Lutein Attenuates Doxorubicin-Mediated Renal Toxicity Via Enhancement of Antioxidant Mechanism and Modulation of Autophagy Proteins.

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Doi: https://doi.org/10.21816/ijfmi.v5i1

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ABSTRACT

Background: Doxorubicin is a potent chemotherapeutic drug used for the treatment of various types of cancers. In spite of its high efficacy in the treatment of tumors, its clinical use is highly restricted due to severe adverse effects. Kidney is one of the numerous organs susceptible to doxorubicin toxicity. Several pharmacological benefits have been attributed to lutein, a natural carotenoid found in fruits and vegetables. This study evaluated the protective mechanism of lutein on doxorubicin induced renal toxicity in male Wistar rat.

Methods: Twenty male Wistar rats weighing between 130 -150g were used and they were randomly selected into four groups: Group 1 was administered with distilled water (10ml/kg i.p); Group 2 was induced with doxorubicin (15mg/kg i.p) for three consecutive daysfollowed by vehicle for 25 days; Group 3 was treated with lutein (40mg/kg i.p) for 28 days; and Group 4 was induced with doxorubicin (15mg/kg i.p) for three consecutive days and then treated with lutein (40mg/kg i.p) for 25 days.

Results: Doxorubicin administration resulted in a significant increase in serum creatinine and urea levels and a significant decrease in Na/K ATPase and H/K ATPase pump activities. It also caused a significant increase in sodium ion and a significant decrease in potassium and bicarbonate ions. Renal malondialdehyde and nitrite were increased but there was a significant decrease in GSH level as well as GST, SOD and catalase activities. A significant increase in TNF-α and IL-6 levels was observed. Renal level of caspase-3 level showed significant increase but a significant decrease in Bcl-2 level. More so, there was a significant increase in beclin-1 and a significant decrease in mTOR expression. The histological examinations revealed disruptions of the normal architectural structure of the kidneys. However, treatment with lutein significantly abated these alterations.

Conclusion: These findings demonstrated that lutein might be a therapeutic agent that can be used to treat doxorubicin associated nephrotoxicity**.** The mechanism of attenuative impact of lutein may be related to suppress oxido-inflammatory burden, inhibition of apoptosis as well as modulation of mTOR/beclin-1 activities.

Keywords: Doxorubicin, Lutein, Antioxidants, Kidney, Autophagy, Apoptosis, Inflammation.

INTRODUCTION

Vitamin-D hydroxylation, plasma potassium, calcium, and phosphorus regulation, erythropoietin secretion to maintain hemoglobin levels, plasma osmolality regulation through sodium excretion and acid-base balance regulation are just a few of the critical biological functions of the kidney Since the kidneys are the primary organ in charge of excreting many medications, they are susceptible to exposure to toxins, However, nephrotoxicity has been linked to 8% to 40% of all occurrences of acute renal damage.[1]

One of the most widely used and effective chemotherapeutic medications for treating various cancer types is doxorubicin, a cytotoxic antibiotic belonging to the anthracycline family.[2] It is believed that doxorubicin's anticancer effects stem from its capacity to intercalate into the DNA helix and/or bind covalently to proteins involved in transcription and DNA replication. DNA, RNA and protein synthesis are reduced, which ultimately leads to cell death.[2]Doxorubicin inhibits topoisomerase II activity when it kills cells, which makes transcription even more difficult.[3]Additionally, doxorubicin function by oxidatively damaging proteins, DNA, and cell membranes.[4]When taking doxorubicin, patients may experience severe adverse effects such as cardiotoxicity, neurotoxicity, hepatotoxicity, and nephrotoxicity.[5] Doxorubicin nearly completely destroys the kidneys due to their low capacity for regeneration and repair. Notably, proteinuria, glomerulosclerosis, and nephropathy are the kidneys' symptoms associated with this injury, which seriously impairs the entire body.[5] There is currently no recognized mechanism for how doxorubicin causes kidney damage.[6]However, according to a number of research, it is linked to oxidative stress.[7] Shi et al.[8]further assert that doxorubicin-induced oxidative stress results in the production of tumour necrosis factor-α (TNF-α), which in turn triggers a number of signaling pathways, including the inflammatory nuclear factor kappa-B (NF-κB) pathway. Elevated levels of the lipid peroxidation markers glutathione and malondialdehyde in renal tissues are indicative of doxorubicin-induced oxidative stress.[9] There is also a decrease in the activities of glutathione peroxidase, superoxide dismutase, and catalase.[9] Doxorubicin poisoning causes oxidative damage and inflammatory alterations in renal tissues.[4] Increased capillary porosity and glomerular shrinkage are the results of doxorubicin-induced nephrotoxicity.[9] Along with increased plasma lactate dehydrogenase activity, it is typified by raised plasma levels of urea, creatinine, and uric acid. [9]Furthermore, renal Ca2+-ATPase, Mg2+-ATPase, and Na/K-ATPase activity is reduced.[10] According to studies, doxorubicin's oxidative damage to the kidney can be avoided or reduced by using natural antioxidants.[6] Therefore, it is crucial to look into different natural sources of antioxidants.

