



The Protective effect of L-Carnitine and kiwi fruit extract against benzopyrene-induced hepatotoxicity in the albino rat

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ABSTRACT

Benzo[a]pyrene, a member of the polycyclic aromatic hydrocarbons, is typically produced during the incomplete combustion of organic matter and has been found to cause liver damage. This investigation explored the protective role of L-carnitine and kiwifruit against hepatotoxicity triggered by benzo[a]pyrene in rats. The study involved albino rats, which were randomly assigned to five treatment groups, each containing eight animals. The groups included a control that received corn oil, benzo[a]pyrene (10mg/kg), benzo[a]pyrene (10mg/kg) combined with L-carnitine (200mg/kg), benzo[a]pyrene (10mg/kg) paired with kiwifruit (500mg/kg), and a combination of benzo[a]pyrene (10mg/kg), L-carnitine (200mg/kg), and kiwifruit (500mg/kg). Benzo[a]pyrene exposure resulted in elevated ($p \leq 0.05$) activities of liver enzymes ALT, ALP, TC, TG and MDA while simultaneously decreasing ($p \leq 0.05$) GSH, CAT and SOD levels in comparison to the control group. Additions of L-carnitine together with kiwifruit produced favorable changes in biochemical indicators that were modified by benzo[a]pyrene exposure. The levels of these parameters in the serum of rats treated with L-carnitine and kiwifruit were nearly identical to those observed in the control group post-treatment. The use of L-carnitine and kiwifruit to counteract the toxic effects of benzo[a]pyrene also contributed to the restoration of the liver's normal histological structure. These findings suggest that L-carnitine and kiwifruit serve as effective protective agents against benzo[a]pyrene-induced changes in the livers of albino rats.

Introduction

The liver serves as a vital organ in the human anatomy. It carries out numerous roles that aid in metabolism, immune function, digestion, detoxification, and vitamin reserves, among various other tasks[1]. This organ is deeply connected with nearly every body system, making it susceptible to numerous diseases[2]. Its main role involves regulating the flow and safety of substances absorbed from the digestive tract prior to their distribution into the systemic circulatory network[3]. The liver is essential for life; no current methods can replace liver function for an extended period, although short-term liver dialysis may be utilized. It has been established that cooking can generate hazardous compounds in foods, conditioned upon the presence of suitable precursors[4]. Among these substances, polycyclic aromatic hydrocarbons (PAH), arising from incomplete combustion, are found in various foods such as those that are charcoal-broiled or smoked[5]. The chemical substance Benzo(a)pyrene [B(a)P] exists as a category member within the polycyclic aromatic hydrocarbon (PAH) family that appears during incomplete burning of carbon-based materials such as cigarettes together with gasoline as well as wood[6]. Benzo(a)P accompanies other PAHs in cigarette smoke as well as foods cooked through grilling or broiling methods and emerges as a manufacturing by-product from diverse industrial procedures[7]. Natural levels of Benzo (a) Pyrene appear in open-air atmospheres and house air as well as selected water reserves. [8]. People who experience [B(a)P] exposure encounter multiple harmful health outcomes that produce cancer development alongside immunosuppression, teratogenic effects and hormonal disturbances[9].

L-carnitine, a compound resembling a vitamin and produced from amino acids, is employed to avert ischemic and reperfusion damage[10]. L-carnitine endogenous production starts from the metabolic combination of lysine and methionine within skeletal muscles together with heart tissue and liver as well as kidneys and brain. [11]. L-

carnitine aids the transference of fatty acids through mitochondrial membranes during β -oxidation as part of fatty acid metabolism[12]. It acts as a transporter for byproducts which arise during peroxisomal fatty acid oxidation along with α -ketoacids obtained from branched-chain amino acids, possesses antiradical and antioxidant properties, and functions as a scavenger of reactive oxygen species (ROS)[13].

Dietary supplementation with fruits now demonstrates benefits for promoting health as well as preventing and managing different diseases[14, 15]. The edible fruits grown from Kiwifruit *Actinidia deliciosa* trees can be found in the Actinidiaceae plant family[16]. The distinctive quality of kiwifruit results from both its delicious taste and its abundance of vitamin C content[17]. The antioxidant properties associated with kiwifruit stem from its biological compounds which produce active compounds[18]. Research shows a clear pattern between the total amount of polyphenols found in the samples and their antioxidant properties[19]. Different research studies show that kiwifruit contains superior polyphenol compounds when compared to other varieties of fruits[20]. Kiwifruit consists of peptides that double as anti-inflammatory agents and antioxidants[21]. The therapeutic value of Kiwi includes several recognized properties which include anti-hypertensive and antidiabetic effects and anti-carcinogenic potential and antifungal benefits and anti-asthma effects and hepatoprotective qualities and anti-platelet properties besides others[22]. Therefore, our current experiment aimed to compare the therapeutic effect of L-carnitine and kiwi fruit extract on the histological and functional changes caused by benzopyrene in male albino rats.

2. Material and Methods:

Chemicals:

The company Sigma-Aldrich (based in St. Louis) provided the purchased chemicals including Benzo[a]pyrene [B(a)P] It was dissolved in corn oil according to[23].

L-carnitine (dietary supplement):

1 mL containing 250 mg L-carnitine was purchased from a pharmacy in Ramadi, Anbar

Governorate, Iraq. Rats received 200 mg/kg. BW as previously reported [24].

Collection of Kiwi Fruit:

Kiwifruits (*Actinidia deliciosa*) were obtained from local market, Ramadi City, Al-anbar Governorate, Iraq.

