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RESEARCH ARTICLE OPEN ACCESS

The Role of Nano Lutein Compound in Mitigating the Impact of Physiological Parameters in Albino Rats Subjected to Oxidative Stress: An Experimental Study

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Abstract

Objective: This study investigated the use of the nano-lutein compound to reduce the negative effect of oxidative stress on some physiological parameters in male white rats.

Methods: Forty-eight male laboratory rats, 12–14 weeks old and 200–250 grams in weight, were used in this study, which was implemented in the animal house of the Department of Biology, College of Education, and the University of Al-Qadisiyah. The experimental animals (48 rats) were divided into six groups of eight rats per group. There were 5 experimental and one control group.

Results: The results of the current study showed a significant (P>0.05) increase in the level of urea, creatinine, and liver enzymes (ALT, AST, ALP), as well as oxidative stress indicators, and a significant (P>0.05) decrease in the level of antioxidants (glutathione, catalase, albumin) in the serum of the first treatment group (T1) under oxidative stress compared to the control and other treatments. In the fourth and fifth treatment groups, which were subjected to oxidative stress and normal or nano-lutein was administered, there was a clear improvement and significant (P>0.05) decrease in liver enzymes, kidney function indicators, and indicators of oxidative stress, and significant (P>0.05) increase in the level of serum antioxidants compared with treatment (T1).

Conclusion: White rats were administered nano-lutein, which exhibited significant efficacy in mitigating the detrimental impacts of oxidative stress on hepatic and renal function. The enhanced bioavailability and higher tissue penetration may elucidate its superior activity when compared with conventional lutein.

Keywords: Nano-lutein compound, Antioxidants, Liver, Kidney, White rats

Plain English Summary

This study assessed the use of a lipophilic (nano-lutein) compound to reduce the negative effect of free radical imbalance and antioxidants on some measurable characteristics that reflect the functions and processes in male white rats. Forty-eight male laboratory rats aged 12- 14 weeks and weighed 200- 250 grams were used for the experiment. The experimental animals were divided into six groups; a group consisting of 8 rats. There were 5 experimental and one control group. White rats administered the lipophilic (nano-lutein) compound exhibited significant efficacy in reducing the detrimental effect of free radicals imbalance and antioxidants (oxidative stress) on liver and kidney functions.

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Background

Phytochemicals are biologically active non-nutrient compounds found in fruits, vegetables, and cereals, and they are often associated with disease prevention (1). Active plant compounds have a variety of biologically active compounds of relevance to nutrition and health, including phytochemicals and secondary metabolites that are accountable for their therapeutic and preventative properties. The majority of these compounds possess polyphenols, flavonoids, terpenoids, and carotenoids which are characterized by their anti-inflammatory and antioxidant properties (2). Among these active compounds are carotenoids, which are lipophilic natural pigments that give fruits and vegetables their vibrant colours. Humans and animals do not produce carotenoids, but access them through their diets (3). The molecular structure of carotenoids primarily dictates their functions and properties because carotenoids are essentially hydrocarbons with acyclic (e.g., lycopene and phytoene) and cyclic (alpha- and beta-carotene) forms (4). Chemically, carotenoids can be classified into hydrocarbon carotenoids (e.g., β-carotene and lycopene) and xanthophylls (e.g., lutein) depending on the absence or presence of oxygen atoms in their molecules (5).

Lutein is a xanthophyll carotenoid, hydrophobic, and is commonly present in dark green leafy vegetables and commercially extracted from marigold flowers (Tagetes erecta). It is a nutritionally essential organic pigment with a molecular formula $C_{40}H_{56}O_2$ and a molecular weight of 568.871 g/mol. It gives a yellow colour to vegetables and fruits and is abundantly found in parsley, spinach, kale, and egg yolk (5). Lutein has various beneficial health effects, including antioxidant, anti-inflammatory, antihypertensive, antidiabetic, anti-ulcer, anticancer, and cardiovascular protective effects (6). Its hydroxyl group-containing chemical structure, one on each end, makes it an excellent RNS and ROS scavenger and is responsible for its antioxidant activity and most of its biological activities, thus explaining its free radical neutralizing ability (7).