According to Alvarado et al. [11], tetraterpenoids, also referred to as lutein ($C_{40}H_{56}O_2$), are present in eggs and dark green leafy vegetables like spinach and kale. Being an antioxidant, lutein helps shield cells from a range of illnesses, primarily those caused by oxidative stress. Lutein protects and promotes the survival and proliferation of photoreceptors by preventing hydrogen peroxide-induced apoptosis.[12] According to Liu et al. [13], lutein delays atherosclerosis by inhibiting inflammatory pathways and MDA levels.

Additionally, when lipopolysaccharide (LPS) caused inflammation in macrophages, it was found that the NF-κB pathway was inhibited and that lutein's anti-inflammatory qualities were strengthened.[14] Despite these pharmacological prospects, no study has reported the impact of lutein on chemotherapy associated renal dysfunction. Hence, the study investigated the effect of lutein on doxorubicin-induced renal injury in male Wistar rat.

Materials and Methods

Chemicals and reagents

Naman Pharma Drugs Mumbai-2 (India) provided the doxorubicin (DOX) (BDRL2302YE), while Puritan Pride Inc. provided the lutein supplement (Lutigold) (B51902-00A), which contains 40 mg of lutein and 1600 mcg of zeaxanthin.

The purchasing and care of animals

A total of 20 male Wistar rats weighing between 130 and 150 g were obtained from the animal house at Delta State University's Faculty of Basic Medical Science, Abraka, for this study. The rats were housed under standard laboratory conditions, which included 12 hours of light and 12 hours of darkness, 45 to 55% humidity, and a temperature of 25 °C. The procedures used to treat the animals were approved by the animal ethics committee of the Faculty of Basic Medical Science at Delta State University in Abraka, Nigeria, with approval number (RBC/FBMS/DELSU/24/593). They were also given unrestricted access to food and clean tap water.

Research design

The study used a randomized controlled experimental design to evaluate lutein's capacity to prevent kidney damage brought on by doxorubicin. Twenty male Wistar rats (n = 5) were randomly assigned to four groups of five animals each after a seven-day acclimatization period. As a vehicle, Group 1 received intraperitoneal injections of distilled water at a dose of 10 millilitres per kilogram of body weight for 28 days. Intraperitoneal injections of DOX (15 mg/kg body weight) were administered to Group 2 for three consecutive days followed by vehicle for 25 days, lutein (40 mg/kg body weight) was administered to Group 3 for 28 days, and doxorubicin (three consecutive days) was administered to Group 4 for 25 days, followed by lutein (40 mg/kg body weight) every day. While the lutein dosage used in this study was slightly different from earlier research by Oyovwi et al.,[15] the doxorubicin dosage was taken from the Asiwe et al. study [16]. At the end of the study, the animals were weighed and sacrificed via cervical dislocation, then after the kidney was removed, it was weighed using sensitive scales. After homogenizing the kidney, it was spined in a cold centrifuge; the supernatant was decanted in order to prepare for the biochemical test. Other kidney tissues were kept in 10% phosphate buffered formalin for histological analysis.

Biochemical assay

Determination of Serum Creatinine

We used the Bonsnes and Taussky[17]method to measure serum creatinine. The Span diagnostic kit supplied the reagents. Three millilitres of picric acid, half a millilitre of filtered water, and half a millilitre of serum were combined, properly mixed, put in a hot water bath for a minute, centrifuged with running tap water, and then promptly chilled. To make the standard, two millilitres of the supernatant were mixed with 0.5 millilitres of 0.75 N sodium hydroxide (Test), and 1.5 millilitres of picric acid were mixed with 0.5

millilitres of 0.75 N sodium hydroxide (Test). Your blank consisted of 0.5 millilitres of 0.75N sodium hydroxide, 1.5 millilitres of picric acid, and 0.5 millilitres of filtered water. Stir thoroughly, then leave for 20 minutes at room temperature. At 520 nm, the optical densities of the blank (B), standard (S), and test (T) were measured in purified distilled water.

Determination Serum Urea

The Chaney and Marbach [18] method was used to test serum urea. The AutoSpan diagnostic kit's components were used. 0.01 ml of serum was added after 1.5 ml of urea reagent had been diluted 1:5 with distilled water. thoroughly combined, then incubated at 37°C for three minutes. The reaction mixture was incubated for five minutes at 37 °C after 1.5 ml of diluted urea chromogen (sodium hydroxide and hypochlorite, 1:5 with distilled water) was added. The same procedure was used to make a standard, except instead of serum, urea standard was added. At 578 nm, absorbance was measured in comparison to a reagent blank.

