Tikrit Journal of Veterinary Sciences (2024) 1(2): 58-65

IRAQI Academic Scientific Journals

TJVS Tikrit Journal of Veterinary Sciences

Tikrit Journal of Veterinary Science

Protective Effect of Olive Leaves Extract on some physiological and biochemical parameters in Male Rats after the Administration of Paracetamol

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ARTICLE INFO.

Article history:

-Received:

-Accepted:

-Available online:

Keywords:
Paracetamol , antioxidant, liver & renal markers.

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ABSTRACT

This study aimed to know the protective effect of olive leaf extract against the side effects of Paracetamol in male rats such as liver & renal functions , antioxidant test (MDA, GSH) s in male rats. The study was conducted in the animal house of the Veterinary Medicine College, Tikrit University during the period (1. Desember.2023 to 30. December.2023). 24 adult male rats were used in this study. They were divided into three groups each group consists of 8 rats. These group are G1 (control), G2 (Paracetamol 1000mg/kg.B.W), G3(Paracetamol 1000mg/kg.B.W,+ Olive leaves extract 500mg/kg.B.W. Result of This study showed significantly increase ($P \leq 0.05$) in liver enzymes, MDA, Urea, creatinine and also a significant decrease in GSH parameter in G2 receiving (Paracetamol 1000mg/kg) after 30 days from treatment compared with control group but significantly graduated decreased ($P \leq 0.05$) in Paracetamol + olive leaf extract treated G3 due to protective effect of OLE .However after treatment with the Paracetamol +OLE group detected a significant increase ($P \leq 0.05$) in GSH parameter compared to Paracetamol group. Conclusion: The OLE has a significant protective effect from Paracetamol on some side effects in the hematological and biochemical parameters the male rats.

1. Introduction

When taken as directed, acetaminophen is one of the most widely used antipyretic and analgesic medications in the world and has no negative side effects. (Kanno et al., 2006). paracetamol is activated by hepatic cytochrome P-450, it forms the highly reactive metabolite N-acetyl-P-benzoquinoneimine (NAPQI), which binds covalently to tissue macromolecules and causes severe hepatic damage (Das et al., 2010). This is the mechanism by which paracetamol is hepatotoxic. (Kumar et al., 2014). The primary and most significant side effect of Paracetamol is hepatotoxicity (Öksüz et al., 2020). While hepatotoxicity is more likely in PCM overdose cases than nephrotoxicity, acute renal failure and renal tubular damage can nonetheless happen without liver damage. (Chinnappan et al., 2019).

Olive leaf extract (Olea europaea) originated from the leaves of the olive tree and has long been used in medicine and is abundant in flavonoid components including tyrosol and hydroxytyrosol, as well as antioxidant phenolic compounds like ligstroside, verbascoside, and oleuropein., protecting cells from oxidative damage by scavenging free radicals (Hannachi., 2020 ; Dehghani, 2021 and Zari,2011). Additionally, it has been demonstrated that OLE can enhance testis antioxidant conditions and sperm parameters in rats treated with rotenone and Oleuropein inhibits oxidative damage by scavenging dangerous free radicals. (Jemai,2008 and Sarbishegi, 2017) . In this work, male rats were used as test subjects to investigate potential therapeutic effects of olive leaf extract on numerous physiological and biochemical parameters.

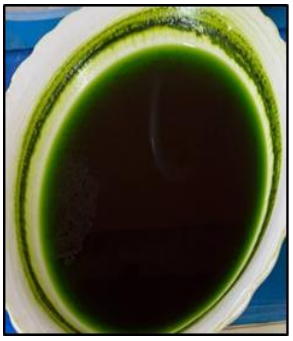
2. MATERIALS AND METHODS

Preparations of crude alcoholic extract (99%) of Olive leave

Fresh olive leaves were picked from a tree in Salah-Adden's Tikrit City and allowed to dry at room temperature for many days Many were crushed using an electric grinder to extract the ethanolic alcohol (99%) concentration, which was then used to generate the alcoholic extract from the olive leaf using soxhlet equipment in accordance with the procedure of (Borjan et al., 2020).

