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Effect Of Chrysin In Liver AST, ALP And ALT And Some Antioxidant Levels In Male Rats Exposed To Oxidative Stress

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Abstract
The present study has been designed to investigate wheather chrysin has its own antioxidant activity and/or it act through induction of the endogenous antioxidants. The study has been conducted on adult male rats at the department of physiology, College of Veterinary Medicine, Al-Qadisiya University , Forty-eight mature male Wistar rats (aged 90 days and weighted 150±10 g) have been randomly assigned to 6 equal groups (8 each), Control group (C): 8 rats were given diet and normal drinking water throughout the duration of the experiment (6 weeks). The first treatment group (T1): 8 rat was dosed with 30% ethanol for a period of 6 weeks. The second treatment group (T2): 8 rat was given chrysin at a dose of 25 mg / kg of body weight for a period of 6 weeks. The third treatment group (T3): 8 rat was given chrysin at a dose of 50 mg / kg of body weight for a period of 6 weeks. The fourth treatment group (T4): 8 rat) chrysin was dosed at a dose of 25 mg / kg of body weight with ethanol 30% for a period of 6 weeks. (half an hour before ethanol). Fifth treatment group (T5): Chrysin was dosed at a dose of 50 mg / kg of body weight with 30% ethanol for a period of 6 weeks. At the end of each treated and control subgroup period, males were anaesthesized (by injection of 0.3ml ketamine + 0.1 ml of xylazine/ kg b.w. *ip*), dissected and blood samples were obtained from heart in non-heparinized tubes. Blood serum samples were separated for assessment of liver enzymes (AST,ALT and ALP), Antioxidants (MDA, SOD, CAT and GSH concentrations. The results of the study showed that there was a positive effect of chrysin in all study groups, compared with a group that received ethanol, on liver enzymes, antioxidants, and MDA. It can be concluded that chyrsin antioxidant activity is of pharmacological value not only as an antioxidant but also as an inducer of endogenous enzymatic and non-enzymatic antioxidants by the mean of increasing of superoxide dismutase, catalase, glutathione activity even in normal intact male rats not only in cases of oxidative stress.

Key words: Chrysin, Rats, Ethanol, Antioxidants, Liver Enzymes

تأثير الكرايسين في الكبد AST و ALP و ALT وبعض مستويات مضادات الأكسدة في ذكور الجرذان المعرضة للإجهاد التأكسدي

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خلاصة

صُممت الدراسة الحالية لمعرفة ما إذا كان للكريسين نشاطه المضاد للأكسدة و / أو أنه يعمل من خلال تحريض مضادات الأكسدة الذاتية. أجريت الدراسة على ذكور الجرذان البالغة في قسم علم وظائف الأعضاء ، كلية الطب البيطري ، جامعة القادسية ، ثمانية وأربعون من ذكور جرذان ويستار البالغة (بعمر 90 يومًا والوزن 150 ± 10 جم) تم تقسيمها عشوائيًا إلى 6 متساوٍ المجموعات (8 كل منها) ، المجموعة الضابطة (C): تم إعطاء 8 جرذان غذاء ومياه شرب عادية طوال مدة التجربة (6 أسابيع). مجموعة العلاج الأولى (T1): تم حقن 8 جرذان بجرعة 30٪ من الإيثانول لمدة 6 أسابيع. مجموعة العلاج الثانية (T2): تم إعطاء 8 جرذان مادة الكريزين بجرعة 25 مجم / كجم من وزن الجسم لمدة 6 أسابيع. مجموعة العلاج الثالثة (T3): تم إعطاء 8 جرذان مادة الكريسين بجرعة 50 مجم / كجم من وزن الجسم لمدة 6 أسابيع. مجموعة العلاج الرابعة (8 :(T4 جرذان) تم جرع الكريزين بجرعة 25 مجم / كجم من وزن الجسم مع 30 ٪ من الإيثانول لمدة 6 أسابيع. (نصف ساعة قبل الإيثانول). المجموعة العلاجية الخامسة (T5): تم إعطاء كريسين بجرعة 50 مجم / كجم من وزن الجسم مع 30٪ إيثانول لمدة 6 أسابيع. في نهاية كل فترة مجموعة فرعية تم علاجها وضبطها ، تم تخدير الذكور (عن طريق حقن 0.3 مل كيتامين + 0.1 مل من زيلازين / كجم وزن حيوي) ، وتم تشريح عينات الدم وأخذ عينات الدم من القلب في أنابيب غير مملوءة بالهيبارين. تم فصل عينات مصل الدم لتقييم إنزيمات الكبد (AST، ALT، ALP) ومضادات الأكسدة (MDA، SOD، CAT، GSH) وأظهرت نتائج الدراسة وجود تأثير إيجابي للكريسين في جميع مجموعات الدراسة مقارنة مع مجموعة تلقت الإيثانول ، على إنزيمات الكبد ، ومضادات الأكسدة ، و MDA. يمكن أن نستنتج أن نشاط مضادات الأكسدة chyrsin له قيمة دوائية ليس فقط كمضاد للأكسدة ولكن أيضًا كمحفز لمضادات الأكسدة الأنزيمية وغير الأنزيمية من خلال زيادة من ديسموتاز الفائق ، الكاتلاز ، الجلوتاثيون حتى في ذكور الجرذان السليمة ، ليس فقط في حالات الإجهاد التأكسدي.