This study aimed to investigate the use of the nano-lutein compound to reduce the negative effect of oxidative stress on some physiological parameters in male white rats.

Materials and Methods

Lutein material was procured from pharmacies in Al-Muthanna province, produced by Newgate (UK), 30 tablets (each 50 mg/kg). The dose of regular lutein material (48 mg/kg body weight) was selected based on the findings of prior studies showing its effectiveness on certain bodily functions, while the dose of nano-lutein loaded onto zinc oxide nanoparticles was 24 mg/kg.

Experimental Animals

Adult male lab rats were obtained from the College of Veterinary Medicine, University of Kufa, with an age range of 12–14 weeks and weighing 200–250g. Rats were kept for two weeks for acclimatization and for health status maintenance. The study was carried out in the animal house of the Department of Biology, College of Education, University of Al-Qadisiyah.

Studied Parameters

The study parameters included the following:

1. Concentration of urea: Measured in serum using the enzymatic method with a ready kit from BioMerieux (France) (8).
2. Concentration of creatinine: Measured in serum using the colorimetric method with protein deproteinization using a kit from RANDOX (UK) (9).
3. Activities of AST and ALT enzymes: Measured using kits from Giesse (Italy) and the colorimetric method (10).
4. Alkaline phosphatase (ALP) activity: Measured enzymatically using Biomerieux kits (France) (11).
5. Malondialdehyde (MDA) concentration: Estimated as per the modified method to quantify lipid peroxidation products by measuring the reaction between MDA and thiobarbituric acid (TBA) in an acidic medium with absorbance at 532 nm (12).
6. Glutathione (GSH) concentration: Estimated as per the modified method (13) using Ellman's reagent.
7. Albumin level: Estimated by utilizing a kit from Biolabo (France) (14).
8. Activity of catalase (CAT) enzyme: Determined by adopting the method of Hadwan (2018) (15), depending on the variation in absorbance as hydrogen peroxide is converted to water and oxygen by the action of catalase.

Experimental Design

Forty-eight rats were distributed into six groups (8 rats each) for 45 days as follows:

1. Control group (C): Received normal drinking water during the experiment.
2. First treatment (T1): Received 1% hydrogen peroxide in drinking bottles.
3. Second treatment (T2): Received normal lutein at 48 mg/kg body weight.

4. Third treatment (T3): Received nano-lutein at 24 mg/kg body weight.
5. Fourth treatment (T4): Received 1% hydrogen peroxide + normal lutein at 48 mg/kg body weight.
6. Fifth treatment (T5): Received 1% hydrogen peroxide + nano-lutein at 24 mg/kg body weight throughout the 45-day experiment.

Results

Kidney function (urea and creatinine level) in serum

Results in Table (1) revealed that there was a significant increase (P>0.05) in the level of urea and creatinine in the first treatment (T1) which was under oxidative stress using hydrogen peroxide compared to the control (C) and other groups. On the other hand, there were no significant differences (P>0.05) in both the second (T2) and the third (T3) treatments compared to the control, as well as to each other. However, the fourth (T4) and fifth (T5) treatments, which were exposed to oxidative stress and administered normal or nano-lutein, showed a clear improvement and a non-significant increase (P>0.05) compared to the control, second (T2), and third (T3) treatments, but a showed significant decrease (P<0.05) when compared to the first treatment (T1). There were no significant differences when T4 and T5 were compared with each other, although the reduction in the level of urea and creatinine in T5 was evident.