Preparation of aqueous Kiwifruit Extracts

Fresh Iraqi kiwifruits were obtained from a market in (Ramadi city) and thoroughly cleansed with water and separated from the peeling skin. Cut into small pieces and dry. Once the fruits had dried completely, they were powdered, and 100 g of ground fruit was soaked in 2.5 L of distilled water before being shaken for ten hours using a mechanical shaker. The extract (combination) had been sieved through a cotton or glass wool sieve. Equal amounts of the extract were diluted with water and given orally to rats[25].

Animal Experiments:

Animals in each group (n = 8) were randomly divided into five groups: group (1) Control group, where rats were treated with corn oil for 90 days; group (2) [B(a)P] -treated group, where rats were administered orally [B(a)P] (10 mg/kg) dissolved in corn oil for 90 days; group (3) co-treatment of [B(a)P] for 30 day and then L-carnitine was administered orally (200 mg/kg) for 60 days, group (4) co-treatment of [B(a)P] for 30 day and then kiwifruit was administered orally (500 mg/kg) for 60 days, and group (5) co-treatment of [B(a)P] for 30 day and then L-carnitine + kiwifruit was administered orally for 60 days, The dosage was three times a week.

Collection of blood samples

The research terminated with a 24-hour period without food followed by euthanasia with chloroform before blood collection through cardiac puncture, then blood put in plane tube and leave in room temperature for 10-15 minutes until it clots. The blood was centrifugation at 2500 rpm for 15 minutes utilizing a centrifuge for obtain serum. The serum was preserved at temperatures (-20C°) until it was required for biochemical analyses. Liver Enzymes:

Liver function test (LFT) as serum Alanine aminotransferase (SALT) and serum Aspartate Transaminase (SAST) were measured by method [26]

Determination of lipid parameters in serum: Total cholesterol (TC) with triglycerides (TG) present in serum were checked through suitable kits from LINEAR, Spain.

Lipid peroxidation (LPO):

The double heating method [27] based on the spectrophotometric measurement of color reproduction during the reaction to thiobarbituric acid (TBA) with malondialdehyde (MDA) was used to assess thiobarbituric acid reactive substances (TBARS), which are used as markers of LPO.

Measuring antioxidant levels:

The antioxidant activity was estimated in this study which included: determination of glutathione peroxidase GSH-Px [28], Catalase (CAT) activity[29], and superoxide dismutase (SOD) activity[30].

Histological examination

After dissection of animals, liver tissues to be histologically examined were taken and fixed in 10% formalin for 24 hours and were dehydrated by ethyl alcohol with increasing concentrations (70) (80), (95), (100) and (100) %, then embedded in Paraffin. All the necessary tissues were paraffin-sectioned at 5 μ m and hematoxylin/eosin-stained. The samples were observed under an optical microscope of 400X power.[31].

Statistical Analysis

The data were analyzed with the Statistical Package for Social Science, (SPSS windows software, version 25) (SPSS Inc., Chicago, IL, USA). Results were presented as mean $\pm$ SD. The differences between study groups were analyzed by one way ANOVA (LSD). P was considered statistically significant when ≤ 0.05 .

3. Results and Discussions:

3-1: Results:

In Figure (1,2), the results of the current study showed a significant increase ($p \leq 0.05$) in the levels of the liver enzymes AST and ALT in the group that received oral benzopyrene (the second positive group) compared to the negative control group. The third group (B(a)P + carnitine) and the fourth

group (B(a)P+ kiwi extract) showed a significant decrease in the levels of the above indicators compared to the second group. The fifth group showed a decrease in the above levels, but it was not significant. Fifth group showed a decrease in the above levels, but it was not significant.

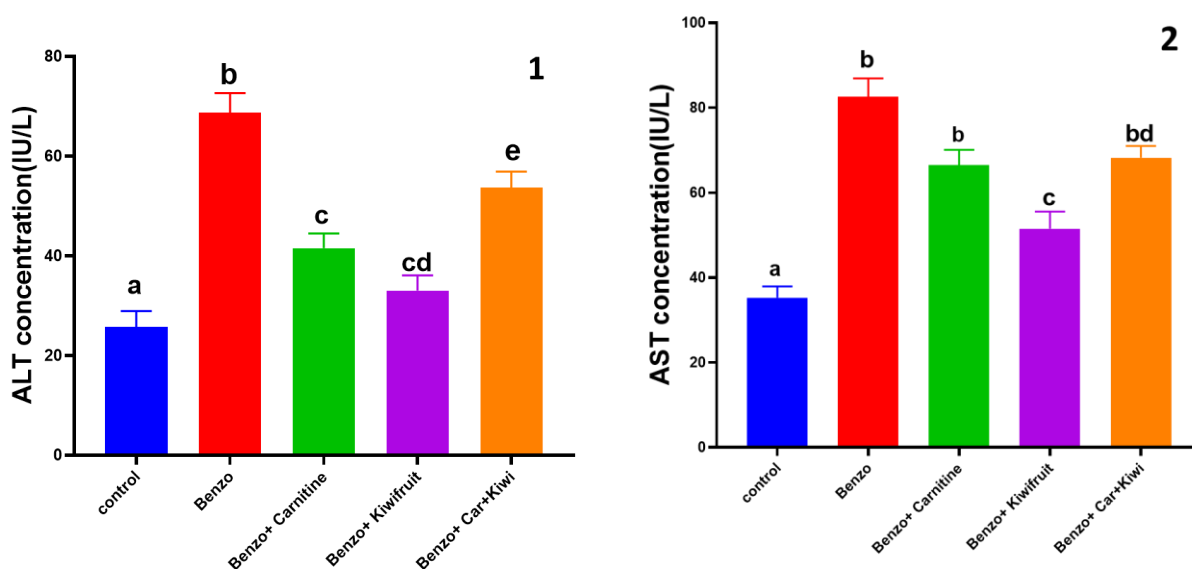


Figure (1,2): Shows the impact of L-carnitine and kiwi fruit extract on ALT and AST concentrations in male rats exposed to benzopyrene. The number of animals (eight), Different lowercase letters indicate a significant difference between groups. Similar lowercase letters indicate no significant difference.