Asaay of sodium-potassium (Na/K ATPase) and hydrogen-potassium (H/K ATPase) adenosine triphosphatase activities

Na/K ATPase and H/K ATPase activities were determined in the renal tissues spectrophotometrically using ELISA techniques. The assay protocol was according to the maufactuer's guideline for its respective kits

Determination of tissue lipid peroxidation

Lipid peroxidation in the tissue was assessed using malondialdehyde (MDA) and the Ohkawa et al [19] approach. The reaction mixture was maintained at 95 degrees Celsius in a boiling water bath for one hour. It contained distilled water, 0.4 millilitres of tissue homogenate, 1.5 millilitres of 0.8% TBA, and 1.5 millilitres of 20% acetic acid (pH 3.5). After the reaction mixture had cooled for an hour, one millilitre of distilled water was added. Five millilitres of the butanol:pyridine (15:1) mixture were added to the reaction tube, and everything was thoroughly mixed. It was centrifuged at 3000 rpm for 10 minutes. The clear supernatant's absorbance at 532 nm was measured in relation to the mixture of pyridine and butanol. A standard graph that was made using various doses (1–10 nmol) of 1'1'3'3'-tetra-methoxypropane in 1 millilitre of distilled water was used to calculate the MDA. Nmol of MDA/mg protein is the unit of measurement for MDA.

Determination of tissue Glutathione s-transferase (GST) activity

Tissue homogenate (approximately 0.5 mg protein) was combined with 2.5 ml of phosphate buffer (0.05 mM, pH 7) and incubated for 10 minutes at 37 °C with 0.1 ml of 1.25 mM H2O2, 0.1 ml of 25 mM NaN3, and 0.1 ml of 5 mM GST to obtain glutathione s-transferase (GST) activity. This method was created by Hafemann et al [20]. The reaction was stopped when two millilitres of 1.65% HPO32-were added to the reaction mixture. For ten minutes, the reaction mixture was centrifuged at 1500 rpm. Two millilitres of 0.4 M Na2HPO4 and one millilitre of 1 mM DTNB were used to dilute the supernatant. The yellow-colored complex was incubated against distilled water for 10 minutes at 37°C prior to the absorbance at 412 nm being measured. One sample was retained as a non-enzymatic reaction; the tissue homogenate was not supplied to it.

Determination of tissue superoxide dismutase (SOD) activity

The McCord and Fridovich[21] approach was used to perform the superoxide dismutase activity assay. 2.6 ml of phosphate buffer (67 mM, pH 7.8), 0.2 ml of 0.1 M EDTA (containing 0.0015% NaCN), and 0.1 ml of 1.5 mM NBT were combined with 0.01 ml of the homogenate in this process. The absorbance of the mixture at 560 nm was measured following the addition of 0.05 ml of riboflavin.One unit of enzyme activity was defined as the volume of the sample required to scavenge 50% of the superoxide anion generated; this value was expressed in U/mg protein. Following fifteen minutes of continuous illumination, the absorbance of the blue hue that developed in each tube was once more measured. By comparing the sample's absorbance to that of the control tube—the tube with no enzyme activity—the percentage of inhibition was determined.

Determination of Catalase activity

The catalase activity was measured using the Goth [22] technique. On a 96-well microtiter plate, a 50-L reaction mixture with varying I/L concentrations and 5 mM H2O2 in phosphate buffer (0.2 M, pH 7.4) was made. After that, it was incubated at 37 °C for five minutes. It was then incubated for 30 minutes at 37 °C with an additional 50 L of catalase solution (50 g mL-1 in phosphate buffer, 0.2 M, pH 7.4). Ammonium molybdate (64.8 mM) in 100 L was utilised to assist in reducing the enzyme's activity. Lastly, we used a microplate reader (SpectraMax M5, Molecular Devices, USA) to detect the absorbance at 405 nm.

Determination of reduced Glutathione (GSH)

Glutathione (GSH) was reduced using Ellman's [23] methods, which include the oxidation of GSH to GSSG and the enzyme-mediated hydroperoxide degradation process. 1.8 millilitres of distilled water, 0.2 millilitres of tissue homogenate, and three millilitres of precipitating agent were centrifuged at 3000 rpm, after which 4.5 millilitres of Ellman reagent were added to the supernatant in a 0.5 millilitre amount. A control mixture was prepared using the same method, and the absorbance at 412 nm within 30 minutes of the colour shift was measured using a microplate reader to compare it to the control mixture.

Nitric oxide measurement

Nitrite is the main byproduct of nitric oxide (NOx) oxidation. To determine a sample's NO level, Griess reagent was used to measure the amount of nitrite present. Asiwe et al.[24] measured the colour changes using a spectrophotometer set to 540 nm in wavelength.

Tumor necrosis factor alpha and interleukin-6 assay

The levels of interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α) in the rat kidney tissue supernatant were measured using an ELISA kit and the manufacturer's instructions. Before use, all reagents, reference solutions, and samples were allowed to reach room temperature. A microplate covered with a biotinylated antibody contained samples, standards, controls, and a streptavidin solution. After that, the microplate was incubated at room temperature for forty-five minutes. Before adding the 50 µl stop solution, each well was filled with 100 µL of TMB one-step substrate and allowed to sit at room temperature for half an hour.The unknown sample concentrations in parts per million were determined using a log–log logistic curve-fit. At 450 nm, a reading was obtained using a Spectramax M-5 (Molecular Devices, Sunnyvale, CA) multipurpose plate reader fitted with Softmax Pro v 5.4 (SMP 5.4).