Table 1: The role of standard and nano lutein compounds on the concentration of kidney enzymes in white rats exposed to oxidative stress induced by hydrogen peroxide

Groups	Parameters	
	Urea Concentration (mg/dL)	Creatinine Concentration (mg/dL)
Control (C)	4.09±0.25 B	1.09±0.04 B
T1 (1% H_2O_2)	7.43±0.77 A	2.20±0.27 A
T2 (Standard Lutein 48 mg/kg)	3.60±0.55 B	1.11±0.06 B
T3 (Nano Lutein 24 mg/kg)	3.41±0.36 B	1.01±0.05 B
T4 (H_2O_2 + Standard Lutein)	4.89±0.28 B	1.28±0.27 B
T5 (H_2O_2 + Nano Lutein)	3.97±0.86 B	1.16±0.33 B
LSD	1.44	0.53

•The values are Mean ± Standard Error (SE); •Different letters indicate significant differences (P<0.05) among groups; •Same letters indicate no significant differences (P>0.05) among groups

Group descriptions: •C (Control Group): Rats who were given normal drinking water throughout the entire 45-day experimental period.; •T1 (First Treatment): Rats exposed to oxidative stress induced by 1% hydrogen peroxide administered via drinking bottles throughout the entire 45-day experimental period.; T2 (Second Treatment): Rats treated with standard lutein compound at 48 mg/kg body weight throughout the 45-day experiment; • T3 (Third Treatment): Rats treated with nano-lutein compound at 24 mg/kg body weight throughout the 45-day experiment; • T4 (Fourth Treatment): Rats treated with 1% hydrogen peroxide + standard lutein at 48 mg/kg body weight throughout the 45-day experiment; • T5 (Fifth Treatment): Rats treated with 1% hydrogen peroxide along with nano-lutein at 24 mg/kg body weight over the 45-day experimental period.

Liver Enzyme Levels

Table 2 shows a significant increase (P<0.05) in levels of liver enzymes (ALT, AST, ALP) among the first treatment group (T1) when compared with that of the control group (C) and others. Statistical analysis also revealed no significant differences (P>0.05) in ALT concentration between the second (T2) and third (T3) treatments and the control group (C), and no significant differences were observed between these two treatments when compared with each other.

Moreover, the fourth treatment (T4) also showed a significant increase (P<0.05) in enzyme levels against the control group (C), but a significant decrease (P<0.05) compared to the first treatment (T1) which was exposed to oxidative stress.

In addition, the fifth treatment (T5) showed noticeable improvement, and although enzyme levels were increased, the increase was not significant (P>0.05) compared to the control group (C), second (T2), and third (T3) treatments. At the same time, no disparities in ALT and ALP content were observed on comparing T5 with T4 despite the registered decrease and correction of these enzymes in T5. However, a significant (P<0.05) decrease in the level of AST was observed on comparing T5 with T4, showing an impressive ameliorating effect.

Table 2: The role of standard and nano lutein compounds on the concentration of liver enzymes in white rats exposed to oxidative stress induced by hydrogen peroxide.

Groups	Parameters		
	ALT Concentration (IU/L)	AST Concentration (IU/L)	ALP Concentration (IU/L)
Control (C)	40.40±3.16 C	33.49±1.80 C	123.80±2.55 C
T1 (1% H_2O_2)	78.20±1.79 A	83.87±2.40 A	197.16±8.70 A
T2 (Standard Lutein 48 mg/kg)	43.73±5.67 C	33.00±1.27 C	124.74±3.32 C
T3 (Nano Lutein 24 mg/kg)	±3.7576.38 C	33.66±1.95 C	125.03±2.61 C
T4 (H_2O_2 + Standard Lutein)	59.87±2.34 B	57.56±6.32 B	148.27±4.13 B
T5 (H_2O_2 + Nano Lutein)	±6.7157.49 BC	40.88±1.92 C	136.33±5.89 BC
LSD	10.82	7.88	12.65

Oxidative Stress Markers and Antioxidant Indicators

The statistical analysis results shown in Table 3 revealed a significant increase ($P<0.05$) in malondialdehyde (MDA) concentration and a significant decrease ($P<0.05$) in the concentrations of antioxidants (glutathione, catalase, and albumin) in the serum of the first treatment (T1) group compared to the control (C) and other treatments. In contrast, the second (T2) and third (T3) treatments showed no significant differences ($P>0.05$) compared to the control group (C) in MDA, catalase, and glutathione levels, and no significant differences were observed between T2 and T3 in MDA and catalase levels. However, both T2 and T3 showed a significant decrease ($P<0.05$) in albumin concentration compared to the control group (C).

