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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 weighing 200- 250 grams were used for the experiment. The experimental animals were divided into six groups, each 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. Most 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 antioxidants, 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 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 caused 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

ALT: Alanine aminotransferase
AST: Aspartate aminotransferase
ALP: Alkaline phosphatase
MDA: Malondialdehyde

GSH: Glutathione
CAT: Catalase
IU: International Units
ROS: Reactive oxygen species
RNS: Reactive nitrogen species

Declarations

Ethical approval and consent to participate

This study was approved by the Ethical Committee at the University of Al-Qadisiyah. All animal care and use procedures were done according to ethical guidelines to ensure that the welfare of the animals was protected, minimizing pain and distress, particularly during euthanasia.

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.

Funding

Nil.

Authors' contributions

AAA: Data collection, conceptualization, methodology, data curation, formal analysis, writing original draft. AAJ: laboratory work, validation and writing – review and editing.

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