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Biochemical Evaluation of The Hepatoprotective Potential of Lemon Oil and Nigella Sativa Oil in Acetaminophen-Induced Liver Injury

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Abstract

Background: A common analgesic and antipyretic medication is acetaminophen. In therapeutic doses, it induces hepatotoxicity over an extended period of time. High dosages of it can cause centrilobular hepatic necrosis. Nigella sativa and citrus lemon both play protective roles in the fight against hepatotoxicity. In order to determine how Nigella sativa oil and lemon oil can treat hepatotoxicity brought on by acetaminophen, the current study was designed.

Method: 20 rats were sorted into 4 subgroups before being used in this investigation one control and 3 treatment groups, liver function tests and histo-pathological examination were used to remark the liver toxicity and the hepatic-protective effects of the herbals medicine.

Results: Consuming acetaminophen raises serum liver enzyme levels while lowering total protein levels. Potential hepatoprotective effects of citrus lemon and Nigella sativa include reversing alterations in ALT, AST, Alkaline Phophatase, bilirubin, and the total protein levels brought on by acetaminophen. Moreover, the acetaminophen-induced histological alterations could be reversed.

Discussion and Conclusion: The antioxidant-rich plant extraction had hepatic protective effects by controlling cell permeation, maintaining cellular stability, and squelching the oxidative impacts. With our result shows pretreatment with lemon oil at dose (500 mg) exhibited a considerable reduction in level of (TSb, AST and significant decrease in level of ALT) this show that liver damage improves when it compared with acetaminophen-treated alone suggesting a protective effect of lemon oil. It is common knowledge that an essential oil incorporates the phenolic, ester, and aromatic and aliphatic acid components, by eliminating anion of superoxide, H_2O_2, hydroxy radical, and reducing the lipid peroxidation, many of these phenolic substances can protect cells. Acetaminophen cause liver injury. lemon oil and black seed oil have hepatoprotective effect so administration of these natural products reduce this damage effectively in a rat model by lowering serum ALT and AST and prevent the hepatotoxicity induced by acetaminophen.

التقييم البيوكيميائي لقدرات زيت الليمون وزيت حبة البركة على حماية الكبد في إصابات الكبد الناجمة عن الأسيتامينوفين

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الخلاصة

المقدمة: عقار الأسيتامينوفين هو أحد الأدوية المسكنة والخافضة للحرارة. وفي الجرعات العلاجية، يسبب سمية الكبد على مدى فترة طويلة من الزمن. والجرعات العالية منه يمكن أن تسبب نخرًا في الفصيصات المركزية للكبد. تلعب كل من حبة البركة والليمون الحامض دورًا وقائيًا في مكافحة سمية الكبد. ولتحديد كيفية قدرة زيت حبة البركة والليمون على علاج سمية الكبد الناجمة عن عقار الأسيتامينوفين، تم تصميم الدراسة الحالية.

المواد وطرق العمل: تم تصنيف 20 فأرًا إلى 4 مجموعات فرعية قبل استخدامها في هذا البحث، مجموعة تحكم وثلاث مجموعات علاجية، وتم استخدام اختبارات وظائف الكبد والفحص النسيجي المرضي لملاحظة سمية الكبد والتأثيرات الوقائية للكبد للدواء العشبي.

النتائج: يؤدي تناول عقار الأسيتامينوفين إلى رفع مستويات إنزيمات الكبد في المصل مع خفض مستويات البروتين الكلي. تشمل التأثيرات الوقائية المحتملة للكبد من الليمون الحامض وحبة البركة عكس التغيرات في مستويات ALT وAST وAlkaline Phophatase والبيليروبين وإجمالي البروتين التي يسببها عقار الأسيتامينوفين. علاوة على ذلك، يمكن عكس التغيرات النسيجية الناجمة عن عقار الأسيتامينوفين.

المناقشة والاستنتاج: كان لمستخلص النبات الغني بمضادات الأكسدة تأثيرات وقائية للكبد من خلال التحكم في نفاذية الخلايا والحفاظ على استقرار الخلايا وإخماد التأثيرات المؤكسدة. أظهرت نتائجنا أن المعالجة المسبقة بزيت الليمون بجرعة (500 مجم) أظهرت انخفاضًا كبيرًا في مستوى TSb) و AST وانخفاضًا كبيرًا في مستوى (ALT وهذا يوضح أن تلف الكبد يتحسن عند مقارنته بالمعالجة بأسيتامينوفين وحده مما يشير إلى التأثير الوقائي لزيت الليمون. من المعروف أن الزيت العطري يحتوي على مكونات الفينول والإستر والأحماض العطرية والأليفاتية، من خلال القضاء على أنيون الأكسجين الفائق، H2O2، والجذور الهيدروكسيلية، وتقليل بيروكسيد الدهون، يمكن للعديد من هذه المواد الفينولية حماية الخلايا. يسبب الأسيتامينوفين إصابة الكبد. زيت الليمون وزيت حبة البركة لهما تأثير وقائي للكبد، لذا فإن إعطاء هذه المنتجات الطبيعية يقلل من هذا الضرر بشكل فعال في نموذج الفئران عن طريق خفض مستويات ALT و ASTفي المصل ومنع السمية الكبدية الناجمة عن الأسيتامينوفين.