Furthermore, the fourth (T4) and fifth (T5) treatments, which were exposed to oxidative stress and administered regular and nano-lutein, respectively, showed a significant increase ($P<0.05$) in antioxidant levels compared to the control group (C). Both treatments also exhibited a significant decrease ($P<0.05$) in MDA concentration compared to the first treatment (T1) that was exposed to oxidative stress alone, indicating a clear improvement due to the administration of regular and nano-lutein. Nevertheless, no significant differences were observed when comparing T4 and T5 to each other ($P>0.05$), although T5 (nano-lutein + hydrogen peroxide) demonstrated more noticeable improvement in oxidative stress markers and antioxidant indicators than T4.

Table 3: The role of standard and nano lutein compounds on oxidative stress markers and antioxidants in white rats exposed to oxidative stress induced by hydrogen peroxide

Groups	Parameters			
	Malondialdehyde (µmol/L)	Glutathione (µmol/L)	Catalase (IU/L)	Albumin (g/dL)
Control (C)	0.15±0.02 C	0.28±0.03 A	0.98±0.01 B	3.90±0.47 A
T1 (1% H_2O_2)	0.26±0.01 A	0.16±0.06 C	0.51±0.12 D	2.57±0.31 C
T2 (Standard Lutein 48 mg/kg)	0.13±0.02 C	0.29±0.03 A	0.99±0.08 B	3.53±0.16 B
T3 (Nano Lutein 24 mg/kg)	0.14±0.06 C	0.30±0.26 A	1.06±0.09 A	3.60±0.28 B
T4 (H_2O_2 + Standard Lutein)	0.19±0.01 B	0.27±0.04 A	0.82±0.037 C	3.76±0.40 A
T5 (H_2O_2 + Nano Lutein)	0.18±0.01 B	0.25±0.018 A	0.86±0.026 C	3.84±0.28 A
LSD	0.027	0.084	0.029	0.166

Discussion

Kidney Function Tests

The results of the present study (Table 3 and Table 4) showed a significant increase (P0.05) from the control, but a significant (P<0.05) drop in comparison to T1, which is per the study done by (16) and (17), emphasizing the protective function of lutein on kidney function.

These enhancements appear to be attributable to lutein's antioxidant properties and its ability to counteract free radicals, thereby protecting renal tissue and attenuating inflammation (18). Additionally, Lutein downregulates pro-inflammatory cytokines IL-6, IL-1β, IFN-γ, TNF-α, and apoptosis markers (Caspase-3) (19). It is noteworthy that T5 (nano-lutein) showed the best efficacy, perhaps because of its greater bioavailability (20), which increases their uptake by the cells and their antioxidant action.

Liver Enzyme Activity

The liver is the main target for toxic elements. Table 4 reveals that the formation of oxidative stress induced by hydrogen peroxide (T1) increased liver enzymes (ALT, AST, ALP) (P<0.05), reflecting hepatic toxicity, which was consistent with studies by Adefegha in 2015 and Bilgiç in 2022 (20, 21). Hydrogen peroxide produces reactive oxygen species (ROS) which are toxic to cellular components such as membrane lipids, resulting in enzyme leakage (22). ALP is a membrane-bound enzyme and is released into serum during biliary obstruction and cellular damage (23, 24). Oxidative stress also triggers hepatocyte necrosis and fibrosis, leading to additional leakage of enzymes (25, 26).

