseed oil.

Histopathological examination:

Selected organs (kidneys and tests) of each rabbit were transported to the Biology Department, College of Medicine, Tikrit University, and stored in 10% formalin solution and then dehydrated with

increasing degrees of alcohol. The samples were embedded in paraffin blocks after being treated with xylene. Serial sections, each 5 µm thick, were cut on a microtome and stained with hematoxylin and eosin (H&E). According to Bancroft and Gamble, a light microscope was used to view the stained sections [14].

Biochemical tests:

The rabbits were subjected to overnight binding, and the scratch was performed by decapitation. They were then divided into two groups for biochemical tests. To determine MDA and GSH.

Oil extraction:

Currently, solvent extraction and mechanical pressing are the most widely used techniques for extracting flaxseed oil. Unrefined, fresh flaxseed oil is yellow to orange in color and has a nutty flavor. It requires refinement via sedimentation, alkali refining, degumming, bleaching, winterization, and deodorization, as with other culinary oils on the market. Homemade cold-pressed oils can occasionally be used straight away for cooking without additional refinement or processing. [15]. There were grape seeds available. It took 15 days from the time the grapes were pressed until the seeds were allowed to dry. By air-drying them at the Distilleries, their moisture content was reduced to approximately 7% (db). Using a system created by Separex (France), supercritical CO2 was used to extract the oil. According to the method, the maximum working pressure, temperature, and flow rate were 70 MPa, 150 °C, and 25 kg CO2/h, respectively, and the extraction vessel had a capacity of 2 L. [16].

Statistical analysis:

The means ± standard errors are used to display all data. A one-way Duncan's Multiple Range Test (DMRT) was used to compare results, and ANOVA was used to assess whether the means of the treatment groups differed significantly. Differences were considered statistically significant at $P<0.05$. The statistical analyses were conducted using SPSS 17 [17].

RESULTS AND DISCUSSION

In the current study, the tramadol group had significantly higher MDA levels ($P < 0.05$). In contrast, the groups treated with flaxseed and grape seed oils exhibited a significant ($P < 0.05$) reduction in MDA levels. GSH levels in male rabbits treated with tramadol significantly decreased after administration. GSH levels, however, were significantly higher in the groups treated with flaxseed and grape seed oils than in the group that received only tramadol, as shown in Table 1.

Table 1: shows the levels of glutathione and malondialdehyde after treatment with tramadol, flaxseed oil, and grape seed oil.

	Control	Tramadol	FSO	GSO
MDA	5.872 ± 0.838 c	10.36 ± 1.036 a	7.358 ± 1.036 B	5.294 ± 0.780 c
GSH	12.206 ± 1.567 A	6.632 ± 0.556 C	8.594 ± 0.513 B	6.462 ± 1.796 c

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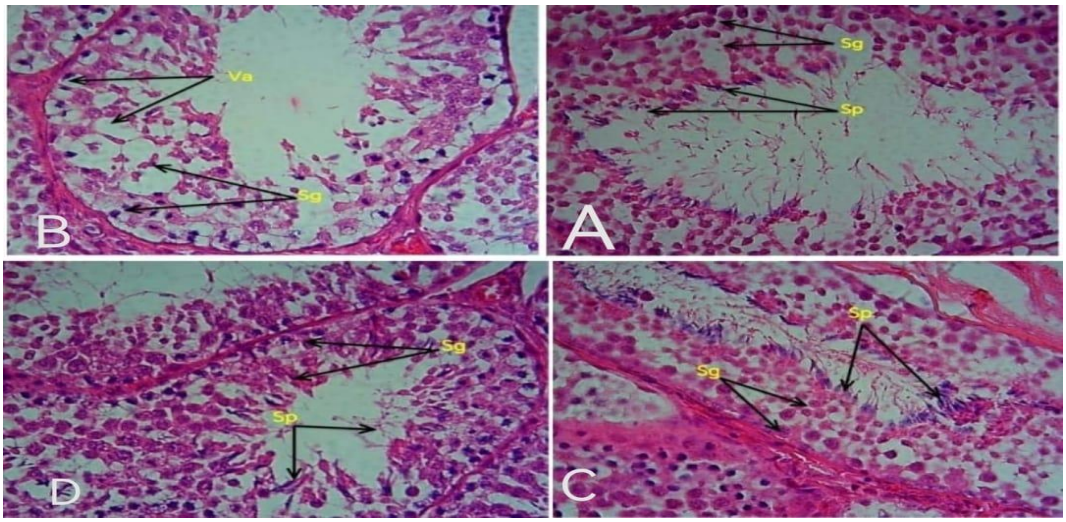

Fig. 1: Histological section of the testicles of male rabbits. The image (A) of the control group shows the stage of sperm formation (Sg) and mature sperm (Sp) within the seminiferous tubule in their normal form. The image (B) of the group treated with Tramadol shows incomplete stages of sperm formation (Sg) and the bursting of sperm-forming cells (Va), with no mature sperm observed. The image (C) of the group treated with Tramadol and flaxseed oil shows the stages of sperm formation (Sg) and mature sperm (Sp) in their normal form. The image (D) of the group treated with Tramadol and grape seed oil shows the stages of sperm formation (Sg) and mature sperm (Sp) in their normal form. H & E 400X,