Determination of serum Sodium

50ul of the serum sample and 0.1ml of NaCl filtrate were thoroughly mixed and allowed to sit for five minutes. After centrifuging the mixture for ten minutes at 1500 rpm, the supernatant was gathered. Then, 0.1 ml of diluted acetic acid, 50 ul of colourant, and 50 ul of the mixed supernatant were added. Reitman and Frankel [25]measured the absorbance at a wavelength of 550 nm.

Determination of serum Potassium

Pipetting was done using twenty microlitres of serum in the same standard, one millilitre of Reagent R in blank, and one millilitre of Reagent R in standard. After adding one millilitre of the reagent and twenty microlitres of the serum to the sample tubes, they were combined and incubated for three minutes at 37°C. The wavelength was determined at 578 nm following extensive mixing.[25]

Determination of Bicarbonate

After five minutes, 0.1 ml of the bicarbonate filtrate was thoroughly combined with 50 ul of the serum sample. After centrifuging the mixture for ten minutes at 1500 rpm, the supernatant was gathered. Next, 50 ul of the combined supernatant, 50 ul of colourant, and 0.1 ml of diluted acetic acid were added, the absorbance was measured at a wavelength of 412 nm.[25]

Determination of Protein concentration

The Biuret method, created by Reitman and Frankel[25], was used to measure the protein content of the exudates from the air pouch experiment. We combined 50 µl of the supernatant with 1.950 ml of distilled water. The contents of the test tube were also supplemented with three millilitres of the biuret reagent. After that, the sample was left to incubate at room temperature for 30 minutes. After measuring the absorbance at 540 nm, the concentration was determined using the standard curve.

Determination of B-cell lymphoma factor-2 level

We measured the kidney's B-cell lymphoma factor-2 (Bcl-2) levels using the ELISA method and the manufacturer's instructions. After filling each well with 100µL of the standard or sample, the wells were incubated at 37°C for 90 minutes. In addition, each well received 100µL of the Biotinylated detection Ab working solution, which was then incubated at 37°C for 60 minutes.

The plate was cleaned three times after the solutions were sucked up. After that, we incubated for 30 minutes at 37°C with 100µL of HRP conjugate working solution.Additionally, the solution was absorbed and performed five times. The microplate was incubated at 37°C for 15 minutes after 90µL of Substrate Reagent was added, and then 50µL of Stop Solution was added, and the microplate reader was read at 450 nm.

Determination of Caspase-3 activities.

The caspase-3 activity was measured using a colorimetric assay, per the manufacturer's instructions. In three millilitres of 10 mM phosphate buffer (pH 7.4), kidney tissues were homogenised. Prior to identifying caspase-3 activity, the peptide substrate acetyl-Asp-Glu-Val-Asp p-nitroanilide had to be hydrolysed. The procedure then released P-nitroaniline, and spectrophotometry at 405 nm was used to measure its concentration.

Determination of Beclin-1 activities

The caspase-3 activity was measured using a colorimetric assay, per the manufacturer's instructions. In three millilitres of 10 mM phosphate buffer (pH 7.4), kidney tissues were homogenised

Prior to identifying caspase-3 activity, the peptide substrate acetyl-Asp-Glu-Val-Asp p-nitroanilide had to be hydrolysed. The procedure then released P-nitroaniline, and spectrophotometry at 405 nm was used to measure its concentration. The colour will turn yellow when Stop Solution is added, and the signal strength will rise by around three times. After stopping the reaction with stop buffer, the end point was measured at 450 nm using a microplate reader.

Determination of mammalian target of rapamycin (mTOR) activities.

The activities of mTOR (mammalian target of rapamycin) were measured using ELISA techniques. After adding 100µL of the standard or sample, the wells were incubated for 90 minutes at 37°C. Each well was then filled with 100µL of Biotinylated Detection Ab working solution, and the wells were incubated at 37°C for 60 minutes. Following aspiration, 100µL of HRP conjugate working solution was added, and the plates were incubated for 30 minutes at 37°C. The mixture was then incubated at 37°C for 15 minutes after adding 90µL of substrate reagent. Finally, 50µL of stop solution was added, and the plate was immediately scanned with a microplate reader at 450 nm.

Histology

Each kidney sample was dried in alcohol, and then immersed in 10% formalin buffered with phosphate saline before being embedded in paraffin. The slide was sliced into five-micrometer pieces, deparaffinized, and then stained with eosin and haematoxylin. Any changes to the histoarchitecture were examined using the ensuing histomicrograph, which was created by imaging the stained slides 400 times larger than previously with a light microscope.[26] Furthermore, pictures of the slices at 40 and 200 times their original size were taken after they were stained for fibrosis using Masson trichrome. The cell nucleus was painted black, the fibrous tissue blue, and the cytoplasm red. Light microscopy was used to study the slices, and 200x magnification pictures were produced. The kidneys' collagen regions were measured using Image-Pro Plus (Media Cybernetics, Rockville, MD, USA), and the average collagen content of each region was calculated. Five visual fields that don't repeat were selected at random.