Groups T4 and T5 had reduced ALT and ALP levels (P<0.05) compared with T1 but they remained higher than control and non-stressed groups consistent with the reports of Mahdi (2024) (27) and Prahalathan (2005) (28). This enhancement was consistent with the antioxidant and anti-inflammatory effects of lutein (29) which can inhibit hepatic lipid accumulation (30), activate endogenous antioxidant pathways and reduce MDA levels (31, 32). Lutein scavenges free radicals and protects against liver toxins including CCl_4, ethanol and paracetamol and reduces lipid peroxidation and increases antioxidants such as SOD, catalase and GSH (33). These effects have been ascribed to lutein's unique structure with conjugated double bonds and hydroxyl groups that can foster electron transfer and ROS neutralization (34). The liver protective effect of nano-lutein (T5) was even greater, which may be attributed to the fact that low-dose lutein can be delivered exactly to the same position and has a better bioavailability (35).

Markers of Antioxidive Stress and Antioxidizes

As shown in Table 4, T1 shows a significant increase (P<0.05) in oxidative stress markers (MDA) and a significant decrease (P<0.05) in antioxidants (GSH, catalase, albumin) compared to control and other groups, following what had been previously published by Balboa (2024) (36) and Li (2015) (37).

Oxidative damage by hydrogen peroxide leads to the depletion of antioxidant reserves, which reduces the antioxidants available (38). H_2O_2 has also been shown to produce distinct mitochondrial ROS (39), increase lipid peroxidation (40), and deplete GSH (41). Catalase reduction represents an overload of reactive oxygen species (ROS), where H_2O_2 breakdown is impaired (42), and albumin depletion represents hepatic malfunction (43).

Although not restored completely to control levels, antioxidant levels were significantly higher and MDA was significantly lower in T4 and T5 compared to T1 (P<0.05). These results are in accordance with Rendón-Ramirez (2007) (18) and Kilicarslan You (2024) (44) which characterised lutein's antioxidant and anti-inflammatory properties (45).

Lutein has shown protective effects against kidney oxidant damage and diabetes-induced nephrotoxicity in a dose-dependent manner (18, 33) via inhibition of lipid peroxidation and upregulation of both enzymatic and non-enzymatic antioxidant defences. The most significant improvement was attributed to nano-lutein in conjunction with H_2O_2 (T5) due to increased water solubility and bioavailability leading to better internalization in the cells (45, 46, 47).

Conclusion

White rats receive nano lutein with remarkable potency against the damaging effects of oxidative stress on the function of the liver and kidney. The increased bioavailability and higher tissue penetration may explain its superior activity as compared to regular lutein (47). Thus, nano lutein may become a useful therapeutic antioxidant agent in the treatment of disorders caused by oxidative stress.

List of Abbreviation

Declarations

Ethical approval and consent to participate

Consent for publication
All the authors gave consent for the publication of the work under the Creative Commons Attribution-Non-Commercial 4.0 license.

Availability of data and materials
The data and materials associated with this research will be made available by the corresponding author upon reasonable request.

Competing interests
The authors declare that they have no competing interests.