As for the figure (3,4), the results showed a significant increase($p \leq 0.05$) in the levels of all cholesterol(TC) and triglycerides(TG) in the second positive group that was treated with benzopyrene compared to control group, while a significant decrease

was observed in these indicators in the third, fourth and fifth groups compared to the second group, noting that the fourth group gave better results than the third and fifth groups of the experiment

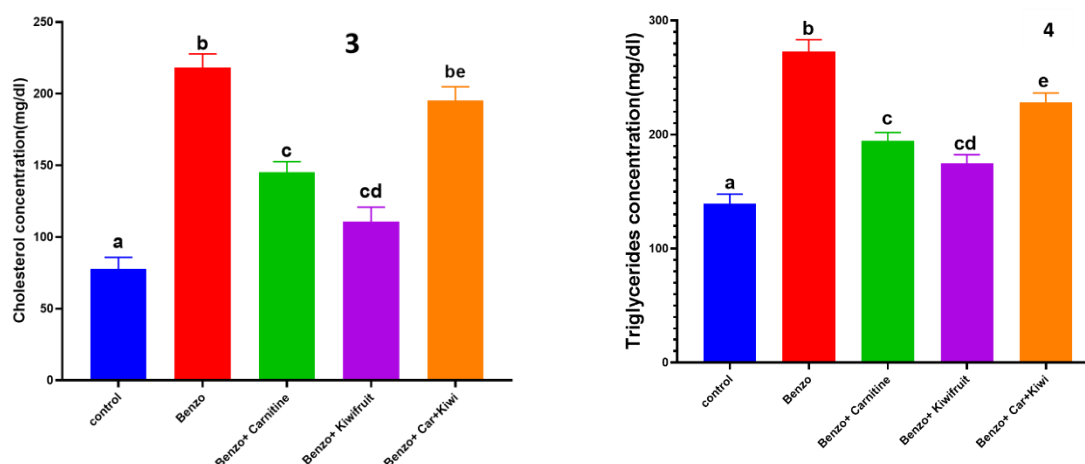


Figure (3,4): Shows the impact of L-carnitine and kiwi fruit extract on Chol and TRI concentrations in male rats exposed to benzopyrene. The number of animals(eight), Different lowercase letters indicate a significant difference between groups. Similar lowercase letters indicate no significant difference.

While the results of the second group showed a significant increase ($p \leq 0.05$) in the level of MDA and a significant decrease in the level of the studied antioxidants, which included GPX, CAT and SOD in benzopyrene group, compared to the control group, the results in the third and

fourth groups showed opposite changes relative to the treatment given to the second group although the fifth group showed no significant variation in their effects along with better results in the fourth group compared to both third and fifth groups. (Figure 5,6,7 ,8)

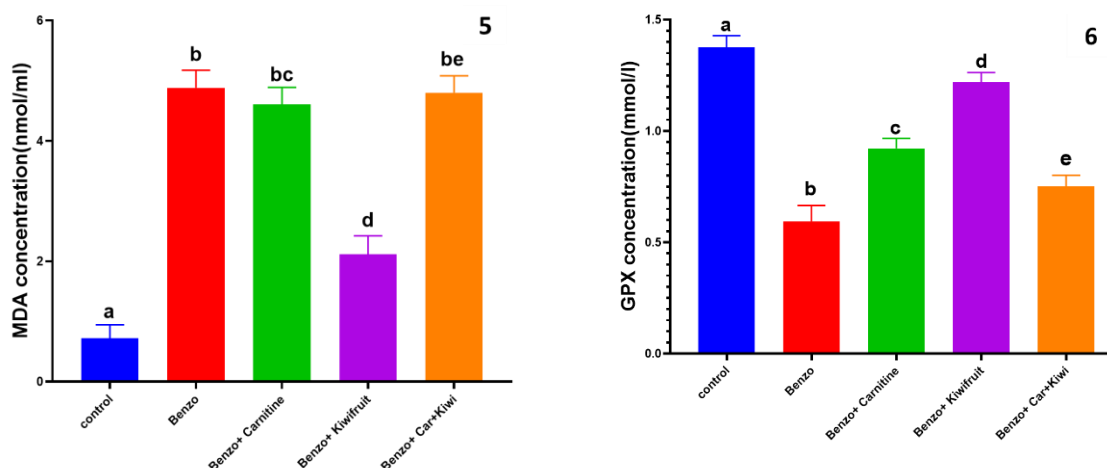


Figure (5,6): Shows the impact of L-carnitine and kiwi fruit extract on MDA and GPX levels in male rats exposed to benzopyrene. Number of animals = 8. Different lowercase letters indicate a significant difference between groups. Similar lowercase letters indicate no significant difference.