Stage1:-
OLE powde

stage:-2
Sexhelt apparatus



stage3:-
Olive leave extract

stage4:-
OLE after dried

Figure 1:- show stages of the Preparations of crude alcoholic extract (99%) of Olive leave

Animals

There are 24 mature male albino rats. (60-75days old) originated from the animal house of the Tikrit University College of Veterinary Medicine; they ranged in age from 60 to 90 days and in weight from 220 to 280 grams. The animals were kept in a room with air conditioning that had a temperature range of 20 to 25 degrees and a 12-hour light cycle every day. The animals were housed in plastic cage enclosures measuring 46 by 28 by 13 cm. Food came in a form of freshly prepared ration pellets., and were fed with the standard specialised lash and water was given freely during the duration of the experiment 30 days, during the period (2023/12/1 to 2023/12/30)

Ethical approve OR data collection permit

The academic board of the University of Tikrit Veterinary Medicine College's Department of Physiology, Biochemistry, and Pharmacology monitored trials, including those involving laboratory rats, in the college's animal house

Experimental design

During the period of 30 days (2023/12/1 to 2023/12/30)), 24 male rats were divided into three groups at random and given distilled water and oral feed in the animal house of the Veterinary Medicine College at Tikrit University., Paracetamol (n=8) group treated with Paracetamol (1000 mg / Kg B.W.) daily dose orally by Gavage needle for 30 days and Paracetamol (1000 mg / Kg B.W.) with doses of OLE 500 mg/kg (n=8) gave daily dose orally by Gavage needle for 30 days .

Olive leaf solution preparation:

To make an olive leaf solution, the extract was dissolve. 500mg/kg B.W(were it was dissolved 1.8g in 20ml of distilled water) given orally one time daily by gavage needle . (Eidi *et al*., 2009). The solution was freshly produced every day before to use, and the dosage was determined based on the body weight of each rat

Preparation of Paracetamol solution:-

The pure powder of Paracetamol of the Chinese company, which was purchased from Samarra Pharmaceutical Factory·(SDI) was used where the pure powder was dissolved in 5 ml of distilled water, the concentration of the treatment is 1000 mg per 1 kg of (B.W) body weight. According to ratio and proportion and B.W each rat was taken 2.8 mg dose of treatment.

Sample collection

After the experiment's thirty days were up, the animals were put unconscious using chloroform, and disposable syringes were used to take blood samples from each anesthetized animal's eye and heart punctures. and collected blood placed in gel tube (with out anticoagulant)and It was left for 15 minutes at room temperature , then Samples were placed in a freezer at (-20ċ) until use after being centrifuged for 15 minutes at 3000 rpm

Liver & Renal markers measurement

A levels AST, ALT , ALP , urea , creatinine was measured using Automated biochemical analyzer type(cobas c 111). The concentrated of liver enzymes within the blood serum was estimated using the Swiss company Agappe Switzerland ready-made analysis kit.

Antioxidant test (MDA, GSH):-

Measurement of Malondialdehyde by using ELISA (MDA):-

The concentrated of MDA within the blood serum was determined based on the method (Mateos *et al*.,2013).

Glutathione Reductase Measuring (GSH):-

The concentration of GSH in animal serum was determined according to using ELISA method (Šinko *et al*.,2007).

Statistical analysis:

The ANOVA Analysis of Variance was used in the statistical analysis. In line with Duncan's multiple ranges, the significant differences were found at a significant level (P≤0.05).. (Sawyer, 2009)

Results and Discussion:-

Estimation of liver enzymes functions (ALT, AST,ALP):-

This study appeared significantly increased (P≤0.05) in liver enzymes in group 2 gaving Paracetamol 1000mg/kg) during 30 days from therapy in comparison with the control group, but decreased significantly (P≤0.05) in Paracetamol + olive leaf extract treated group3,ALT was(96.3080 ± 12.04017) in G2 but decreased due to protecting influence of olive leaf extract in G3(68.1880 ± 5.80534).also in AST was(146.6200 ± 5.03309) in G2,and (133.0620 ± 5.60766) in G3, and in ALP was (283.9000 ± 22.18479) in G2 and 9223.2200 ± 26.72652) in G3 (Tab. 1) .

(Table 1): - The effect of *Olive leaves* extract on liver enzyme in male rat exposed to Paracetamol..

Groups / Parameters	1st group	2nd group	3rd group
ALT (IU/L)	54.13 ± 7.77 C	96.30 ± 12.04 A	68.18 ± 5.80 B
AST (IU/L)	115.92 ± 5.55 C	146.62 ± 5.03 A	133.06 ± 5.60 B
ALP (IU/L)	217.90 ± 30.87 C	283.90 ± 22.18 A	223.22 ± 26.72 B