الكلمات المفتاحية: كريسين ، جرذان ، إيثانول ، مضادات الأكسدة ، إنزيمات الكبد

Introduction

Flavonoids are the major group of plant secondary metabolites with broad spectrum biological activities desirable for human health. Research on flavonoids has continued to develop interest because they act through physiological mechanisms and many signaling pathways that are involved in many medical disorders (Samarghandian et al., 2016). Flavonoids are also major polyphenol constituents that express a wide range of biological activities, such as anti-inflammatory, anticoagulant, antioxidant, antibacterial, analgesic, and angiogenic effects (Farkhondeh and Samarghandian, 2019). Nowadays, there is increasing interest in elucidating the role and mechanism of phytochemicals as free radical scavengers and inhibitors of oxidative stress. In fact, the pharmacological effects of many traditional medicines have been attributed to the presence of flavonoids (Zhao et al., 2019). Chrysin has been shown to

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possess several beneficial pharmacological properties, such as antioxidants (Xue et al., 2018). And anti-hypertensive (Khan et al., 2021), and anti-diabetic. (Lukacinova et al., 2008), anti-inflammatory (Wang et al., 2017). Alcohol-related disorders are one of the current health problems associated with social and economic consequences. It is known that oxidative stress plays an important role in the pathogenesis of ethanol hepatic injury (Guillot et al., 2019). Ethanol administration can perturb the delicate balance between pro- and antioxidant leading to oxidative stress. Several studies have demonstrated the involvement of cytokines and oxidative stress in alcohol-induced liver damage. It is known that ethanol induces the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Jiang et al., 2020). Alcohol-induced oxidative stress is associated with ethanol metabolism. Ethanol metabolism occurs primarily in the liver, which sustains the greatest amount of organ damage from heavy drinking (Gao et al., 2019).

Material and Methods

Experimental Design

In this experiment, 48 adult male white rats of the Worcester breed were used, and their weight ranged between (150 ± 10) g. They were divided randomly into 6 groups, each group containing 8 rats, as follows:

- **Control group (C):** 8 rats were given diet and normal drinking water throughout the duration of the experiment (6 weeks).
- **The first treatment group (T1):** 8 rat was dosed with 30% ethanol for a period of 6 weeks.
- **The second treatment group (T2):** 8 rat was given chrysin at a dose of 25 mg / kg of body weight for a period of 6 weeks.
- **The third treatment group (T3):** 8 rat was given chrysin at a dose of 50 mg / kg of body weight for a period of 6 weeks.
- **The fourth treatment group (T4):** 8 rat) chrysin was dosed at a dose of 25 mg / kg of body weight with ethanol 30% for a period of 6 weeks. (half an hour before ethanol).
- **The Fifth treatment group (T5):** Chrysin was dosed at a dose of 50 mg / kg of body weight with 30% ethanol for a period of 6 weeks.