الكلمات المفتاحية: الأسيتامينوفين، السمية الكبدية، زيت الليمون، إنزيمات الكبد، زيت حبة البركة.

1. Introduction

One of the most common and often used as antipyretics and painkillers and medications, (acetaminophen) acetaminophen is available over _the_ counter either alone or in combination with other component form. More calls to poison are made as a result of acetaminophen overdose than any other overdose .(Jóźwiak-Bebenista and Nowak, 2014) acetaminophen are responsible for more than 100,000 calls, 56,000 emergency room visit, 2,600 hospital admissions, with 458 deaths related to acute liver failure. (Lee, 2020) Fig.1 illustrated acetaminophen mechanism of action.

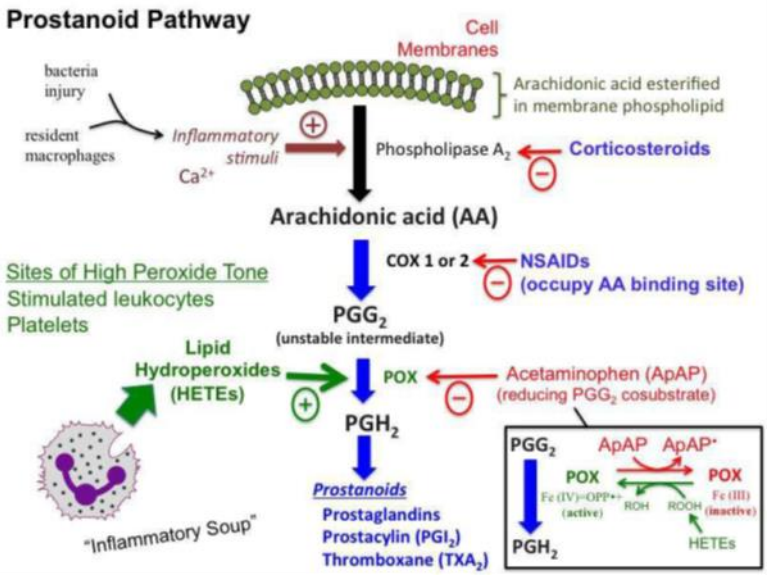

Figure 1: The Prevailing Hypothesis on The Mechanism of Action of Acetaminophen

According to the Food and Agricultural Organization (FAO), citrus is the most significant crop in the world. Citrus fruits, including lemons, are indeed widely cultivated and consumed around the world. The genetic ancestry of the lemon being a hybrid of the bitter orange and the citron is a fascinating finding. It highlights the complexity and diversity of plant breeding and evolution .(Elbagerma *et al.*, 2011). When determining the biological activity of Citrus Limon fruit and oils, Flavonoids that containing flavonones like eriodictyol, Naringin and Hesperetin, Flavones like Diosmin and Apigenin, while Flavonols like quercetin with their derivatives were crucial. (Ben Hsouna *et al.*, 2017) Fig.2. Nigella sativa (NS), which has long been revered as one of the plants with highest nutrition in human history evidence has been made to support the historically asserted usage of this species' tiny seeds. (Yimer et al., 2019)Due to its high concentration of vegetable protein, minerals, fibers and vitamins, black seeds have a high nutritional value. (Hassanien, 2007). According to a number of sources, the nutritional breakdown varied in the following order: 20–85% proteins, 38.20% fats, 7-94% the fiber, while 31.94% carbohydrates in total are included in a diet. Among the amino acids discovered were Arginin, Glutamate, and Aspartate, but Methionin and Cystein were the minor and major amino acids. (Khalil et al., 2021)Approximately forty distinct chemicals were represented by essential makeup oil (0.4-0.45%) reported in Multiple inquiries, among the plentiful. The quantity of the volatile oil's most important

bioactive component, thymoquinone that was extracted using various techniques from the seeds of N. sativa ranged widely, respectively .(Shahid *et al.*, 2022) Furthermore, Black seeds have high levels of folic acid, thiamin, niacin, copper, zinc, phosphorus, calcium, and phosphorus .(Chouhan, Sharma and Guleria, 2017)