Statistical Analysis

The data was analysed using GraphPad Prism 8.3 (GraphPad software, San Diego, CA, USA). The data is expressed using means ± SEM. The groups were compared using the Tukey Post hoc test and one-way analysis of variance (ANOVA). $P<0.05$ was established as the cutoff point for statistical significance.

RESULTS

Lutein prevents weight alteration in doxorubicin renal toxicity.

Figure 1 shows a substantial decrease in kidney weight (%) {Time; [F (1, 12)=0.5597, P=0.4688], Column factor; [F (3, 12) = 10.27, p=0.0012], Subject [F (12, 12) = 2.700, p=0.0492], Time × column factor; [F (3, 12) = 0.02933, p=0.9929] in both the left and right kidneys of the DOX group as compared to the control group. Lutein therapy led to a significant increase in both the left and right kidney compared to the DOX group.

Lutein abates Glomerular Dysfunction

As seen in Figure 2A-B, DOX significantly increased the serum levels of urea [F (3, 12) = 10.24, P=0.0013] and creatinine [F (3, 12) = 374.1, P<0.0001] in comparison to the control group. Compared to animals given DOX, lutein treatment significantly reduced blood urea and creatinine levels.

Lutein abates tubular Dysfunction

Figure 3A-C's figures While concomitant lutein therapy considerably reduced sodium [F (3, 12) = 25.99, P<0.0001] and significantly increased potassium [F (3, 12) = 79.18, P<0.0001] and bicarbonate [F (3, 12) = 32.77, P<0.0001] ions, DOX exposure caused a considerable increase in sodium ions and a significant decrease in potassium and bicarbonate ions relative to control. Figure 3D-E shows that the DOX Group's H/K ATPase [F (3, 12) = 12.02, P=0.0006] and Na/K ATPase [F (3, 12) = 74.84, P<0.0001] activity significantly decreased when compared to the control group. The administration of lutein also increased the activity of H/K ATPase and Na/K ATPase in comparison to the DOX group.

Antioxidant effect of lutein on doxorubicin-induced oxidative stress

As Fig. 4A–D shows, DOX significantly decreased kidney catalase [F (3, 12) = 13.09, P=0.0004], SOD [F (3, 12) = 19.10, P<0.0001], GST [F (3, 12) = 14.89, P=0.0002], and GSH [F (3, 12) = 31.52, P<0.0001] levels in comparison to control, while kidney MDA [F (3, 12) = 29.02, P<0.0001] and nitrite [F (3, 12) = 19.57, P<0.0001] levels were significantly elevated following doxorubicin exposure in comparison to control. This study evaluated the effect of lutein on DOX-induced oxidative stress. Lutein administration dramatically decreased oxidative stress compared to the DOX-exposed group by raising GSH, SOD, GST, and catalase levels and decreasing MDA and nitrite levels.

Pro-inflammatory cytokine release is inhibited by lutein

As shown in Fig. 5A–B, doxorubicin exposure significantly enhances the release of interleukin-6 [F (3, 12) = 61.23, P<0.0001] and tumor necrosis factor-alpha [F (3, 12) = 11.53, P=0.0008]. However, this cytokine release was significantly decreased by concomitant lutein treatment, which stopped inflammatory reactions.

Lutein abates doxorubicin induced cell death

This study investigated the reversal effect of lutein on doxorubicin-induced apoptosis. When compared to control, rats given DOX exhibited a substantial decrease in Bcl-2 [F (3, 12) = 50.86, P<0.0001] levels and a rise in caspase-3 [F (3, 12) = 18.73, P<0.0001] levels, as seen in Fig. 6A–B. Moreover, lutein treatment markedly reduced caspase-3 activity and raised Bcl-2 in rat treated with DOX, suggesting that apoptosis was inhibited.

Lutein modulates doxorubicin associated autophagy

As shown in Fig. 7A–B, lutein treatment modulated these autophagic processes by significantly inhibiting beclin-1 [F (3, 12) = 23.02, P<0.0001] and increasing mTOR [F (3, 12) = 52.38, P<0.0001] activities when compared with animals exposed to doxorubicin, whereas doxorubicin significantly increased kidney beclin-1 activities and decreased mTOR activation when compared with control.

Effect of Lutein on Doxorubicin- Induced Fibrosis.

Figure 8 illustrates the application of Mason trichrome staining of histological specimens to identify renal fibrosis. The findings showed that doxorubicin-treated rats had increased collagen and extracellular matrix protein deposition, leading to fibrotic plague [F (3, 12) = 94.16, P<0.0001], when compared to control. However, lutein treatment significantly decreased renal fibrosis by reducing collagen deposition.

Effect of lutein treatment on kidney histology

This study found that the treatment of doxorubicin resulted in histomorphological alterations, including tubular necrosis and substantial glomerular degeneration. On Plate 9, however, the lutein therapy seemed typical in comparison to the DOX group.