After the end of the experiment, the animals were sacrificed, as the animals were anesthetized using a mixture of 0.3 ml of ketamine and 0.1 ml of xylazine per kg of body weight under the peritoneum I.P. Then, blood samples were drawn from the heart directly and then placed in tubes that did not contain EDTA anticoagulant. It was placed in an inclined manner and then placed in a centrifuge at a speed of 3000 revolutions / minute for 15 minutes for the purpose of obtaining blood serum. The tubes were kept at a temperature of -20 °C until the tests required in the current study were performed, which included measuring the concentrations of liver enzymes (AST, ALT and ALP) and the levels of oxidants and antioxidants. Oxidative stress (SOD, GSH, CAT and MDA)**,** Spectrophotometer technique was using for estimation (AST, ALT and ALP) levels in the serum, using measurement kits obtained from (USA, BioSystem) Company , and To estimate of serum (SOD, GSH, CAT and MDA) concentration used colorimetric method, an examination based on the manufacturer Reagents by (China.BioSolar) Company.

Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). Comparisons were performed using one way analysis of variance (ANOVA1) and newman- keuls to test all groups unpaired values. Differences were considered to be significant at the level of $P<0.05$. All statistical analysis were carried out using the GraphPad Prism (SAS Institute, Inc., USA).

Results

The results of the current study (Figure 1,2 and 3) indicated that the T1 that dosed 30% ethanol achieved a significant increase ($P<0.05$) in the levels of liver enzymes (ALT, AST, ALP) compared with the control and the rest of the experimental treatments, while the two treatments recorded T2 and T3 doses of chrysin 25 and 50 mg/kg, respectively, showed a significant decrease ($P<0.05$) in the levels of ALT, AST, and ALP compared with the control and the rest of the experimental treatments. T3 recorded a significant decrease ($P < 0.05$) compared with T2, and the results of the analysis showed The statistical results showed that T4 and T5 doses of chrysin 25 and 50 mg/kg, respectively, in conjunction with 30% ethanol, achieved a significant increase ($P < 0.05$) in the levels of ALT, AST, and ALP compared with control, T2 and T3, and T5 showed a significant decrease ($P < 0.05$) compared to T4.

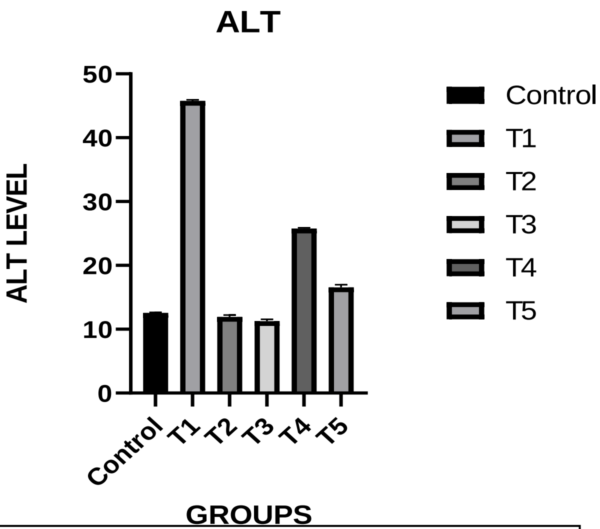

Fig. 1: Effect of chrysin on ALT enzyme level in rats exposed to alcoholic stress

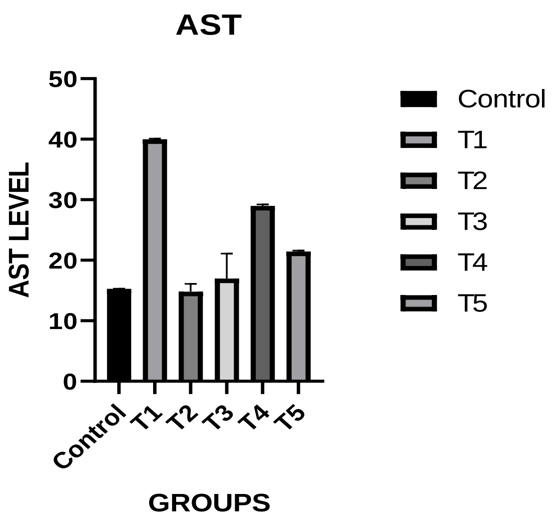

Fig. 2: Effect of chrysin on AST enzyme level in rats exposed to alcoholic stress