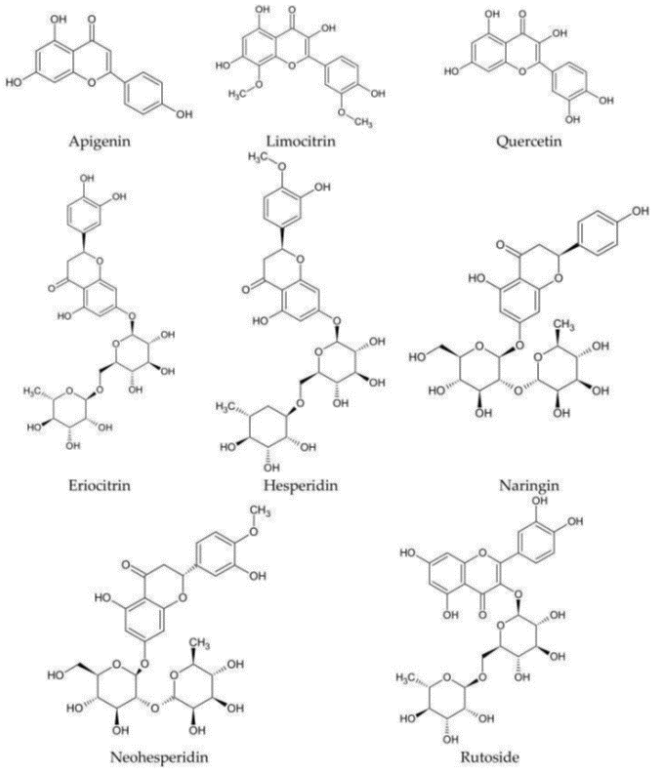

Figure 2: Citrus Oil Active Constituents (Guerra *Et Al.*, 2020)

2. Materials and Methods

The present research complied with all guidelines provided by the animal ethics committee and was carried out in December 2023 at the pharmacy college of University of Kerbala.

2.1. Animals

20 adult rats of 6 weeks old and approximately 140-270 mg weight were acquired from the college's pharmacology department's animal house. To ensure uniform rat growth and performance, all the rats were kept in typical light-dark circles, fed a standard diet in laboratory with free access to water.

2.2.Inclusion Criteria

1. Weight and age: rats weighing approximately 200-230grams and age around 8–12 weeks were only included in the study to ensure uniformity and comparability of results.
2. Gender: the study included only male rats, to mitigate the effects of hormonal changes in females during the experimental period.
3. Health status: The only healthy rats without pre-existing liver diseases that didn’t participate in a previous research experiment were included.

2.3.Exclusion Criteria

1. Pre-existing liver disease: The rats with pre-existing liver diseases or any other conditions that may affect the outcomes of the study were excluded from the study.

2. Gender, age and weight: The females, and male rats that do not fall with the specified age and weight were excluded.

3. Unwillingness to participate: rats that didn't comply with the procedures of the study and showed distress signs were excluded.

2.4.Experimental Design

Rats have been subdivided randomly into four subgroups one for control and the other three as the treatment groups and as the following Table 1.

Table 1: Experimental Design

Group number	Group name	Rats number	Categorization
1	Control	6	Didn't take any medications
2	Acetaminophen group	6	Received acetaminophen only
3	Acetaminophen + limon oil	4	Received acetaminophen and limon oil
4	acetaminophen + black seed oil	4	Received acetaminophen and black seed oil

2.5.Hepatotoxicity Induction and Biochemical Analysis

The research continues for 14 days, Acetaminophen syrups 250mg/5ml of Julphar Pharmaceuticals company UAE were used to prepare the dose used to induce toxicity, which is 500 mg|gm. Lemon oil in dose of 400 mg\1kg and Black seed oil in dose of 4 ml\1kg (each from Emad factory, Iraq) were given by gastric lavage. 24 hours following the last dosage, rats have been undergoing anesthesia by the aid of chloroform and then sacrificed by cervical dislocation. then the blood separated with centrifugation by (3000 rpm) for 10 min, the serum carefully aspirated and liver function tests for Alanine-aminotransferases (ALT) and Aspartate Aminotransferases (AST) biochemical measurements.

The biochemical analysis Using Dri-Chem- NX500 auto-analyzer (Fujifilm Corporations, Japan), were assessed in the blood samples. The liver was excised and fixed in saline formaldehyde solution for histological examination.

3. Results

3.1.Biochemical Analysis

For the measured biochemical parameters, the values are within normal range for the control group and in high levels for the acetaminophen group revealed acute liver injury and toxicity. Additionally, lemon oil and black seed administration have shown a positive result concerning the acetaminophen toxicity protective effects indicated by lowering the measuring enzymes levels and as shown in Table 2 and Fig.4.

