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Evaluation of the antioxidant activity of *Zingiber officinale* alcoholic extract and vitamin e on liver damage induced by paracetamol drug in males of New Zealand rabbits

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Abstract

The aim of study is to reduce hepatic damage from paracetamol will be funded using alcohol extract for ginger and vitamin E as antioxidants in male New Zealand rabbits. Paracetamol (acetaminophen) is a widely used over-the-counter analgesic and antipyretic drug which is known to cause liver injuries in both humans and experimental animals when administered in overdose. The current study was conducted at the animal house of the College of Veterinary Medicine, Tikrit University to detect certain side effects developed with the use of the drug paracetamol, some physiological values resulting from liver damage through the use of 40 male New Zealand rabbits aged 5-7 months, randomly divided into four equal groups, including: The control group were given the normal physiological solution and the second group were given the paracetamol drug was given orally 400 mg/kg per rabbit while the third group was given vitamin e 50 mg/kg body weight as well as 400 mg/kg of the paracetamol drug was given orally. The fourth group was dosed with ginger alcoholic extract of 150 mg/kg body weight, which was given 400 mg/kg of the paracetamol drug was given orally. The study's findings demonstrated a significant decrease in the levels of catalase (CAT), super oxidase dismutase (SOD) and glutathione (GSH) in the treatment with a paracetamol drug compared to the control group, as well as a significant increase in the levels of liver enzymes and malondialdehyde. The study's results also found a significant decline in the levels of liver enzymes and malondialdehyde while revealing a significant increase in the levels of CAT, SOD and GSH in 3rd and 4th group compared to 2nd group. From the aforementioned findings, it can be concluded that vitamin e and ginger alcohol extract both reduce the unfavorable and harmful effects in some physiological parameters coming from liver damage caused by Paracetamol drug usage.

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Introduction

The paracetamol drug will be funded from widely used drugs for the treatment of various headaches and body pain, and is also used heavily in the treatment of severe colds and flu as well as antitheater and painkillers (1). In several studies suggested that overland use would be funded with a high dose that would fatally break down hepatocytes and be called a major cause of acute liver failure (2,3), is one of the most important laboratory drugs used for hepatotoxic events (4). Hepatotoxicity from the drug paracetamol will be funded from its high efficacy in the formation and stimulation of Cyp3A4, Cyp1A2, Cyp2E1 and cell content consumption of glutathione (GSH) and oxidative stress events (3). The

Zingiber plant is first of the most used nutrients in the world. The rhizome of ginger contains important ingredients including phenolic, zingerol, shawagol and zingeron compounds (5). The main principle of ginger, have many interesting physiological and pharmacological activities including, anti-tumor, antioxidants and anti-inflammatory activities (6). Bhandari *et al.* (7) reported that alcohol extract ginger plant can protect tissue from fat oxidation. Vitamin e is a fat-soluble antioxidant that ceases to produce ROS interactions that are formed when fat levels increase and help prevent or delay chronic diseases associated with free radicals (8). Vitamin E is one of the most important natural antioxidants that work against oxidative stress (9,10). Studies have suggested that the dosing of laboratory animals' paracetamol results in damage to hepatocytes proven by the moral rise in Malondialdehyde levels (MDA- Effectiveness of liver enzymes Aspartate transaminase (AST), Alkaline phosphatase (ALP) and Alanine transaminase (ALT). In contrast, a significant decrease in antioxidant levels was observed Glutathione (GH), Super oxidase dismutase (SOD) and Catalase (11,12).

The aim of the study is to reduce hepatic damage from paracetamol will be funded using alcohol extract for ginger and vitamin E as antioxidants in male New Zealand rabbits.

Materials and methods

The current study was conducted at the animal house of the College of Veterinary Medicine, Tikrit University from February to March 2022 to detect some of the side effects of paracetamol use on some physiological measurements resulting from liver damage through the use of 40 male New Zealand rabbits aged 5-7 months and weighing 1000-1200 g, which were randomly divided into four groups. The first group served as the control group and received a daily dose of physiological solution, second group administration with 400 mg/kg paracetamol while the third group administered 400 mg/kg of the medication paracetamol together with 50 mg/kg of vitamin e. The fourth group gave was dosed with ginger plant alcohol extract of 150 mg/kg body weight which was with 400 mg/kg of the drug paracetamol was given orally daily.

Ethical approve

Trials, including laboratory rats, in animal house of the College were followed-up by the academic board of the Department of Physiology, Biochemistry, and Pharmacology at the University of Tikrit Veterinary Medicine College, register number 7in 24-1-2022.

Plant and chemicals compounds

Paracetamol was purchased from Samarra Pharmaceutical Company. Preparation of plant materials, the *Zingiber officinale* rhizome was extracted using Soxhlet extraction according to the procedure used by Harborne JB (13). Z. officinal solution was made by melt of dry plant extract in warm distilled water at 60°C (14). Vitamin E was used from the Indian company and at a concentration of 50 mg / g body weight, according to Ourique *et al.* (15).