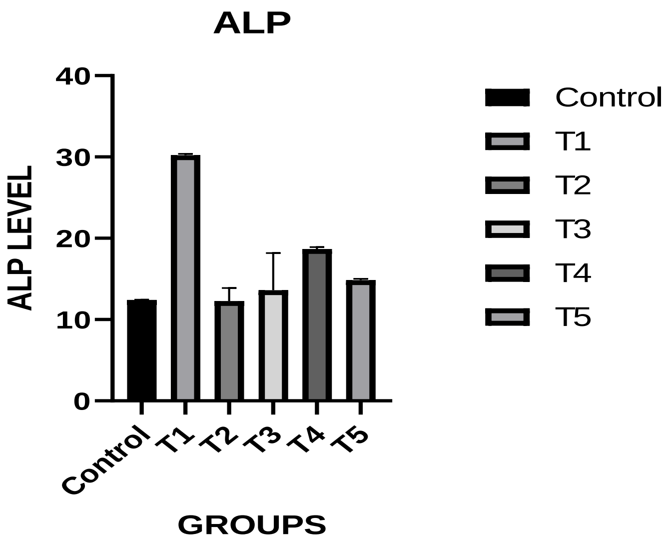

Fig. 3: Effect of chrysin on ALP enzyme level in rats exposed to alcoholic stress

- **Control group (C):** 8 rats were given diet and normal drinking water throughout the duration of the experiment (6 weeks).
- **The first treatment group (T1):** 8 rat was dosed with 30% ethanol for a period of 6 weeks.
- **The second treatment group (T2):** 8 rat was given chrysin at a dose of 25 mg / kg of body weight for a period of 6 weeks.
- **The third treatment group (T3):** 8 rat was given chrysin at a dose of 50 mg / kg of body weight for a period of 6 weeks.
- **The fourth treatment group (T4):** 8 rat) chrysin was dosed at a dose of 25 mg / kg of body weight with ethanol 30% for a period of 6 weeks. (half an hour before ethanol).
- **The Fifth treatment group (T5):** Chrysin was dosed at a dose of 50 mg / kg of body weight with 30% ethanol for a period of 6 weeks.

The results of the current study (Figure 4) showed that T1 that dosed 30% ethanol achieved a significant increase (P<0.05) in the level of MDA compared with the control and the rest of the experimental treatments, while T2 and T3 that dosed chrysin 25 and 50 mg/kg, respectively, recorded a decrease. Significantly (P<0.05) in the level of MDA compared with T1 and T4 that dosed chrysin 25 mg/kg of body weight in conjunction with 30% ethanol and T5 that dosed chrysin 50 mg/kg of body weight in conjunction with 30% ethanol, T3 also achieved a significant decrease (0.05). P> compared with T2 and control and the rest of the treatments, and the results showed that the treatments T4 and T5 achieved a significant increase (P<0.05) compared with control and with T2 and T3

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As for antioxidants, fig 5,6 and 7 the results of the statistical analysis of the current study indicated that T1 achieved a significant decrease ($P<0.05$) in the levels of antioxidants (SOD, GSH and CAT) compared with control and the rest of the treatments in the experiment, while T3 recorded a significant increase ($P>0.05$) in the levels of SOD, GSH and CAT compared to control and all treatments of the experiment, and the two treatments T4 and T5 achieved a significant decrease (0.05) (P>) in the levels of SOD, GSH and CAT compared with control and T2 and T3 while achieving a significant increase (0.05). >P) compared with T1.