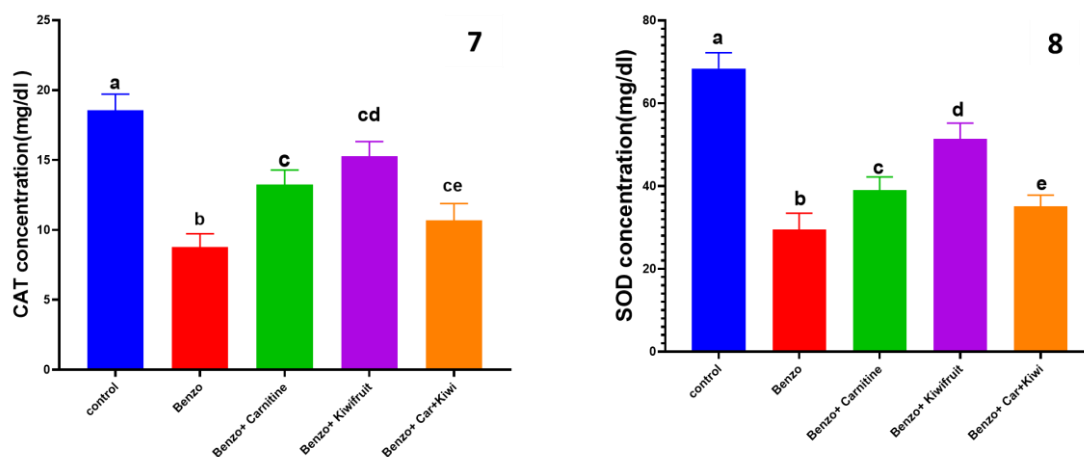


Figure (7,8): Shows the effect of L-carnitine and kiwi fruit extract on CAT and SOD enzymes levels in male rats exposed to benzopyrene. Number of animals = 8. Different lowercase letters indicate a significant difference between groups. Similar lowercase letters indicate no significant difference.

Histological results of liver sections:

The histological results showed the normal microscopic pattern of liver tissue in the control group as in the figure (9), while the second group that was orally dosed with benzopyrene showed severe histopathological changes represented by severe congestion (CON), increased wall thickness (TW), cellular degeneration (D), massive inflammatory infiltration (LI), and deposition of necrotic materials (NM) as in figures (10, 11 and 12) compared to control, while the histological pattern of hepatocytes in the third group was observed to be similar to the control group with the presence of residual histological changes which included the

presence of degeneration (D) of some cells, expansion of the sinusoids (S), and slight inflammatory infiltration (LI) as in figures (13 and 14), while the fourth group gave better results as the normal histological pattern of the liver was observed with the presence of simple histological changes represented by the presence of simple degeneration (D) of cells, sloughing of the blood vessel lining SE, and slight inflammatory infiltration (LI) as in figures (15 and 16). As for the fifth group, clear histological changes were observed represented by the lack of Hepatocyte HC regularity, dilatation of sinusoids, congestion, cell necrosis as shown in figures (17 and 18).

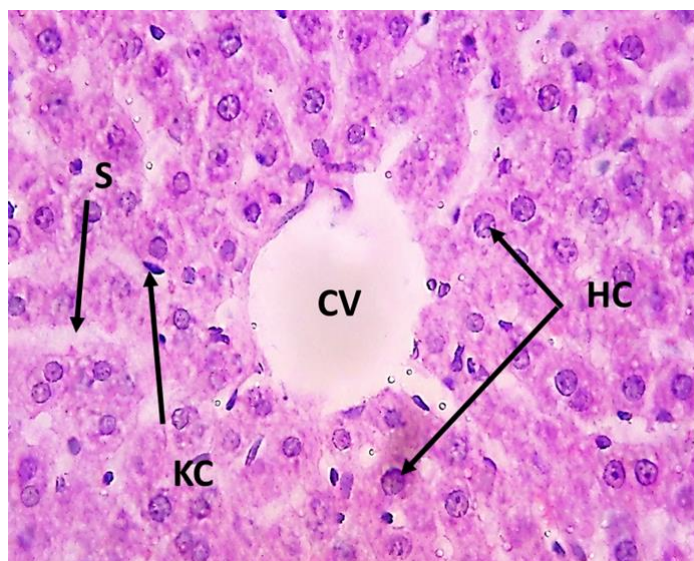


Figure (9) A cross specimen of the liver of the control group showing the central vein C.V and around it are arranged strips of hepatocytes HC and sinusoids S and inside them are kupffer cells KC (hematoxylin and eosin stain. 400X).

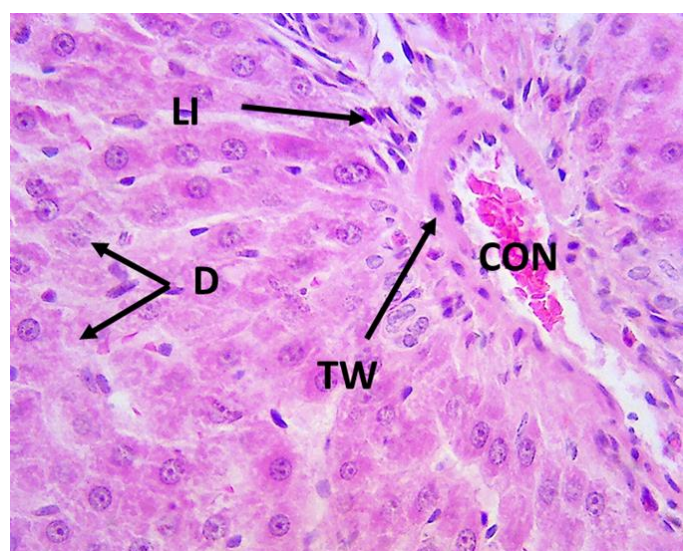


Figure (10) Cross specimen of the liver of the benzopyrine group. Congestion of the central vein (CON), thickness of the wall (TW), degeneration of D cells, inflammatory infiltration (LI) (hematoxylin and eosin stain. 400X).