At a significant level of P≤0.05, a statistically significant difference appears in the same row
The results of the current study on liver enzyme activity were consistent with (Mahmoud, 2014),and (Wadee & Hadree, 2022) by studying that the toxic effect of Paracetamol for 14 days observed an increase in ALT and AST in comparison with the control group, as well as the findings of the (El Faras & Elsawaf, 2017). The reason for the high liver binding is that it is a clear indicator of liver dysfunction increased in AST and ALT indicating cellular damage and the hepatocytes' lack of functional efficiency. and a sign of fat oxidation in liver cells and high leakage (AST, ALT, ALP,) Enzymes through the cellular membrane of hepatocytes (ALP) are membrane bound enzymes, which lose them These changes lead to changes in the influence of the cell membrane leading to leakage of liver enzymes from hepatocytes and can be used to assess liver injury (Rashid *et al.,* 2016).
After olive leaf administration, hepatocytes appear to be normal. This outcome supports the widely held belief that when the hepatic parenchyma improves and the hepatocytes regenerate, the blood levels of transaminases correct. (Taha *et al.,* 2020) , Hepatic marker activities were dramatically enhanced by treatment with olive leaf extract. Previous research demonstrated the hepatoprotictive efficacy of olive leaf aqueous extract against the overdosing of Paracetamol in male albino rats, which is consistent with the present data. (Al-Attar & Abu Zeid, 2013). The protective effect of the extract containing various phytoconstituents, such as fatty acids (oleanolic and Pentaadecanoic acid, Octadecenoic acid, Cis-Vaccenic acid 11E, and Stearic acid) and flavonoides (e.g., Isovitexin, Astibin, Quercetin, B-Sitosterol), which exhibit hepatoprotective properties and have a role in preserving the structural integrity of the hepatocellular membrane and may be responsible for the membrane stabilizing activity, was confirmed by a significant decrease in the activities of hepatic enzymes in OLE-treated rats.
(Taha *et al.,* 2020).

Estimation Renal function test(urea , creatinine):-

This investigation revealed a marked rise in (P≤0.05) in Urea in group 2 receiving Paracetamol (1000mg/kg) after 30 days from treatment compared with control group but significantly graduated decreased (P≤0.05) in Paracetamol + olive leaf extract treated group compared with G2 was (58.4820 ± 2.78128)and G3 was (46.3340 ± 2.50668). Also study showed graduated increased (P≤0.05) in creatinine in G2 compared with control group, while G3was decreased compared with G2 (1.3240 ± 0.11971) and G3 (1.0240 ± 0.01673) Tab.2.

Table 2):- The effect of Olive leaves extract on Renal markers in male rat exposed to Paracetamol.

Groups / Parameters	1st group	2nd group	3rd group
urea (mg⁄dl)	**39.38 ± 2.17 C**	**58.48 ± 2.78 A**	**46.33 ± 2.50 B**
creatinine (mg⁄dl)	**0.838 ± 0.44 C**	**1.32 ± 0.119 A**	**1.02 ± 0.016 B**

At a significant level of P≤0.05, a statistically significant difference appears in the same row
In this study appear Insufficient glutathione in the renal parenchyma causes nephrotoxicity, which happens regardless of cause nephrotoxicity that is dependent on the kidney's glutathione reserves and metabolic balance (Boutis & Shannon, 2001). The current investigation found that giving of paracetamol significantly raised serum urea and creatinine levels, which resulted in a decline in kidney function.. An increase in these parameters' plasma concentration appears to be a reliable sign when examining drug-induced nephrotoxicity in animals. (Roy *et al.,* 2015). According to earlier research, these results were reported (Canayakin 2016; Reshi, 2017). Urea and creatinine levels in serum decreased after treatment with olive leaf extract. These results were reported of (Zari and Al-Attar, 2011) who presented the report that olive leaves extract ameliorated renal toxicity induced by carbendazim in rats. Additionally, (Al-Sowayan and Mousa, 2014) also OLE administration significantly improved kidney function, reduced kidney damage, and alleviated oxidative stress. These results highlight the role of oxidative stress in renal dysfunction and suggest that OLE's antioxidant properties contribute to its protective effect against glycerol-induced acute

kidney damage in rats (Badawy 2024 ; Abugomaa,, 2020).

Estimation antioxidant markers:

This study showed significantly increase (P≤0.05) in MDA in group 2 receiving Paracetamol (1000mg/kg) after 15 days from treatment compared with control group but significantly graduated decreased (P≤0.05) in Paracetamol + olive leaf extract treated group compared withG2 was (354.34 ± 87.27) and G3 was (292.34 ± 12.24).Also study showed graduated decreased (*P≤0.05*) in GSH in G2 compared with control group, while G3was increased near by normal compared with G2 (256.68 ±9.99) and G3(276.2000 ± 5.37) Tab.3.