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Authors' contributions

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References

1. Moosavi MA, Haghi A, Rahmati M, Taniguchi H, Mocan A, Echeverría J, et al. Phytochemicals as potent modulators of autophagy for cancer therapy. Cancer Letters. 2018;424:46-69. https://doi.org/10.1016/j.canlet.2018.02.030
2. Shahidi F, Chandrasekara A, Zhong Y. Bioactive phytochemicals in vegetables. Handbook of vegetables and vegetable processing. 2018:181-222. https://doi.org/10.1002/9781119098935.ch8
3. Saini RK, Prasad P, Lokesh V, Shang X, Shin J, Keum Y-S, et al. Carotenoids: Dietary sources, extraction, encapsulation, bioavailability, and health benefits—A review of recent advancements. Antioxidants. 2022;11(4):795. https://doi.org/10.3390/antiox11040795
4. Faure H, Fayol V, Galabert C, Grolier P, Le Moel G, Steghens J-P, et al., editors. Carotenoids: 1. Metabolism and physiology. Annales de Biologie Clinique; 1999.
5. Maoka T. Carotenoids as natural functional pigments .Journal of natural medicines. 2020;74(1):1-16. https://doi.org/10.1007/s11418-019-01364-x
6. Akkewar AS, Mishra KMA, Kamble MG, Kumar S, Dey J, Sethi KK. A mechanistic review on growing multiple therapeutic applications of lutein and its global market research. Phytotherapy Research. 2024;38.217-3190:(6) https://doi.org/10.1002/ptr.8197
7. Arunkumar R, Gorusupudi A, Bernstein PS. The macular carotenoids: A biochemical overview. Biochimica et b\Biophysica Acta (BBA)-Molecular and cell biology of lipids. 2020;1865(11):158617 https://doi.org/10.1016/j.bbalip.2020.158617.
8. Patton CJ, Crouch S. Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. Analytical chemistry. 1977;49(3):464-9. https://doi.org/10.1021/ac50011a034
9. Islam MA, Shahriar M, Rahman M. Studies of Neurotransmitter Mediating Enzyme Dopamine-β-Hydroxylase, Its Cofactors and Other Biochemical Parameters in the Serum of Non-Diabetic Heart Disease Patients. Bangladesh J. 2004;16(1):53-60.
10. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American journal of clinical pathology. 1957;28(1):56-63. https://doi.org/10.1093/ajcp/28.1.56
11. El-Anany AM, Ali RF. Biochemical and histopathological effects of administration various levels of Pomposia (Syzygium cumini) fruit juice as natural antioxidant on rat health. Journal of food science and technology. 20.95-50:487;13 https://doi.org/10.1007/s13197-011-0372-6
12. Mishra J, Srivastava R, Shukla S, Raghav C. Antioxidants in aromatic and medicinal plants. Science tech entrepreneur. 2007;7:1-16.
13. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Analytical biochemistry. 1968;25:192-205. https://doi.org/10.1016/0003-2697(68)90092-4
14. Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromcresol green. Clinica Chimica Acta. 1971;31(1):87-96. https://doi.org/10.1016/0009-8981(71)90365-2
15. Hadwan MH. Simple spectrophotometric assay for measuring catalase activity in biological tissues. BMC biochemistry. 2018;19:1-8. https://doi.org/10.1186/s12858-018-0097-5
16. Ibrahem MK. Effect of aqueous extract Lawsonia inermis leaves on Urea, Creatinine and Histological of kidneys in white male Rats