Blood sampling

After 30 days of experiment, blood samples from rabbits were collected. Blood was drawn using the heart puncture technique by disposable medical injection 5 ml. Blood samples were kept in sterile tubes, kept for no more than two hours before the serum was assembled by centrifuge at 3,000 rpm for 15 minutes and frozen at -18°C for biochemical tests consisting of hepatic activities including alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and alkaline phosphates (ALP), estimated the Kinetic method of International Federation of Clinical Chemistry (IFCC). The concentration of glutathione (GSH) and malondialdehyde (MDA) by the method the effect of the TBA-based interaction with MDA (16,17). Antioxidant enzymes of CAT and SOD were appreciated in accordance with Kakkor *et al.* (18) and Sinha (19).

Statistical analysis

All the group data was statistically evaluated with SPSS program. The results were expressed as mean ± standard errors and analyzed by factorial analysis of variance (ANOVA) the level of statistically significance was set at $P<0.05$.

Results

As showed in table 1 that paracetamol increased significantly the levels of hepatic enzymes in 2nd group comparative with 1st group. Meanwhile In 3rd and 4th group, treated paracetamol with vitamin e extract of *Z. officinale*, significantly reduced paracetamol effects and reduced MDA level and significantly reduced liver enzymes compared to those in experimental positive 2nd group.

As showed in table 2 that paracetamol decreased significantly the levels of liver enzymes CAT, SOD and GSH in 2nd group comparative with 1st group while increased significantly in MDA level. Meanwhile In 3rd and 4th group, treated paracetamol with vitamin e and extract of *Z. officinale*, significantly attenuated the effects of paracetamol and reduced level MDA and significantly increased liver enzymes CAT, SOD and GSH as compared to that in 2nd group.

Table 1: Effect of ginger plant and vitamin E on liver enzyme indices in rabbits exposed to paracetamol

Parameters	1st group	2nd group	3rd group	4th group
AST (IU/L)	93.27±9.17 E	203.57±10.5 A	134.16±9.14 B	127.70±9.95 C
ALP (IU/L)	132.37±8.65 E	252.81±9.50 A	181.58±11.0 B	174.38±25.7 C
ALT (IU/L)	24.71±5.53 E	59.06±3.33 A	36.85±7.29 B	29.88±7.33 C

A statistically significant difference appears in the same row at a significant level at P≤0.05.

Table 2: Effect of ginger plant and vitamin E on antioxidant statues biomarkers liver indices in rabbits exposed to paracetamol

Parameters	1st group	2nd group	3rd group	4th group
CAT (mmol/dl)	0.68±0.12 E	0.39±0.08 A	0.65±0.92 B	0.79±0.02 C
SOD (mmol/dl)	56.54±6.76 E	31.63±3.09 A	42.88±3.35 B	52.41±4.96 C
GSH (mmol/dl)	1.68±0.13 E	0.89±0.16 A	1.51±0.11 B	1.63±0.09 C
MDA (mmol/dl)	3.19±0.23 E	7.84±0.96 A	4.73±0.37 B	3.78±0.34 C

A statistically significant difference appears in the same row at a significant level at P≤0.05.

Discussion

The most popular and safest antipyretic medication is paracetamol, a non-steroidal anti-inflammatory medicine When taken at the therapeutic dose, but when taken in excess, it will cause liver damage (20-22), through the affects the liver pharmacologically by interacting with the harmful protein sulfhydryl group that is formed when cell necrosis and fat peroxide occur (3). Results of present study revealed that paracetamol administration causes a significant increase in liver enzymes compared with the control group. Paracetamol causes a significant increase in liver enzyme levels.

In group treated with paracetamol compared to 1st group. A fast increase in AST, ALP and ALT activity in paracetamol-treated rabbit serum may be caused by increased plasma membrane permeability or cellular necrosis that results in enzyme leakage into the bloodstream (23). These biochemical markers AST, ALP and ALT are utilized to diagnose early liver injury (24). It elevates serum levels of AST, ALP and ALT, and total bilirubin when the liver cell membrane is destroyed, which can happen in a number of common illnesses (25,26).

By promoting lipid peroxidation and other oxidative damage, the majority of the hazardous substances in the liver kill hepatocytes. Meanwhile, liver enzyme levels were decreased by treatment with vitamin E and *Z. officinale* extract. this result is agreement with recent studies that showed that ginger had better therapeutic that prophylactic detoxification effect on to liver, and improve hepatic enzyme due to potent antioxidant activities (27) and agreement with a study performed by Olfat and Enas (20), which confirmed the protective effect of ginger extract against 6-mercaptopurin, liver toxicity caused by the drug Ginger has antioxidant activity because of its polyphenol components that have the ability to be resistant to hydrogen atoms or electrons and capture free radicals, so they act as radical scavengers (22), where there was a significant decrease in AST, ALP and ALT after giving ginger along with 6-MP. The reduction of previously mentioned liver enzymes by ginger extract has also been reported by Bhandari *et al*. (21).