Table 3):- The effect of *Olive leaves* extract on antioxidant markers in male rat exposed to Paracetamol

Groups / Parameters	1st group	2nd group	3rd group
MDA ((µmoil)	252.70 ± 14.65 B	354.34 ± 87.27 A	292.34 ± 12.24 AB
GSH (µmoil)	286.94 ±6.40 A	256.68 ±9.99 C	276.20 ± 5.37 B

At a significant level of P≤0.05, a statistically significant difference appears in the same row

The increased MDA level is accordance with previous investigations. of Farghaly& Hussein, (2010), It shown the link between MDA and PCM toxicity. Other investigations showed a considerable increase in the level of MDA in the liver, which weakens the function of glutathione reductase (GSH). (Saheed 2016 ; Ajiboye 2016; Amin,2017).

It is known that glutathione reduces the toxicity of Paracetamol in the liver mainly through the contributory association of the toxic compound (NAPQI) The oxidative metabolism of paracetamol that involves the sulfhydryl groups of GSH and other proteins. may increase the production of free radicals and increase the oxidation of lipids, thereby increasing the levels of MDA, as well as increasing the composition of the compound (H2O2) which is a free radical that inhibits catalyst enzyme and super oxides causing the loss of liver cells to function, overdose of Paracetamol significantly reduced the effectiveness of GSH (Bkhairia *et al*.,2018) contrast the olive leaf extract-treated groups had a marked decrease in MDA levels and an increase in GSH., because found some of the phenolic compound and vitamins which in turn act as antioxidants, this result similar to (Motawea, *et al*.,2020). The administration of OLE considerably reduced these alterations, indicating that the chronic inflammation and oxidative stress are reversed by OLE extract that contains phytoconstituents such oleuropein and hydroxytyrosol.. This is according to several investigations. (Bulotta 2013; Ben ,2015).

Conclusion

In regards to specific adverse changes in the physiological and biochemical parameters of the male rats, OLE significantly protects against the effects of Paracetamol

Acknowledgments

The College of Veterinary Medicine at Tikrit University is acknowledged by the authors for all of its help in making this work possible.

Conflicts of interests

The publishing of this paper does not present any conflict of interest, according to the authors.

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التأثير الوقائي لمستخلص أوراق الزيتون على بعض المعايير الفسلجية والكيموحيويه في ذكور الجرذان بعد اعطاء الباراسيتامول

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الملخص

هدفت الدراسة إلى معرفة التأثير الوقائي لمستخلص أوراق الزيتون ضد بعض التأثيرات الجانبية لعقار الباراسيتامول في ذكور الجرذان ، مثل وظائف الكبد والكلية، ومضادات الأكسدة (مالون الديهايد، كلوتاثيون) في ذكور الجرذان. أجريت الدراسة في البيت الحيواني التابع لكلية الطب البيطري جامعة تكريت خلال الفترة (2023/12/1 إلى 2023/12/30). تم استخدام 24 من ذكور الجرذان البالغة في هذه الدراسة. وتم تقسيمهم عشوائيا إلى ثلاث مجاميع كل مجموعة تتكون من 8 جرذان هي (مجموعة السيطرة)، مجموعة (باراسيتامول 1000 ملغم/كغم من وزن الجسم)، مجموعة (باراسيتامول 1000 ملغم/كغم من وزن الجسم، + مستخلص أوراق الزيتون 500 ملغم/كغم من وزن الجسم. أظهرت نتائج الدراسة زيادة معنوية ($P\leq0.05$) في إنزيمات الكبد، مالون الديهايد ، اليوريا، الكرياتين وكريات الدم البيضاء وكذلك انخفاض في كلوتاثيون في المجموعة الثانية (باراسيتامول 1000 ملغم/كغم) بعد 30 يوم من العلاج مقارنة مع مجموعة السيطرة ولكن انخفضت بشكل ملحوظ ($\mathbf{P\leq0.05}$). في مجموعة الباراسيتامول + مستخلص أوراق الزيتون المعالج بسبب التأثير الوقائي لـ مستخلص اوراق الزيتون .كما أظهرت الدراسة ارتفاعا معنويا ($P\leq0.05$) في كلوتاثيون في المجموعة الثالثة مقارنة بالمجموعة الثانية. نستنتج من هذه الدراسة أن المعاملة بالمستخلص الكحولي أوراق الزيتون له تأثيرا إيجابيا في إصلاح ضد التأثيرات الجانبية لعقار الباراسيتامول على بعض المعايير الفسيولوجية والكيموحيويه في ذكور الجرذان.

الكلمات المفتاحية: الباراسيتامول ,مضادات الاكسدة, وضائف الكبد والكلية.