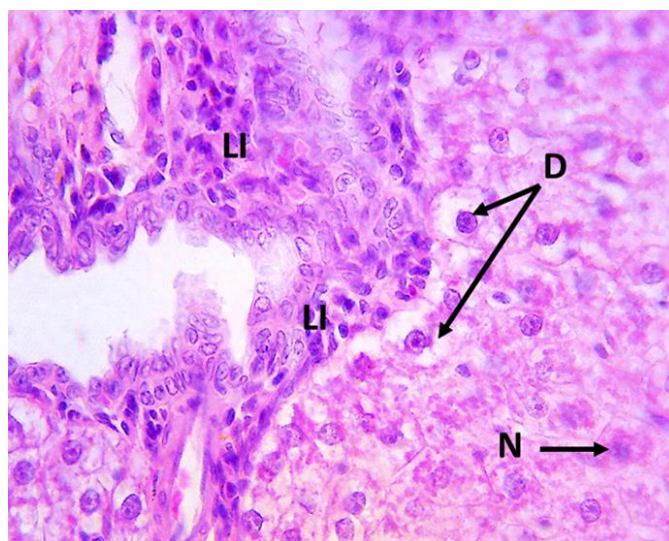


Figure (11) Cross specimen of the liver of the benzopyrene group. Severe inflammatory infiltration LI, degeneration D, necrosis N (hematoxylin and eosin stain. 400X).

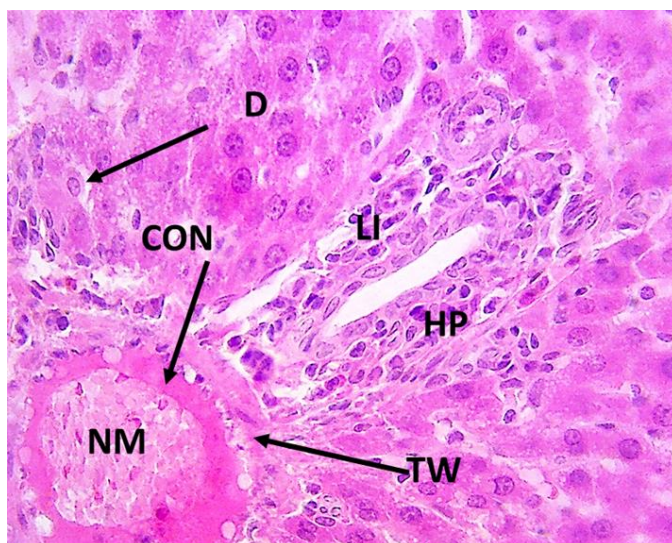


Figure (12) Cross specimen of the liver of the benzopyrene treatment. Severe inflammatory infiltration LI, degeneration D, hyperplasia HP, thickening of wall TW, congestion CON and necrotic material NM deposition in the central vein (hematoxylin and eosin stain. 400X).

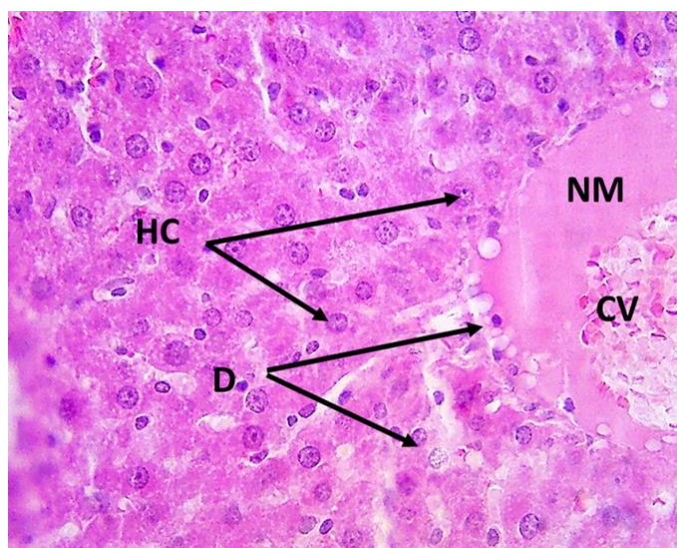


Figure (13) A cross-section of the liver of benzopyrene + L-carnitine group shows the normal arrangement of HC hepatocytes around the central vein CV, degeneration D, and deposition of necrotic material NM (hematoxylin and eosin stain. 400X).

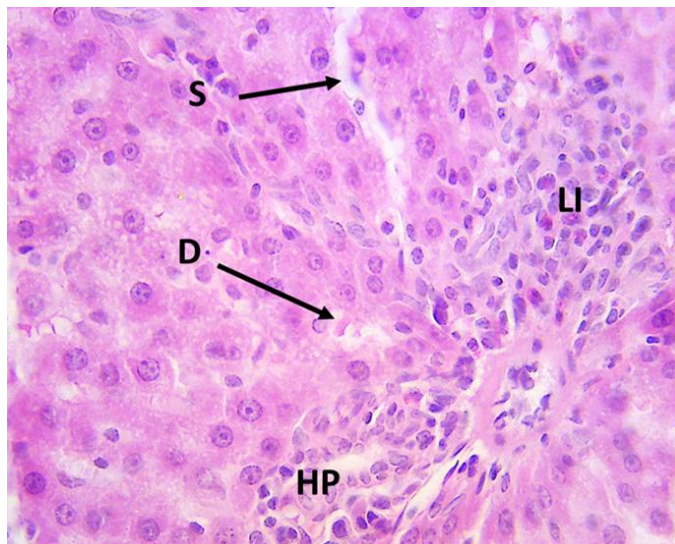


Figure (14) Cross-section of the liver of benzopyrene + L-carnitine group, showing degeneration D, inflammatory infiltration LI, dilatation of the blood sinusoids S, and hyperplasia HP (hematoxylin and eosin stain. 400X).