The protective effects of ginger against liver toxicity are mainly due to its antioxidant and anti-inflammatory effect. Ginger has antioxidant activity because of its polyphenolic components that have the ability to be resistant to hydrogen atoms or electrons and capture free radicals, so they act as radical scavengers (22). Vitamin E is an antioxidant that has the ability to inhibit the production of free radicals resulting from fat oxidation (28). While the study confirmed the ability of the vitamin to improve antioxidants glutathione and reduce the concentrations of malonaldehyde and these results agree with what reached the researchers (29). This result is in agreement with a study performed by El-Gawish *et al.* (30), Wesam and Amira (31).

The result showed that paracetamol induced decreased significantly the levels of the CAT, SOD and GSH while increase significantly in MDA level in 2nd group comparative with 1st group. These antioxidants play important role in removing reactive oxygen species (ROS), reducing lipid peroxidation and maintaining oxidation balance and reducing the biological system (32).

Increased levels of ROS can attack biological molecules such as phosphorous fat, leading to superoxidation of fat and reduction of antioxidant enzymes that lead to more oxidative stress (33).

Meanwhile treatment with vitamin e and extract of *Z. officinale*, increased the levels of antioxidant enzymes while decreased significantly in MDA level comparative with in 2nd group. Saber (34) showed that ginger water extract removed free radicals and improved liver damage and the reduce level of MDA serum, which acts as a lipid peroxide marker, and increase the serum level of antioxidant enzyme,

superoxide dismutase. Ginger treatment was found to exhibit antioxidative effect by enhanced activity of GSH and decrease of free radical and diminished amount of lipid peroxidation induced oxidative stress (26).

While the study confirmed the ability of vitamin e with the plant extract to reduce fat oxidation and improve the antioxidants of glutathione and this study agreed with, that the water extract of leaves of plant has antioxidants So effective vitamin e supplementation with these compounds reduced MDA levels and increased antioxidant enzymes levels (35). This result is in agreement with a study performed by Wesam and Amira (31), Rafi *et al*. (36).

Conclusion

According to this study, administering paracetamol to rabbits at a dose of 400 mg/kg for 30 days will result in oxidative stress in the liver as shown by changes in liver function signs and oxidative stress studies. As a result, the anti-liver and antioxidant activity with vitamin e 50 mg/kg and *Zingiber officinale* alcoholic extract 150 mg/kg which can serve as an effective scavenger for free radicals

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Conflicts of interests

The authors declare that there is no conflict of interest in the publication of this paper.

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تقييم الفعالية المضادة للأكسدة للمستخلص الكحولي لنبات الزنجبيل وفيتامين هـ على تضرر الكبد المستحدث بعقار الباراسيتمول في ذكور الأرانب النيوزلندية

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الخلاصة

هدفت الدراسة لمعرفة دور فيتامين هـ والمستخلص الكحولي لنبات الزنجبيل في التقليل من سمية الباراسيتمول على الكبد في ذكور الأرانب النيوزلندية. يعتبر الباراسيتمول دواء مسكنا للألم وخافض للحرارة وان استخدامه بجرعات عالية يسبب ضرر في الخلايا الكبدية.أجريت الدراسة الحالية في البيت الحيواني العائد لكلية الطب البيطري، جامعة تكريت للكشف عن بعض التأثيرات الجانبية المستحدثة بتعاطي عقار البارا سيتمول على بعض القياسات الفسيولوجية الناتجة من تضرر الكبد وذلك من خلال استعمال أربعون ذكر من الأرانب النيوزلندية بعمر ٥-٧ أشهر، قسمت عشوائيا الى أربع مجاميع متساوية تضمنت :مجموعة سيطرة أعطيت محلول الفسيولوجي، أما المجموعة الثانية أعطيت عقار الباراسيتمول بمقدار ٤٠٠ ملغم /كغم لكل أرنب بينما المجموعة الثالثة فأعطيت فيتامين هـ ٥٠ ملغم /كغم من وزن الجسم مع ٤٠٠ ملغم /كغم من عقار الباراسيتمول يوميا أما المجموعة الرابعة جرعت بمستخلص الكحولي لنبات الزنجبيل بمقدار ١٥٠ ملغم / كغم من وزن الجسم مع ٤٠٠ ملغم /كغم من عقار الباراسيتمول. وأظهرت نتائج الدراسة انخفاضا معنويا في مستويات في تركيز الكاتليز وسوبر أكسيد ديسميتوتاز الكلوتاثيون في المجموعة المعاملة بعقار الباراسيتمول مقارنة مع مجموعة السيطرة بينما أظهرت ارتفاعا معنويا في مستويات أنزيمات الكبد والمالوندالديهايد كذلك أوضحت نتائج الدراسة انخفاضا معنويا في مستويات أنزيمات الكبد والمالوندالديهايد في المجموعة الثالثة والرابعة مع زيادة معنوية في مستويات في تركيز الكاتليز وسوبر اكسيد ديسميتوتاز الكلوتاثيون مقارنة مع المجموعة الثانية. نستنتج من نتائج الدراسة أن هناك دوراً إيجابيا لفيتامين هـ والمستخلص الكحولي لنبات الزنجبيل في تقليل الأثار السمية للباراسيتامول على الكبد والتي تؤثر سلبا على بعض المعايير الفسيولوجية.