Table 2: The Measured Parameters for The Experimental Groups

Group	GPT	GOT	TSB
Control	35.6 ±3.42 a	130.4 ± 9.76 a	0.15 ± 0.034 a
acetaminophen	74 ±3.42 b	208.6 ± 8.76 b	0.544 ± 0.24 b
Lemon oil	58 ± 8.55 ab	133 ± 54.73 a	0.3955 ± 0.14 ab
Black seed	39 ± 2.33 a	139 ± 28.29 a	0.2025 ± 0.11 a
Biochemical values have been expressed as mean ± SEM and different small letters mean the significant difference when ($p \leq 0.05$)			

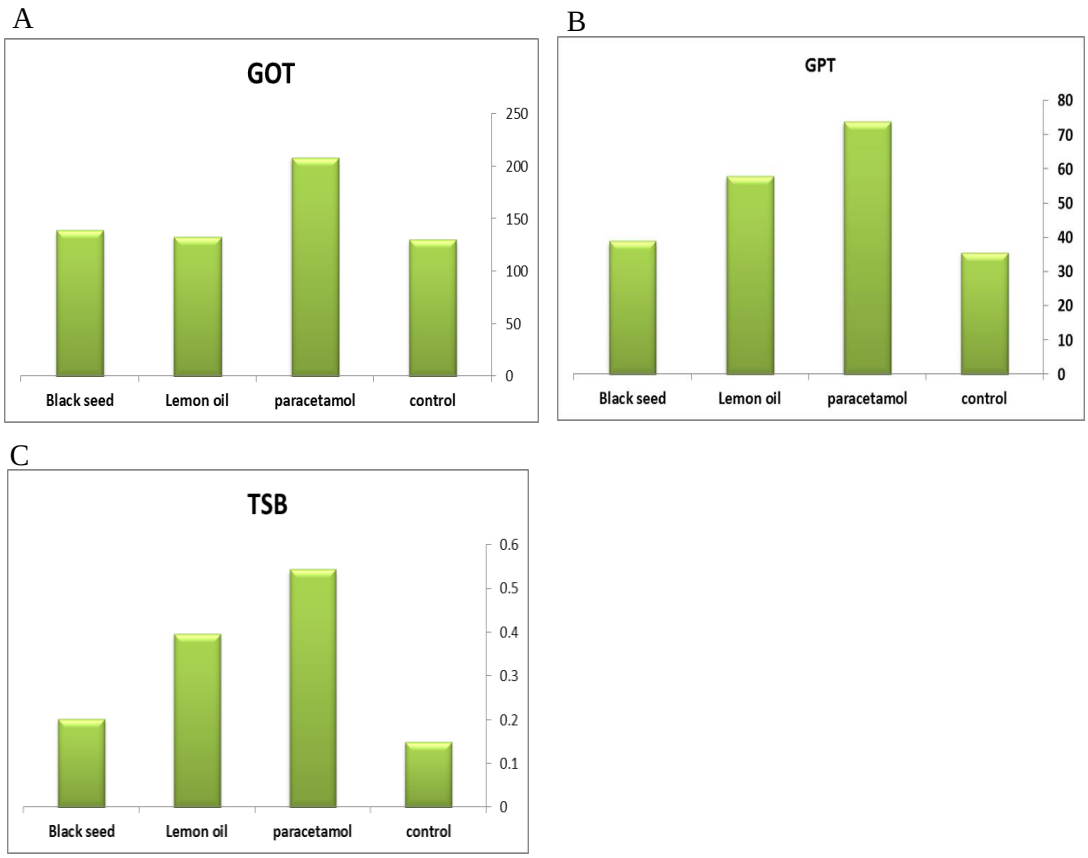

Figure 4: Presents the Measured Biochemical Parameters.
A) GOT (Glutamate Oxaloacetate Transaminase)
B) GPT (Glutamate Pyruvate Transaminase)
C) TSB (Total Serum Bilirubin)—in the experimental groups. These parameters were assessed to evaluate liver function following treatment.

3.2.Histological Analysis

The control group liver analysis showing normal tissue characteristics while the acetaminophen group section at 100 X microscopic strength presenting showed because hepatic circulatory system has been deteriorating, there is a stagnation of blood within the liver parenchyma that results in congestion and when the microscopic strength was increase to 400x, the degradation of hepatocytes was clearly appeared_some hepatocyte was with irregular cytoplasm and some hepatocyte was without nuclei which mean they exposed to necrosis because of acetaminophen toxicity.as explained by Fig.5A-B. While for rats treating with lemon oil, the histological examination showed a few cells was exposed to degeneration on 400x microscopic strength and clarified in Fig.5C. For the black seed receiving group, the histological examination showed that there is no any congestion or degeneration and look like a normal liver slide. This indicates the strength of the effect of black seed oil on protecting the liver from Acetaminophen toxicity and as shown in Fig.5D-E.