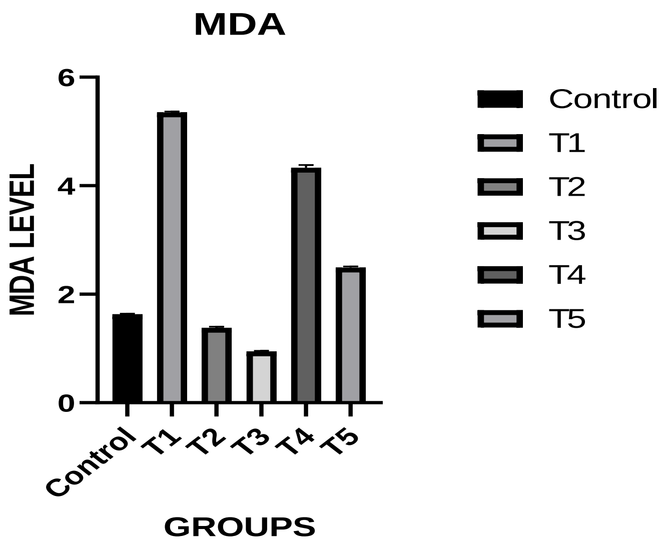

Fig. 4: Effect of chrysin on MDA level in rats exposed to alcoholic stress

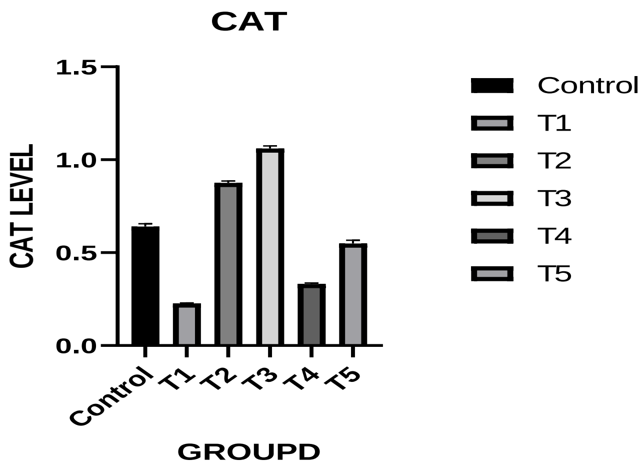

Fig. 5: Effect of chrysin on CAT level in rats exposed to alcoholic stress

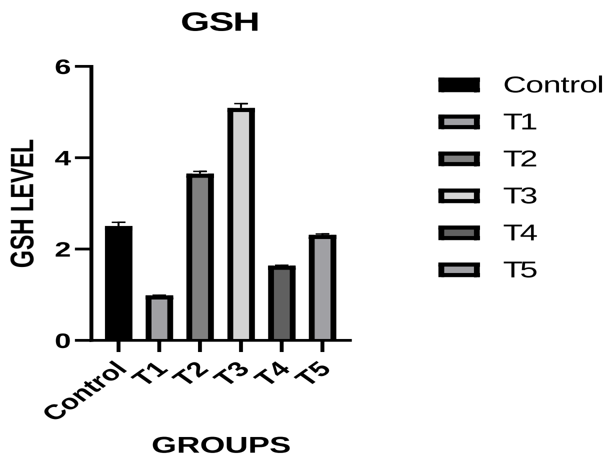

Fig. 6: Effect of chrysin on GSH level in rats exposed to alcoholic stress

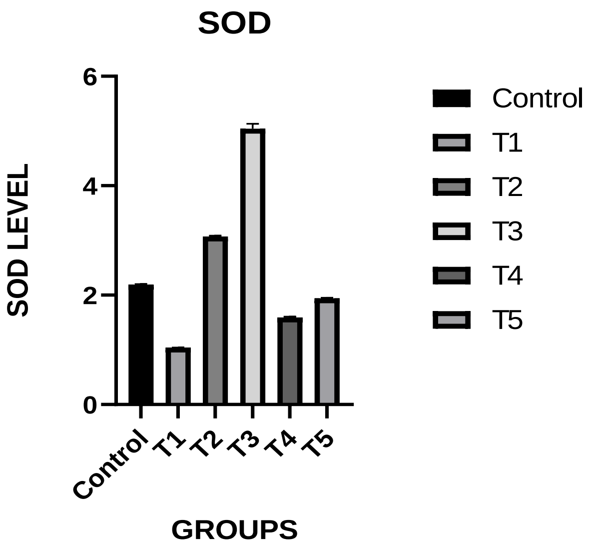

Fig. 7: Effect of chrysin on SOD level in rats exposed to alcoholic stress

- **Control group (C):** 8 rats were given diet and normal drinking water throughout the duration of the experiment (6 weeks).
- **The first treatment group (T1):** 8 rat was dosed with 30% ethanol for a period of 6 weeks.
- **The second treatment group (T2):** 8 rat was given chrysin at a dose of 25 mg / kg of body weight for a period of 6 weeks.
- **The third treatment group (T3):** 8 rat was given chrysin at a dose of 50 mg / kg of body weight for a period of 6 weeks.
- **The fourth treatment group (T4):** 8 rat) chrysin was dosed at a dose of 25 mg / kg of body weight with ethanol 30% for a period of 6 weeks. (half an hour before ethanol).
- **The Fifth treatment group (T5):** Chrysin was dosed at a dose of 50 mg / kg of body weight with 30% ethanol for a period of 6 weeks.

Discussion

The current study was conducted for the purpose of investigating the effect of chrysin on rats treated with ethanol. Natural medicines are widely circulated at the present time by focusing on the active substances in plants and using them in the pharmaceutical industry as one of the options for treating various diseases (Biglar et al., 2014, Roshanravan et al. ., 2020, Pourbagher-Shahri et al., 2021). Free radicals theory has been known for more than 50 years, however, its role in the development of diseases was only discovered in the past two decades, and thus the beneficial effects of antioxidants have been extensively studied (Liu, 2019). Free radicals play an essential role. In many biological processes in the body and many of these elements are necessary for life, such as the destruction of intracellular bacteria by phagocytes, especially by neutrophils and macrophages (Finkel and Holbrook, 2000). Alcohol causes a variety of short- and long-term adverse effects. Short-term adverse effects include generalized