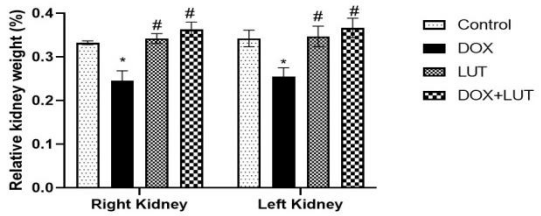

Figure 1: Lutein modulates doxorubicin induced weight alteration.

Graph is presented as mean ±SEM, n=5, *p<0.05= Significant when compared with Control Group, #p<0.05 = Significant when compared with Doxorubicin Group, DOX=Doxorubicin Group, LUT= Lutein Group, DOX+LUT= Treatment group.

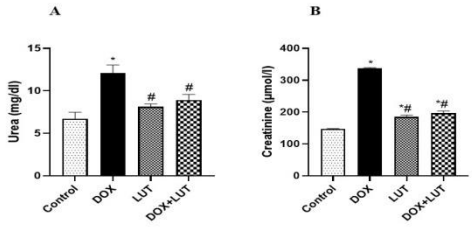

Figure 2: Lutein abates renal functional glomerular dysfunction induced by doxorubicin. (A)Serum urea (B) Serum creatinine.

Graph is presented as mean ±SEM, n=5, *p<0.05= Significant when compared with Control Group, #p<0.05 = Significant when compared with Doxorubicin Group, DOX=Doxorubicin Group, LUT= Lutein Group, DOX+LUT= Treatment group.

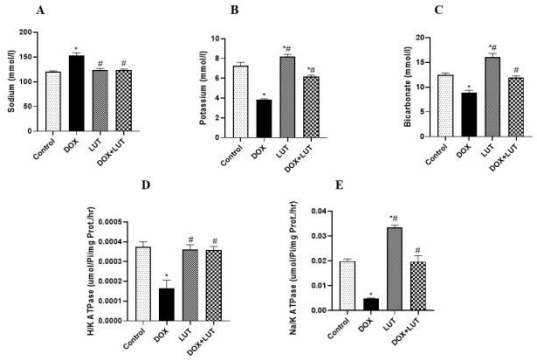

Figure 3: Lutein abates renal functional tubular dysfunction induced by doxorubicin. (A)Sodium(B)Potassium (C) Bicarbonate (D) H/K ATPase (E) Na/K ATPase.

Graph is presented as mean ±SEM, n=5, *p<0.05= Significant when compared with Control Group, #p<0.05 = Significant when compared with Doxorubicin Group, DOX=Doxorubicin Group, LUT= Lutein Group, DOX+LUT= Treatment group.

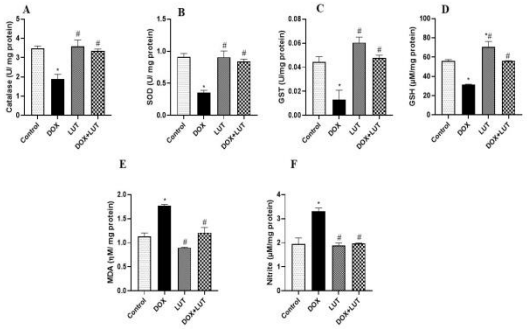

Figure 4: Antioxidant effect of lutein on doxorubicin-induced oxidative stress. (A)Catalase (B)Superoxide Dismutase (C) Gluthathione S-transferase (D) Gluthathione (E) Malondialdehyde (F) Nitrite.

Graph is presented as mean ±SEM, n=5, *p<0.05= Significant when compared with Control Group, #p<0.05 = Significant when compared with Doxorubicin Group, DOX=Doxorubicin Group, LUT= Lutein Group, DOX+LUT= Treatment group.

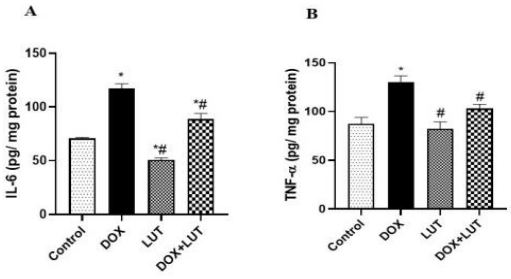

Figure 5:Lutein suppresses the release of proinflammatory cytokines. *(A)Interleukin - 6(B)Tumour necrotic factor alpha.*

Graph is presented as mean ±SEM, n=5, *p<0.05= Significant when compared with Control Group, #p<0.05 = Significant when compared with Doxorubicin Group, DOX=Doxorubicin Group, LUT= Lutein Group, DOX+LUT= Treatment group.

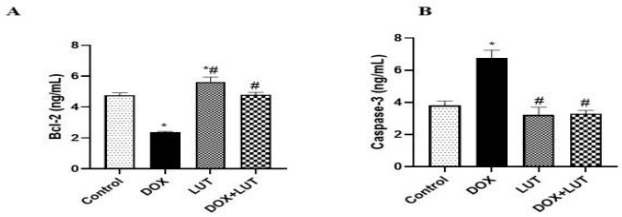

Figure: 6Lutein abates doxorubicin induced cell death. (A) B cell lymphoma factor 2(B)Caspase-3.