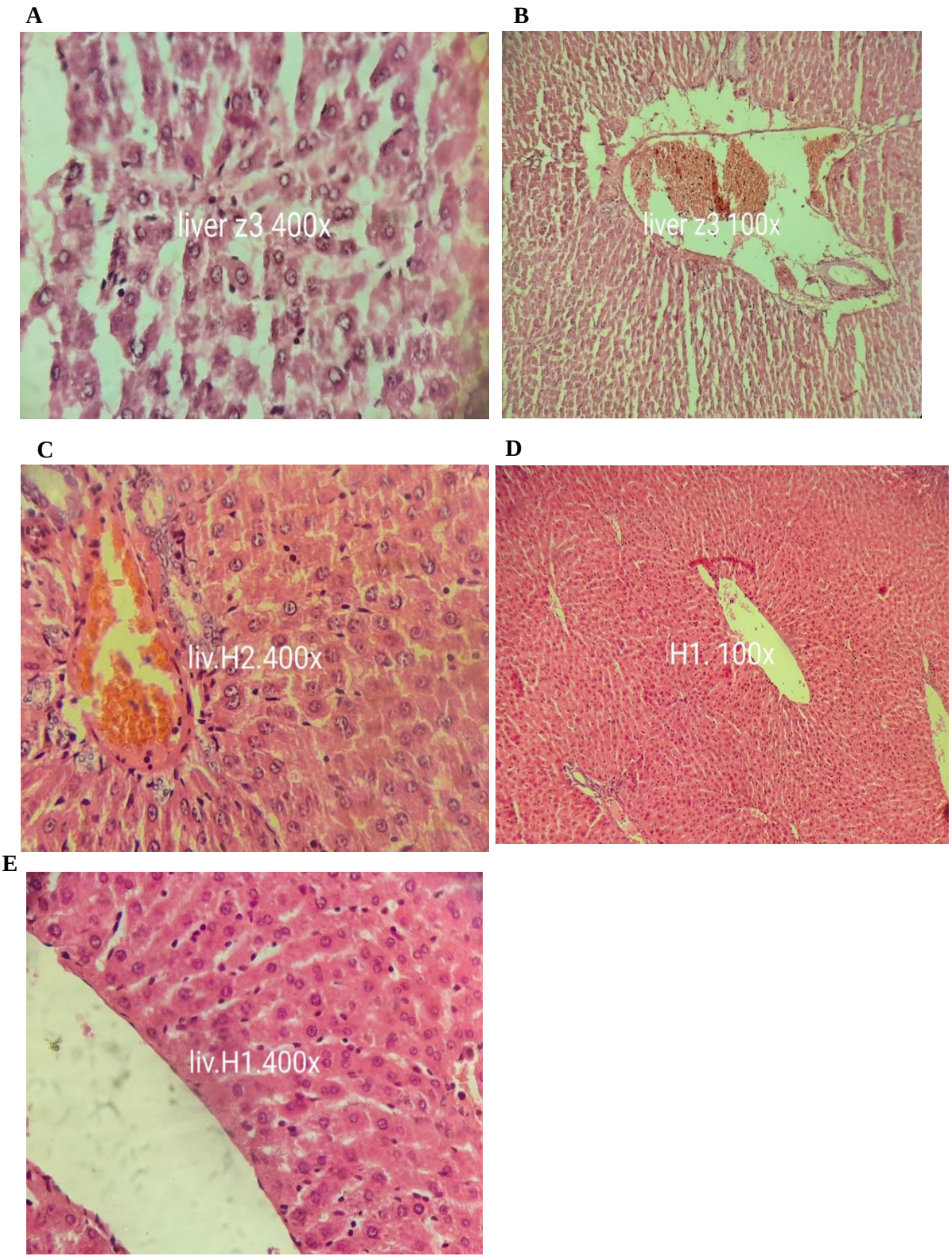

Figure 5: The Effects of Acetaminophen on The Liver of Rats in the 2nd Group. (A and B) illustrate liver histopathological changes induced by acetaminophen toxicity, including potential signs of hepatocellular damage, such as inflammation, necrosis, and cellular degeneration. The observed damage is consistent with acetaminophen-induced hepatotoxicity, which disrupts normal liver architecture. **C)** Histological analysis under reveals fewer necrotic areas and improved cellular morphology, indicating hepatoprotective properties of lemon oil. **D and E)** show improved hepatocyte integrity and fewer areas of cellular degeneration or congestion.