impairment of neurocognitive function, dizziness, nausea, vomiting, and hangover-like symptoms. It can also have a variety of long-term harmful effects, such as liver and brain damage (Rossi et al., 2019). The only alcohol present in alcoholic beverages or commonly used, other alcohols such as methanol and isopropyl alcohol are more toxic than ethanol, as previous studies indicated that exposure to methanol in an amount (10-15 ml). The harmful effects of alcohol on health appear when it is used in excessive amounts. Antioxidants break down radical chain reactions, preventing damage associated with oxidative stress (Da Pozzo et al.2018). Chrysin (dihydroxy flavone 5,7) is a natural flavonoid that belongs to the class of flavones that contains two benzene rings (A, B) and a heterocyclic ring (C) that contains oxygen. Previous studies indicated that the pharmacological activity of chrysin is due to the side moieties on the A ring and the C ring, where hydroxyl can exert anti-toxic effects (Siddiqui et al., 2018). Previous studies indicated that chrysin has an important role in protecting nerves in many neurological diseases, including epilepsy, oxidative stress, apoptosis in neurons, inflammation of the nerves, anxiety, depression, Guillain-Barre syndrome, and multiple sclerosis. sclerosis, Huntington’s disease, Parkinson’s disease, Alzheimer’s disease, hippocampal neurogenesis depletion, spinal cord injury, and cerebral ischemia (Monteiro etal., 2018, Zhang and Hölscher, 2017). Chrysin has also shown protective properties in hepatic physiological disorders including hepatotoxicity, non-alcoholic fatty liver disease (NAFLD), hepatic encephalopathy, liver fibrosis, and biliary smooth muscle contraction (He et al. al., 2019). Previous studies have demonstrated the protective role of chrysin in nephrotoxicity, renal ischemia, glomerulosclerosis, chronic kidney disease, diabetic nephropathy, and hyperammonemia (Samarghandian et al., 2017 Pingili et al., 2019).

Conclusion

We conclude through the results of our study that ehtanol has a negative effect on liver enzymes and antioxidants statue in this study, and we also concluded that chrysin had a positive effect on liver enzymes and antioxidants statue taken in this study.

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