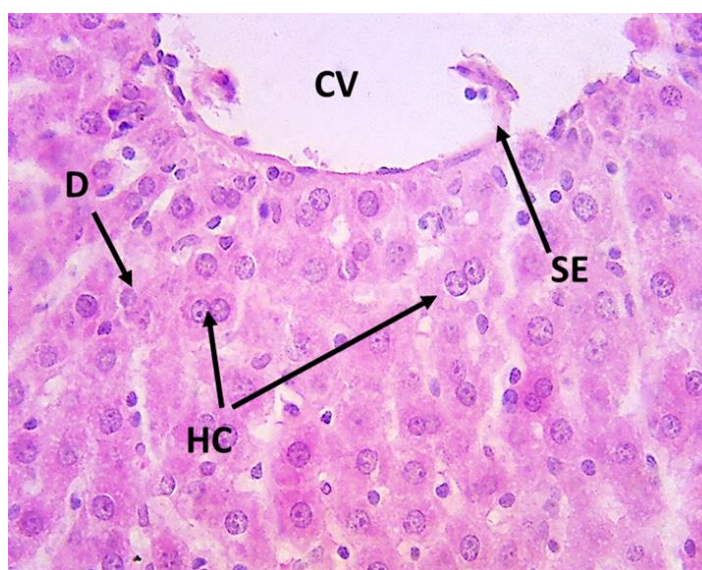


Figure (15) A cross section of the liver of benzopyrene + kiwi extract group shows the normal pattern of hepatocytes (HC) around the central vein (CV), slight degeneration (D), and endothelial sloughing (SE) in the central vein (hematoxylin and eosin staining. 400X).

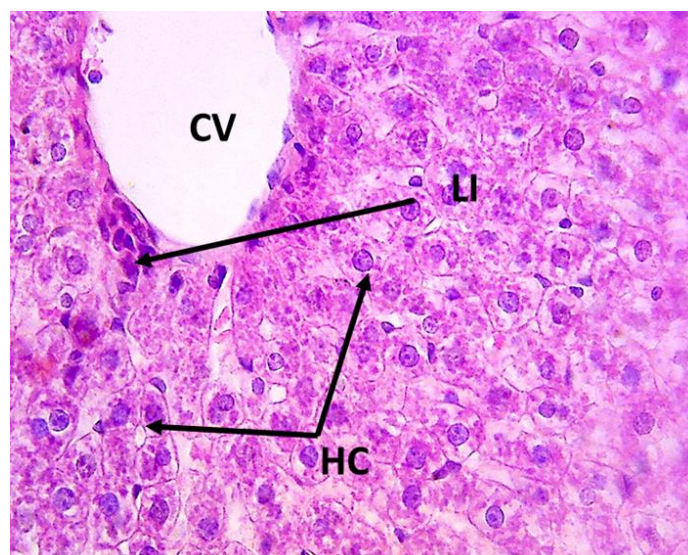


Figure (16) A cross-section of the liver of the benzopyrene + kiwi extract group shows the normal arrangement of hepatocytes (HC) around the central vein (CV), a slight inflammatory infiltrate (LI), (hematoxylin and eosin stain. 400X).

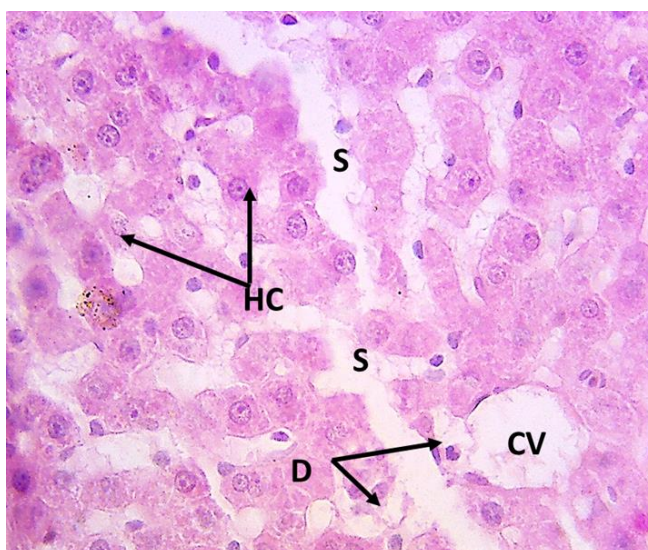


Figure (17) A cross-section of the liver of benzopyrene + carnitine + kiwi group, showing irregularity of the hepatocytes HC around central vein CV and their disintegration, degeneration D, expansion of the blood sinusoids S, (hematoxylin and eosin stain. 400X).