4. Discussion

Acetaminophen had been known as the most typical reason for sudden liver failure. Numerous over-the-counter and prescribed medications include acetaminophen and There is a substantially higher risk of overdose because to its widespread availability and comparable high toxicity when compared to ibuprofen and aspirin among the consumers.(Rotundo and Pyrsopoulos, 2020) Since then, a large number of research investigations have linked acetaminophen consumption to dose-related liver damage. When there is concurrent alcohol addiction, starving ketosis, or concurrent illnesses, these consequences are exacerbated. The microsomal cytochrome of P450 (CYP450) in hepatocyte cells breaking down acetaminophen into byproducts with harmless effect. The reactive oxygen species are yielded by this CYP450 metabolism pathway, particularly by cytochrome P450 2E1 (CYP2E1), which was initially believed to be the primary source of liver damage in acetaminophen abuse.(Ramachandran and Jaeschke, 2018) That long-held misconception has recently been disproved, and now the primary cause of the free radicals and oxidative damage in acetaminophen hepatotoxicity is thought to be mitochondrial malfunction. Reactive metabolite of acetaminophen as N-acetyl-p-benzoquinone imine (NAPQI), forms drug-protein adducts with the proteins in mitochonria that contribute to chain of electron transporting, as a first sign of mitochondrial malfunction. Furthermore, an overdose of acetaminophen results in an elevated level of activity of mitochondrial complex I, a site renowned for producing free radicals. It has been found out that the degree of activity related to the severity of liver damage. Peroxynitrite and Superoxide produced by the mitochondria are chiefly responsible for the Oxidative Stress that achieved by an extreme usage dose of acetaminophen.(Kheradpezhouh *et al.*, 2014) Main cause of oxidative and nitrosative stress is the extreme reactive peroxy-nitrite species that is created when nitric oxide and superoxide combined. When it taken by oral-route, over 80% of acetaminophen medication has a high bioavailability .(Li *et al.*, 2015) Half-life of acetaminophen in people who have not experienced hepatocyte liver damage is about 2-3 hours. 90% of acetaminophen is transformed into not harmful metabolites by both sulfidation and glucuronidation pathways at the therapeutic blood level before being eliminated through kidneys. Still, these pathways reach saturation at overdose stages, which causes CYP450 to convert significant amounts of acetaminophen into its hazardous metabolite (NAPQI). After being converted to innocuous metabolites by glutathione conjugated system, the NAPQI is subsequently eliminated .(Kadhim, Mosa and Ubaid, 2022)The main metabolic processes for acetaminophen are the glucuronidation and sulfation system. Approximately 5% of it is processed by CYP2E1. During this process, superoxide and NAPQI had been produced. NAPQI is quickly detoxified via conjugation when there is a sufficient amount of glutathione present. Glutathione is quickly reduced when there is a severe overload. NAPQI combines covalently with multiple proteins as its concentration rises, resulting in toxicity. The collapse of ATP synthesis, the onset of lactic acidosis, and the dissolution of calcium in ionized form from the mitochondrial reserves are all effects of the uncoupling oxidative phosphorylation, which is of particular interest. Hepatocellular apoptosis and necrosis are the result .(Ubaid, 2019) The loss of liver cells caused by acetaminophene excess raises the blood levels of the enzyme aminotransferases. the evaluation of particular liver enzyme levels in the serum as AST, ALT and TSB, were mostly the common used biochemical parameters for hepatotoxic screen (Ubaid, 2019)Therefore, study of serum hepatic biomarkers is crucial for identifying damage to the liver. In our study work, acetaminophene administered in (500 mg/kg) for two weeks to the rat, cause increased in the level of the transaminases and phosphatase which established