Graph is presented as mean ±SEM, n=5, *p<0.05= Significant when compared with Control Group, #p<0.05 = Significant when compared with Doxorubicin Group, DOX=Doxorubicin Group, LUT= Lutein Group, DOX+LUT= Treatment group.

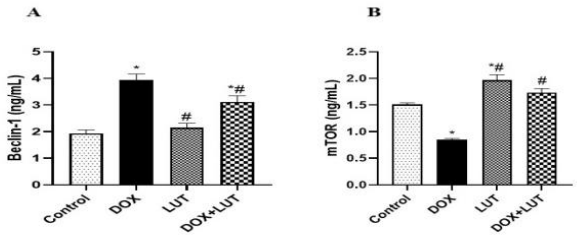

Figure 7:Lutein modulates doxorubicin associated autophagy. (A) Beclin-1(B)mTor.

Graph is presented as mean ±SEM, n=5, *p<0.05= Significant when compared with Control Group, #p<0.05 = Significant when compared with Doxorubicin Group, DOX=Doxorubicin Group, LUT= Lutein Group, DOX+LUT= Treatment group.

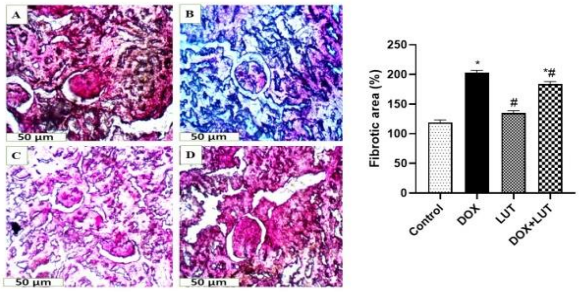

Figure 8: Effect of Lutein on Doxorubicin-Induced Fibrosis. Lutein modulates doxorubicin associated autophagy. (A) Control group(B)Doxorubicin group (C)Lutein group (D) Treatment group.

Graph is presented as mean ±SEM, n=5, *p<0.05= Significant when compared with Control Group, #p<0.05 = Significant when compared with Doxorubicin Group, The slide was stained with mason trichrome and captured at X400 magnification. DOX=Doxorubicin Group, LUT= Lutein Group, DOX+LUT= Treatment group.

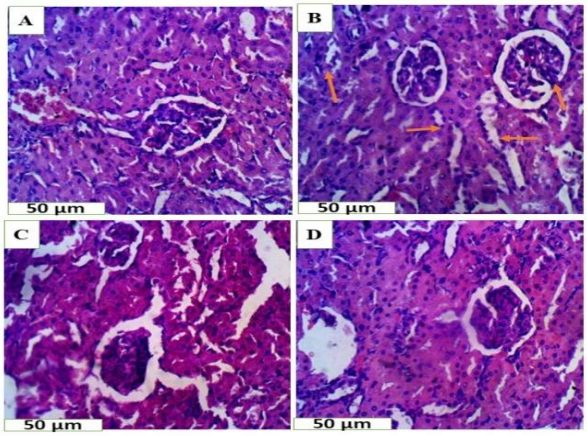

Figure 9: Effect of lutein treatment on kidney histology. Lutein modulates doxorubicin associated autophagy. Red arrows indicates areas of significant lesion on a H&E stain at X400 magnification. (A) Control group(B)Doxorubicin group (C)Lutein group (D) Treatment group.

DISCUSSION

Doxorubicin is a powerful chemotherapy medication used to treat various malignancies. Its therapeutic use is severely limited due to significant adverse effects, despite its tremendous efficiency in treating tumours.

Doxorubicin poisoning can affect a variety of organs, including the kidney.[27]However, lutein, a naturally occurring carotenoid found in fruits and vegetables, has been linked to specific pharmacological advantages.[28] The selective destruction of proximal tubule cells, the induction of oxidative stress caused by an increase in reactive oxygen species, which triggers further pathological sequelae like inflammation, apoptosis, dysregulated autophagy and histological abnormalities, are the primary causes of the detrimental effects of doxorubicin-induced nephrotoxicity, according to this study. Interestingly, lutein treatment significantly abated these aberrant functional changes.

Differences in organ weight can be utilized to determine the extent of harm produced by medicines and associated substances.[29] Acute organ damage, physiologic abnormalities, enzyme induction, and animal sensitivity to toxicity are all assessed in toxicological studies that use animal models.[16] In this study, the weight of the kidney was dramatically reduced, indicating that tissue fiber atrophy and retraction were induced by the necrotic effect of doxorubicin. It is interesting to note that kidney weight returned following lutein treatment, which could be connected to its nutritional benefits. Lutein has been shown to affect weight fluctuations and improve testicular atrophy, which is in line with the current study.[15]

The kidneys are responsible for eliminating waste products and toxins, such as urea, creatinine, and uric acid. Apart from producing hormones including erythropoietin, renin, and 1,25 dihydroxyvitamin D, they also control serum osmolality, electrolyte levels, and extracellular fluid volume. The glomerulus and tubular portion (proximal and distal tubules as well as collecting duct) comprise the nephron, the kidney's functional unit. The glomerulus selectively permits the excretion of drug metabolites, acting as a gatekeeper for toxins.