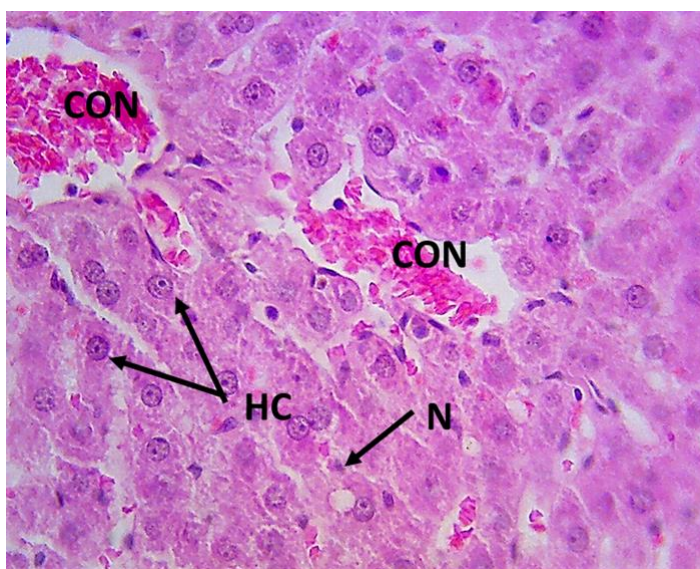


Figure (18) Cross section of the liver of the benzopyrine + carnitine + kiwi group, showing regularity of hepatocytes HC around central vein CV, cell necrosis N, severe congestion CON in the vein (hematoxylin and eosin stain. Magnification power 400X).

Discussion

Benzo[a]pyrene [B(a)P] is a type of PAHs to which humans are mainly exposed through the consumption of grilled or smoked meat[32]. [B(a)P] is recognized for its detrimental effects on cellular development, proliferation, and survival by elevating oxidative stress levels[6]. The notable rise in mean plasma ALT and AST activities observed in the [B(a)P] treated rats indicates [B(a)P]-induced liver damage and the subsequent leakage of these enzymes from the liver due to compromised plasma membrane integrity of hepatic cells into the bloodstream[33, 34]. This could also stem from an enhanced production of these enzymes due to organ dysfunction. [35]noted increased plasma transaminase levels (AST, ALT) resulting from oxidative stress, which leads to changes in membrane integrity, ultimately affecting permeability and causing a gradual decrease in intracellular enzymes. Oxidative stress, driven by elevated free fatty acids, has been closely associated with lipoapoptosis in liver cells. In our current research, the levels of TG and TC were significantly heightened in the [B(a)P] group, suggesting that liver function impairment directly impacts lipid metabolism within the body.

The L-carnitine treatment administered via intragastric route for six weeks produced marked reductions in AST and ALT and TG and TC levels in rats from the L-carnitine group thus indicating the protective effects of L-carnitine on liver damage along with lipid improvement and reduction of lipotoxicity[36]. The oxidation of lipids occurs more efficiently because of L-carnitine which limits both the hepatocytes infiltration and the elevation of triglycerides and

total lipids[37]. The protective activities of L-carnitine on free radicals and the upregulation of enzymatic/non-enzymatic antioxidants can combine to produce these effects or exist independently to generate these outcomes[38].

Post-benzo[a]pyrene exposure the mean activity levels of serum liver enzymes decreased noticeably in the kiwi fruit extract treated group because of kiwi fruit's antioxidant properties[39]. The consumption of Kiwi fruit extract leads to better functioning liver. Kiwifruit shows its potential to lower the activity levels of both ALT and AST enzymatic compounds[40]. The identified substances within kiwi fruit extract include naringin along with ferulic acid, gallic acid, rutin, caffeic acid and syringic acid and protocatechuic acid[41]. These compounds protect the liver from damage and inhibit lipid peroxidation, which protects unsaturated fatty acids in cell membranes from oxidation processes, keeping the cell contents intact, and preventing the leakage of enzymes into the blood, decrease TC and TG levels this is due to the kiwi fruit containing polyphenols, carotenoids, vitamin C, and flavonoids, which work to reduce the concentration of hepatic cholesterol by inhibiting the enzymes responsible for building cholesterol in the liver, such as HMG-CoA reductase, the metabolic processes of fatty acids remain under regulation through their activity[42, 43].

The intake of Benzo[a]pyrene leads to breakdown of antioxidant defense mechanisms. The experimental data confirms previous findings because benzo[a]pyrene strongly reduced enzyme activities of renal SOD, CAT and GPx as well as elevated MDA content

compared to control rats. The high MDA levels emerge from free radical production when rats receive [B(a)P] treatment which subsequently oxidizes cell membrane lipids[44]. The prone targets for free radical reactions in cell membranes are unsaturated fatty acids due to their double bonds acting as primary free radical targets[33]. Acute toxic reactions in the body produce MDA through free radical breakdown of fatty acids during lipid peroxidation[45]. The activities of free radical accumulation-associated enzymes decrease since it creates harm to cellular membranes by causing damage to both their structure and functionality. The exposure to [B(a)P] activates a peroxy radical that results in inactivation of GPX, CAT and SOD enzymes[46]. The probable cause for lower CAT and SOD enzyme activities exists in the rats[47]. The experimental evidence revealed that rats treated with different doses of L-carnitine before receiving [B(a)P] exposure showed substantial ($P<0.05$) recovery of changes in antioxidant enzymes[48]. L-carnitine possesses antioxidant properties thus could provide hepatoprotection through these activities which activate antioxidant mechanisms to counteract oxidative stress according to our study findings[24]. L-carnitine prevents hepatocytes enzyme infiltration[49]. Kiwi fruit extracts demonstrate the ability to manage lipid metabolism thus supporting prevention of lipid-related medical issues[50].