exposed to oxidative stress of H2O2. Tikrit Journal of Pure Science. 2017;22.(1)
17. Khudair N, Al-Okaily B. Renal ameliorating effect of resveratrol in hydrogen peroxide induced male rats. Iraqi Journal of Veterinary Sciences. 2022;36(3):571-7. https://doi.org/10.33899/ijvs.2022.130939.1898
18. Rendón-Ramirez A, Cerbón-Solórzano J, Maldonado-Vega M, Quintanar-Escorza M, Calderón-Salinas J. Vitamin-E reduces the oxidative damage on δ-aminolevulinic dehydratase induced by lead intoxication in rat erythrocytes. Toxicology in vitro. 2007;21(6):1121-6. https://doi.org/10.1016/j.tiv.2007.04.019
19. Heidari-Soreshjani S, Asadi-Samani M, Yang Q, Saeedi-Boroujeni A. Phytotherapy of nephrotoxicity-induced by cancer drugs: an updated review. Journal of nephropathology. 2017;6(3):254. https://doi.org/10.15171/jnp.2017.41
20. Adefegha SA, Omojokun OS, Oboh G. Modulatory effect of protocatechuic acid on cadmium induced nephrotoxicity and hepatoxicity in rats in vivo. Springerplus. 2015;4:1-7. https://doi.org/10.1186/s40064-015-1408-6
21. Bilgiç S, Gür FM, Aktaş İ. Biochemical and histopathological investigation of the protective effect of lutein in rat kidney exposed to cisplatin. Medical Records. 2022;4.8-433:(3) https://doi.org/10.37990/medr.1142424
22. Adefola ET, John AA, Sunday ES, Ugochukwu U. Histological and Biochemical Effects of Lutein on Paraquat-induced Renal Toxicity in Wistar Rats. 2024. https://doi.org/10.9734/ajmah/2024/v22i111117
23. Fatani AJ, Al-Rejaie SS, Parmar MY, Ahmed OM, Abuohashish HM, Ahmed MM. Lutein attenuates diabetic-induced renal damage via inhibiting oxidative and nitrosative stresses. Progr Nutr. 2017;19:57-66.
24. Emeka PM, Rasool ST, Morsy MA, Islam MIH, Chohan MS. Protective effects of lutein against vancomycin-induced acute renal injury in mice via upregulation of peroxisome proliferator-activated receptor gamma/nuclear factor erythroid 2-related factor 2 and inhibition nuclear factor-kappaB/caspase 3. The Korean Journal of Physiology & Pharmacology: Official Journal of the Korean Physiological Society and the Korean Society of Pharmacology. 2021;25(4):321-31. https://doi.org/10.4196/kjpp.2021.25.4.321
25. Dai Z, Song J, Chen Y, Feng L, Xu Y, Li D, et al. Study on the bioavailability of stevioside-encapsulized lutein and its mechanism. Food Chemistry. 2021;354:129528. https://doi.org/10.1016/j.foodchem.2021.129528
26. Ahmed-Farid OA, Rizk HA ,Shehata AM. Hydrogen peroxide modulates redox status, energy metabolism, and gene expression in a dose-and time-dependent manner in rat liver. Journal of Biochemical and Molecular Toxicology. 2018;32(10):e22199. https://doi.org/10.1002/jbt.22199
27. Mahdi SS, Al-Kennany ER. Programmed Cell Death Induced by Hydrogen Peroxide. Iraq Medical Journal. 2024;8.(3) https://doi.org/10.22317/imj.v8i3.1288
28. Prahalathan C, Selvakumar E, Varalakshmi P. Protective effect of lipoic acid on adriamycin-induced testicular toxicity. Clinica Chimica Acta. 2005;360(1-2):160-6. https://doi.org/10.1016/j.cccn.2005.04.025
29. de Vries EM ,Wang J, Leeflang MM, Boonstra K, Weersma RK, Beuers UH, et al. Alkaline phosphatase at diagnosis of primary sclerosing cholangitis and 1 year later: evaluation of prognostic value. Liver International. 2016;36(12):1867-75. https://doi.org/10.1111/liv.13110
30. Kumar V, Gill KD, Kumar V ,Gill KD. To estimate the activity of alkaline phosphatase in serum. Springer; 2018. https://doi.org/10.1007/978-981-10-8186-6_26
31. Tan HK, Yates E, Lilly K, Dhanda AD. Oxidative stress in alcohol-related liver disease. World journal of hepatology. 2020;12(7):332. https://doi.org/10.4254/wjh.v12.i7.332
32. Yu S, Liu F, Wang C, Zhang J ,Zhu A, Zou L, et al. Role of oxidative stress in liver toxicity induced by nickel oxide nanoparticles in rats. Molecular Medicine Reports. 2018;17(2):3133-9. https://doi.org/10.3892/mmr.2017.8226
33. Du S-Y, Zhang Y-L, Bai R-X, Ai Z-L, Xie B-S, Yang H-Y. Lutein prevents alcohol-induced liver disease in rats by modulating oxidative stress and inflammation. International journal of clinical and experimental medicine. 2015;8(6):8785.
34. Asiwe JN, Yovwi GD, Alawode MO, Isola T, Umukoro EK, Igbokwe VU, et al. Lutein protection against doxorubicin-induced liver damage in male Wistar rat is associated with inhibition of oxido-inflammatory stress and