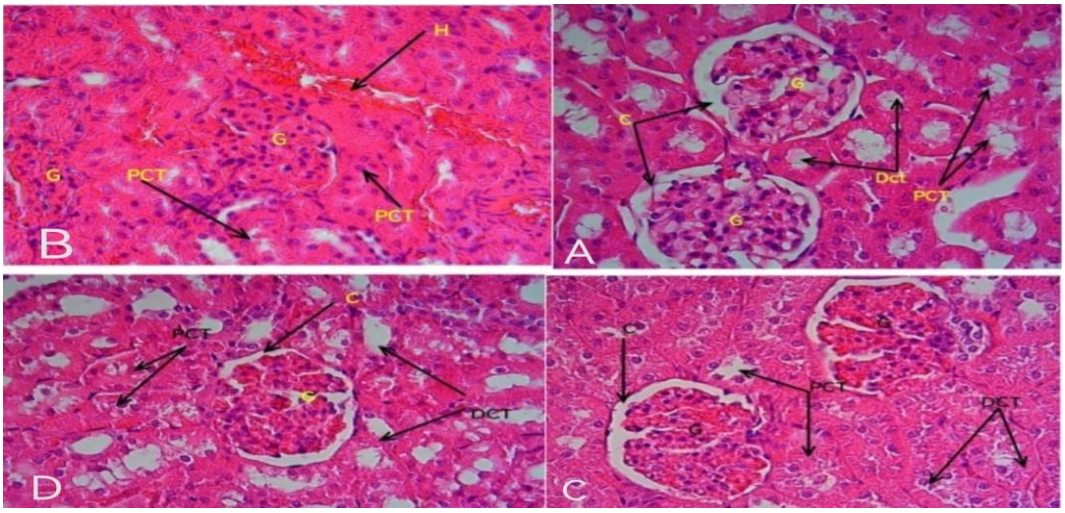

Fig. 2: Histological slice of the kidneys of a male rabbit. The control group's picture (A) displays the periglomerular space (C), the renal glomerulus (G), and the proximal and distal convoluted tubules (PCT and DCT). While the image (C) of the kidney section of the group treated with Tramadol with flaxseed oil shows the renal glomerulus (G), the periglomerular space (C), and swelling of the proximal convoluted tubule (PCT) and distal convoluted tubule (DCT) cells with bleeding within (H) the kidney tissue, the image (B) of the group treated with Tramadol shows swelling of the renal glomerulus (G) and the disappearance of the periglomerular space. While the image (D) of the group treated with Tramadol with grape seed oil displays the renal glomerulus (G), the periglomerular space (C), and the proximal convoluted tubule (PCT) and distal convoluted tubule (DCT) in their normal shape, H & E 400X,

Centrally acting analgesics such as tramadol can significantly increase MDA levels, increase the lipid peroxidation index in tissues, and disrupt the antioxidant balance. [18]. GSH levels decreased as a result of tramadol use. Furthermore, its low content after tramadol use may be due to its use to neutralize the free radicals produced.[19]. Whereas flaxseed oil consumption raises GSH levels, one of the significant antioxidants, which ROS oxidizes to GSSG [20]. A study showed that consuming flaxseed oil significantly reduced malondialdehyde (MDA) levels, an indicator of oxidative stress resulting from lipid peroxidation. [21]. The active compounds in grape seed oil inhibit certain enzymes involved in the formation of free radicals, such as xanthine oxidase, which generates them [22]. Researchers have been interested in GSO because of its diverse biological actions, including antioxidant.[23] or by giving these free radicals electrons to stabilize and thus inhibit the process of lipid peroxidation, thus reducing the resulting indicators such as MDA and raising the levels of antioxidant enzymes such as glutathione and others [24].

The structure and function of the renal and testicular tissues in male Sperg-Dawley albino rats were shown to be adversely affected by tramadol in both acute and long-term toxic effects. Tramadol is therefore seen to be more effective at treating pain, but its harmful effects should be taken into account.[25]. The histological study of the Tramadol-treated group also indicated that the stages of sperm formation (Sg) were not completed, and sperm-forming cells (Va) were observed without the formation of mature sperm. This is because Tramadol affects male hormones, including testosterone, so that it can affect the functions of the testicles, including changes in sperm production.[26]. Given that 30% of the medication is eliminated intact by the kidney, this could be explained by tramadol's toxicokinetics. The liver, however, transforms the remainder into active metabolites. Drug metabolites eliminated by the kidneys may harm cells, potentially leading to renal disease.[27]. Previous studies have also indicated that there are significant morphological changes observed in any of the epididymal tubule regions of mice that ate flax seeds due to the antioxidant compounds they contain [28]. A study by Diab *et al.* [29]. Demonstrated that feeding flaxseed oil to animals significantly improved both the kidney's histology and function. Consequently, this oil might help prevent kidney damage. It was discovered that grape seed Oil (GSO) significantly reduced the diabetic rats' serum Cr and BUN levels [30].

CONCLUSIONS

Uncontrolled use of tramadol can disrupt the redox balance and cause damage to tissues such as the kidneys and tests. These risks associated with this medication can be avoided by administering grape seed and flaxseed oil orally to the kidney and testicular tissues.

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