The efficacy of ultrasound-treated Kiwi fruit extracts [B(a)P] plays a crucial role in lowering enzyme concentrations[51]. This extract is rich in flavonoids (like quercetin, luteolin, epicatechin, and epigallocatechin gallate) and phenolic acids (including ellagitannin, caffeic

acid, and chlorogenic acid), which are naturally occurring antioxidants[52]. These compounds possess the ability to neutralize free radicals, prevent the degradation of cell membranes, safeguard liver tissue, and obstruct the leakage of enzymes into the bloodstream[53]. Scientific studies acknowledge Kiwi fruit because it contains more antioxidant polyphenols than numerous other types of fruit. The consumption of polyphenols leads to increased production of endogenous antioxidant enzymes which includes superoxide dismutase and catalase.[54, 55].

The transformation of liver tissues due to B[a]P exposure requires reactive oxygen species (ROS) as a key factor leading to histological modifications[56]. The activities of liver enzymes increased in rats subjected to B[a]P exposure indicating hepatocytes damage that resulted in cell membrane permeability[57]. B[a]P-induced membrane damage of hepatocytes led to the raised activities of these enzymes which researchers observed[23, 58]. This evidence supports the finding that L-carnitine restored normal activity levels in liver enzymes used as membrane damage indicators following B[a]P exposure[59]. Histological analysis showed degenerative liver changes in rats given B[a]P. L-carnitine functions as a hepatoprotector and therapeutic agent against B[a]P-mediated toxicity because it removes free radicals while monitoring oxidative stress transitions to establish healthy antioxidant conditions and chelates metal ions to prevent Fenton reaction occurrences[60]. Research indicates that kiwi fruit extract demonstrates three principal mechanisms which protect

damaged livers due to B[a]P by holding radical molecules while also functioning as an antioxidant and anti-inflammatory agent. The extract prevents peroxidative chain reactions in lipids through its metal-sequestering function.[20]. The bioactive compounds of kiwi use their combined effects to function as antioxidants thanks to polyphenols and flavonoids and vitamin C and carotenoids.

Conclusion:

We conclude from the results of our experiment that the therapeutic ability of both L-carnitine and kiwi extract has

a significant effect in improving most of the histological and functional variables caused by benzopyrene intake in male rats, noting that treatment with kiwi extract was better than L-carnitine, in addition to the synergistic use of L-carnitine and kiwi did not give positive results.

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الدور العلاجي للكارنيتين ومستخلص فاكهة الكيوي ضد السُمِّيَّة الكبدية المستحثة بالبنزو [a] بيرين في الجرذان البيضاء

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الملخص

يُعدُّ البنزو [a] بيرين (Benzo[a]pyrene) ، وهو أحد أفراد الهيدروكربونات العطرية متعددة الحلقات، من المركبات التي تنتج عادةً عن الاحتراق غير الكامل للمواد العضوية، وقد ثبتت قدرته على إحداث أضرار كبدية. هدفت هذه الدراسة إلى استقصاء الدور العلاجي لكلٍ من الكارنيتين (L-Carnitine) ومستخلص فاكهة الكيوي ضد السُمِّيَّة الكبدية المستحثة بالبنزو [a] بيرين في الجرذان.

اشتملت الدراسة على جرذان بيضاء وُرِّعت عشوائيًا على خمس مجموعات تجريبية، ضُمَّت كل مجموعة ثمانية حيوانات. شملت المجموعات: مجموعة السيطرة التي تلقت زيت الذرة، ومجموعة البنزو [a] بيرين بجرعة 10 ملغم/كغم، ومجموعة البنزو [a] بيرين بجرعة 10 ملغم/كغم مع الكارنيتين بجرعة 200 ملغم/كغم، ومجموعة البنزو [a] بيرين بجرعة 10 ملغم/كغم مع مستخلص فاكهة الكيوي بجرعة 500 ملغم/كغم، ومجموعة التوليفة التي تلقت البنزو [a] بيرين بجرعة 10 ملغم/كغم والكارنيتين بجرعة 200 ملغم/كغم ومستخلص فاكهة الكيوي بجرعة 500 ملغم/كغم.

أدى التعرض للبنزو [a] بيرين إلى ارتفاع معنوي ($p \leq 0.05$) في فعالية إنزيمات الكبد، وهي ناقلة أمين الألانين (ALT) والفوسفاتاز القلوي (ALP) ، وفي مستويات الكوليسترول الكلي (TC) والدهون الثلاثية (TG) والمالون ثنائي الألدريد (MDA)، بالتزامن مع انخفاض معنوي ($p \leq 0.05$) في مستويات الكلوتاثيون المختزل (GSH) وإنزيمي الكاتالاز (CAT) وفوق أكسيد الديسميوتاز (SOD) ، مقارنةً بمجموعة السيطرة.

أدى إعطاء الكارنيتين مع مستخلص فاكهة الكيوي إلى إحداث تغيرات إيجابية في المؤشرات الكيميائية الحيوية التي تأثرت بالتعرض للبنزو [a] بيرين. كما أصبحت مستويات هذه المؤشرات في مصل الجرذان المعاملة بالكارنيتين ومستخلص فاكهة الكيوي، بعد المعاملة، قريبة جدًا من المستويات المسجلة في مجموعة السيطرة. كذلك أسهم استعمال الكارنيتين ومستخلص فاكهة الكيوي في مواجهة التأثيرات السامة للبنزو [a] بيرين واستعادة التركيب النسيجي الطبيعي للكبد.

تشير هذه النتائج إلى أن الكارنيتين ومستخلص فاكهة الكيوي يُعدَّان عاملين وقائيين فعالين ضد التغيرات المستحثة بالبنزو [a] بيرين في أكباد الجرذان البيضاء.

الكلمات المفتاحية: بيروكسيداز الكلوتاثيون، الهيدروكربونات العطرية متعددة الحلقات، الضرر التأكسدي، الخلايا الكبدية