by induced acetaminophen hepatotoxicity in the experimental rats. Numerous investigations shown that antioxidant-rich plant extraction had hepatic protective effects by controlling cell permeation, maintaining cellular stability, and squelching the oxidative impacts. With our result shows pretreatment with lemon oil at dose (500 mg) exhibited a considerable reduction in level of (TSb, AST and significant decrease in level of ALT) this show that liver damage improves when it compared with acetaminophen-treated alone suggesting a protective effect of lemon oil. It is common knowledge that an essential oil incorporates the phenolic, ester, and aromatic and aliphatic acid components, by eliminating anion of superoxide, H_2O_2, hydroxy radical, and reducing the lipid peroxidation, many of these phenolic substances can protect cells. (Ubaid, 2019) Possibly, the Eriocitrin exhibits stronger anti-oxidant properties than other citrus flavonoids components. It also protects rats' livers from oxidative damage, scavenges the reactive oxygen species, and inhibits the production of both superoxide and hydroperoxide and it could be employed to boost liver tissue levels of catalase and glutathione as anti-oxidant enzymes, in addition to the presence of citrates, flavonoids, vitamin E, vitamin C, and lemonades which shown powerful antioxidant effect. Researchers noticed anti clastogenic and antimutagenic agents in vitamin C that worked as strong anti-oxidant and as scavengers for the free radicals, keeping the cells and tissues from being destroyed by them .(Quita, 2016) There are several studies concerning the effects of the lemon oils such as that introduced by Zhou, T., et al. Our update study shown that the hepatic protection effect were observed by lemon juice administered in liver damage induction in alcohol consumption mice groups by decreasing serum GOT, GPT level and this result agreed by additional study confirming the beneficial effect of citrus limon essential oil against aspirin toxic effect in rat model.(Zhou *et al.*, 2017) It was experiential in the ongoing study that Nigella sativa maintained the defense system in rats liver that exposure to acetaminophen toxicity through several mechanism: Firstly, Nigella sativa oil contains various bioactive compounds, with thymoquinone being one of the most studied and prominent. Thymoquinone has been found to possess antioxidant properties, meaning it can neutralize harmful free radicals and reduce oxidative stress in the liver. Acetaminophen-induced liver toxicity is partly mediated by the production of reactive oxygen species (ROS) and oxidative stress, which can damage liver cells. By reducing oxidative stress, thymoquinone helps protect the liver from acetaminophen-induced damage. One of the causes of oxidative stress is the accumulation of harmful lipids, measured by the lipid profile. (Ashraf *et al.*, 2011), (Abed Al-Kareem, Aziz and Ali Zghair, 2022). Secondly, Nigella sativa oil has been shown to possess anti-inflammatory effects. Acetaminophen-induced liver toxicity is associated with inflammatory response, characterized by releasing the inflammatory mediators and infiltrating the immune cells into the liver. The anti-inflammatory features of Nigella sativa oil, again attributed to compounds like thymoquinone, can help suppress this inflammatory response and reduce liver damage (El-Sayed, 2011) Additionally, Nigella sativa oil has been found to modulate various signaling pathways involved in liver health and regeneration. For example, it has been shown to upregulate the expression of certain enzymes with antioxidant effect such as glutathione peroxidase and superoxide dismutase, which can further enhance the liver's ability to combat oxidative stress. It has also been found to inhibit the activation of certain pro-inflammatory signaling molecules, such as nuclear factor-kappa B (NF-κB), which can help dampen the inflammatory response in the liver .(Hamed, El-Rigal and Ali, 2013).

5. Conclusion

Acetaminophen cause liver injury. lemon oil and black seed oil have hepatoprotective effect so administration of these natural products reduce this damage effectively in a rat model by lowering serum ALT and AST and prevent the hepatotoxicity induced by acetaminophen.

6. Limitations of The Study

The research may have some limitations to consider. Firstly, the sample size used in the study could be small, which restricts the hypothesize of these findings to a larger population. Additionally, while rats can provide useful insights, their behavior may not fully represent human behavior, so the findings may not directly apply to humans. Methodological limitations, such as the specific parameters measured and the doses administered, could also affect the study's conclusions. Secondly, it's essential to acknowledge that there may be bias or confounding factors present, and ethical guidelines regarding animal experimentation must be followed. Understanding these limitations is crucial when interpreting the study's results.

References

Abed Al-Kareem, Z., Aziz, N. D. and Ali Zghair, M. (2022) 'Hepatoprotective Effect of Coenzyme Q10 in Rats with Diclofenac Toxicity.', *Archives of Razi Institute*, 77(2), pp. 599–605. doi: 10.22092/ARI.2022.357210.1998.

Ashraf, S. S. *et al.* (2011) 'Nigella sativa extract as a potent antioxidant for petrochemical-induced oxidative stress.', *Journal of chromatographic science*, 49(4), pp. 321–326. doi: 10.1093/chrsci/49.4.321.

Chouhan, S., Sharma, K. and Guleria, S. (2017) 'Antimicrobial Activity of Some Essential Oils-Present Status and Future Perspectives.', *Medicines (Basel, Switzerland)*, 4(3). doi: 10.3390/medicines4030058.

El-Sayed, W. M. (2011) 'Upregulation of chemoprotective enzymes and glutathione by Nigella sativa (black seed) and thymoquinone in CCl4-intoxicated rats.', *International journal of toxicology*, 30(6), pp. 707–714. doi: 10.1177/1091581811420741.