modulation of Beclin-1/mTOR activities. 2024. https://doi.org/10.21203/rs.3.rs-4641525/v1
35. Prathyusha P, Viswanathan G, Tomcy AT, Binitha PP, Bava SV, Sindhu ER. Lutein and inflammation: a comprehensive review of its mechanisms of action. Exploration of Drug Science. 2025;3:100885. https://doi.org/10.37349/eds.2025.100885
36. Balboa E, Saud F, Parra-Ruiz C, De La Fuente M, Landskron G, Zanlungo S. Exploring the lutein therapeutic potential in steatotic liver disease: mechanistic insights and future directions. Frontiers in Pharmacology. 2024;15:1406784. https://doi.org/10.3389/fphar.2024.1406784
37. Li S, Ding Y, Niu Q, Xu S, Pang L, Ma R, et al. Lutein has a protective effect on hepatotoxicity induced by arsenic via Nrf2 signaling. BioMed Research International. 2015;2015(1.315205:(https://doi.org/10.1155/2015/315205
38. Kim JE, Clark RM, Park Y, Lee J, Fernandez ML. Lutein decreases oxidative stress and inflammation in liver and eyes of guinea pigs fed a hypercholesterolemic diet. Nutrition research and practice. 2012;6(2):113-9. https://doi.org/10.4162/nrp.2012.6.2.113
39. Sindhu ER, Firdous AP ,Preethi KC, Kuttan R. Carotenoid lutein protects rats from paracetamol-, carbon tetrachloride-and ethanol-induced hepatic damage. Journal of Pharmacy and Pharmacology. 2010;62(8):1054-60. https://doi.org/10.1111/j.2042-7158.2010.01123.x
40. Murillo AG, DiMarco DM, Fernandez ML. The potential of non-provitamin A carotenoids for the prevention and treatment of non-alcoholic fatty liver disease. Biology. 2016;5(4):42. https://doi.org/10.3390/biology5040042
41. Wang J, Zhu R, Sun X, Zhu Y, Liu H, Wang S-L. Intracellular uptake of etoposide-loaded solid lipid nanoparticles induces an enhancing inhibitory effect on gastric cancer through mitochondria-mediated apoptosis pathway. International journal of nanomedicine. 2014:3987-98. https://doi.org/10.2147/IJN.S64103
42. Hamad RH. Effects of bee glue on oxidant-antioxidant balance and renal funication in the male rat exposed to oxidative stress induced by hydrogen peroxide. Central Asian Journal of Medical and Natural Science. 2024;5(2):657-66.
43. Sulaiman YA, Al-saeedy K, Al-Anzy MM. Effect of aqueous extract of Asparagus officinalis on some Antioxidants in Rats Exposed to Oxidative Stress induced by Hydrogen peroxide. Tikrit Journal of Pure Science. 2017;22(5):103-9. https://doi.org/10.25130/tjps.v22i5.775
44. Kilicarslan You D, Fuwad A, Lee KH, Kim HK, Kang L, Kim SM, et al. Evaluation of the protective role of vitamin E against ROS-driven lipid oxidation in model cell membranes. Antioxidants. 2024;13(9):1135 https://doi.org/10.3390/antiox13091135.
45. Montserrat-Mesquida M, Ferrer MD, Pons A, Sureda A, Capó X. Effects of chronic hydrogen peroxide exposure on mitochondrial oxidative stress genes, ROS production and lipid peroxidation in HL60 cells. Mitochondrion. 2024;76:101869. https://doi.org/10.1016/j.mito.2024.101869
46. Varzakas T. 5 Food Waste Management. Handbook of Food Processing: Food Safety, Quality, and Manufacturing Processes. 2015:141. https://doi.org/10.1201/b19398-6
47. Hsu C-H, Chi B-C, Liu M-Y, Li J-H, Chen C-J, Chen R-Y. Phosphine-induced oxidative damage in rats :role of glutathione. Toxicology. 2002;179(1-2):1-8. https://doi.org/10.1016/S0300-483X(02)00246-9