Elbagerma, M. A. *et al.* (2011) 'Identification of a new cocrystal of citric acid and paracetamol of pharmaceutical relevance', *CrystEngComm*, 13(6), pp. 1877–1884. doi: 10.1039/C0CE00461H.

Guerra, S. *et al.* (2020) 'Karyotype variability of sour orange (Citrus aurantium L.) and the origin of its heteromorphic karyotypes', *Tree Genetics & Genomes*, 16, p. 78. doi: 10.1007/s11295-020-01471-x.

Hamed, M. A., El-Rigal, N. S. and Ali, S. A. (2013) 'Effects of black seed oil on resolution of hepato-renal toxicity induced bybromobenzene in rats.', *European review for medical and pharmacological sciences*, 17(5), pp. 569–581.

Hassanien, M. (2007) 'Nutritional value, functional properties and nutraceutical applications of black cumin (Nigella sativa L.): An overview', *International Journal of Food Science & Technology*, 42, pp. 1208–1218. doi: 10.1111/j.1365-2621.2006.01417.x.

Ben Hsouna, A. *et al.* (2017) 'Citrus lemon essential oil: chemical composition, antioxidant and antimicrobial activities with its preservative effect against Listeria monocytogenes inoculated in minced beef meat', *Lipids in Health and Disease*, 16(1), p. 146. doi: 10.1186/s12944-017-0487-5.

Jóźwiak-Bebenista, M. and Nowak, J. Z. (2014) 'Paracetamol: mechanism of action, applications and safety concern.', *Acta poloniae pharmaceutica*, 71(1), pp. 11–23.

Kadhim, S. H., Mosa, A. U. and Ubaid, M. M. (2022) 'Hepatorenal protective activity of Artemisia against diclofenac toxicity in male rats.', *The Pan African medical journal*, 43, p. 192. doi: 10.11604/pamj.2022.43.192.36160.

Khalil, P. *et al.* (2021) 'Proximate and sensory analysis of wheat bread supplemented with Nigella sativa oil and Nigella sativa extract', *Pure and Applied Biology*, 10, pp. 1158–1165. doi: 10.19045/bspab.2021.100122.

Kheradpezhouh, E. *et al.* (2014) 'TRPM2 channels mediate acetaminophen-induced liver damage', *Proceedings of the National Academy of Sciences*, 111(8), pp. 3176–3181. doi: 10.1073/pnas.1322657111.

Lee, W. M. (2020) 'Acetaminophen Toxicity: A History of Serendipity and Unintended Consequences.', *Clinical liver disease*, 16(Suppl 1), pp. 34–44. doi: 10.1002/cld.984.

Li, S. *et al.* (2015) 'The Role of Oxidative Stress and Antioxidants in Liver Diseases.', *International journal of molecular sciences*, 16(11), pp. 26087–26124. doi: 10.3390/ijms161125942.

Quita, S. M. (2016) 'Evaluation of lemon fruit extract as an antioxidant agent against histopathological changes induced by cyclophosphamide in the testes of albino mice.', *Electronic physician*, 8(1), pp. 1824–1831. doi: 10.19082/1824.

Ramachandran, A. and Jaeschke, H. (2018) 'Oxidative Stress and Acute Hepatic Injury.', *Current opinion in toxicology*, 7, pp. 17–21. doi: 10.1016/j.cotox.2017.10.011.

Rotundo, L. and Pyrsopoulos, N. (2020) 'Liver injury induced by paracetamol and challenges associated with intentional and unintentional use.', *World journal of hepatology*, 12(4), pp. 125–136. doi: 10.4254/wjh.v12.i4.125.

Shahid, M. A. *et al.* (2022) 'Development of antibacterial nanofibrous wound dressing and conceptual reaction mechanism to deactivate the viral protein by Nigella sativa extract', *Advances in Traditional Medicine*, 22(2), pp. 283–291. doi: 10.1007/s13596-020-00538-3.

Ubaid, M. (2019) 'Using of Omega 3 to Reduce the Toxic Effect of Antituberculosis Therapy on Hepatorenal Function and Some of Blood Parameters of Albino Male Rats', *Indian Journal of Public Health Research & Development*, 10, p. 2100. doi: 10.5958/0976-5506.2019.03868.3.

Yimer, E. M. *et al.* (2019) 'Nigella sativa L. (Black Cumin): A Promising Natural Remedy for Wide Range of Illnesses.', *Evidence-based complementary and alternative medicine : eCAM*, 2019, p. 1528635. doi: 10.1155/2019/1528635.

Zhou, T. *et al.* (2017) 'Protective Effects of Lemon Juice on Alcohol-Induced Liver Injury in Mice.', *BioMed research international*, 2017, p. 7463571. doi: 10.1155/2017/7463571.