However, doxorubicin exposure in this study resulted in a significant rise in serum urea and creatinine levels, which could be a sign of a glomerulus dysfunction. Numerous studies indicate that urea, creatinine, and uric acid are helpful markers of renal failure. Interestingly, the significant increase in serum concentrations of these biomarkers strongly suggests renal damage.[15,30,31] Lutein therapy restored glomerular function by significantly lowering blood levels of creatinine and urea in comparison to the DOX-exposed group. This was consistent with previous studies that discovered lutein had a renoprotective impact on cisplastin-induced kidney damage.[32]

Electrolytes provide essential ionic charge that drives cell signalling, controls extracellular content, and promotes muscular contraction and relaxation. However, an imbalance in serum electrolytes may alter the physiology of the body.[33] In this study, the doxorubicin group exhibited a significant increase in blood sodium and a significant decrease in serum bicarbonate and potassium when compared to the control group. Optimal sodium, potassium, and bicarbonate levels are necessary for blood pressure control, heart chronotropic and ionotropic function, and acid-base balance. Specifically, exposure to doxorubicin markedly decreased the activity of the pumps that propel electrolyte transport within cells, Na/K ATPase and H/K ATPase. This contributed significantly to the electrolyte imbalance seen in this investigation. These changes in blood levels of renal biomarkers are caused by tubular cell necrosis and altered renal architecture.[34]

Curiously, lutein administration significantly increased the activities of H/K-ATPase and Na/K-ATPase, which led to a decrease in serum sodium levels and an increase in blood potassium and bicarbonate concentrations. Previous studies by Widomska et al.[35] have shown that lutein has a membrane-stabilizing effect.

Oxidative stress is a complex phenomenon that has detrimental effects on the organism through a number of different processes and on multiple levels. It is marked by an imbalance between pro-oxidants and a network system of antioxidant defenses, according to Asiwe et al.[16] It happens when the production of reactive oxygen species (ROS) overwhelms the renal intrinsic antioxidants. When ROS continuously attack renal tissues, the intricate antioxidant defense system usually keeps things in balance, preventing oxidative damage.[36] Oxidative stress triggers several intracellular signalling pathways that might lead to cell growth, apoptosis, or inflammation, ultimately resulting in organ failure.[37] This study showed a large rise in pro-oxidants malondialdehyde, which is a byproduct of lipid peroxidation, and nitrite, which is a byproduct of nitric oxide oxidation. Several studies have shown that an increase in MDA levels stimulates leukocyte recruitment and NF-kB expression in tissues exposed to harmful metabolites.[38-40] Additionally, it has been demonstrated that cell membranes are harmed by peroxynitrite, which is produced when nitric oxide and superoxide anion combine.[41] Remarkably, it has been shown that when glutamate and NF-kB work together, high nitrite levels aggravate kidney damage by encouraging renal excitotoxicity.[16] According to Abdel-Razek et al.[42], lutein treatment dramatically decreased renal MDA and nitrite levels, indicating that both lipid peroxidative and peroxynitrite damage was lessened. Furthermore, several studies have connected increased pro-oxidants to inadequate antioxidants.[43,44] Consistent with previous study [45], exposure to doxorubicin significantly depleted renal antioxidant defense system as evidence in significant reduction in GSH level as well as GST, SOD and catalase activities. The increase in pro-oxidants and decrease in antioxidants suggest that Doxorubicin caused oxidative stress in the renal tissue. Nevertheless, lutein administration resulted in a marked reduction in nitrite and malondialdehyde levels along with a corresponding rise in GSH, GST, SOD, and catalase activity, indicating that lutein may be able to prevent and lessen oxidative stress brought on by doxorubicin.

According to Stone et al. [46], inflammation is an essential immune system response that ensures life during infection and tissue injury. The discovery of certain molecular patterns connected to infection or tissue damage is the first step in the intricate process of understanding the molecular mechanism of inflammation.[47] Liu et al. [48] state that the entire inflammatory response process is mediated by several key regulators that are involved in the selective synthesis of pro-inflammatory chemicals. According to Antar et al.[49], doxorubicin directly damages the glomerular base membrane, podocytes, and glomerular endothelial cells, resulting in tubular interstitial inflammation and fibrosis. Fang et al.[50] claim that DOX produces cytokines and chemotactic factors locally in response to cellular injury, plasma protein filtration, and glomerular inflammatory mediators, resulting in a severe tubulointerstitial inflammation. We observed a significant increase in TNF-α and Il-6 levels following doxorubicin treatment.

According to previous studies, disruption of the cardiac lipid membrane might have triggered an immunological response that resulted in the release of pro-inflammatory cytokines such TNF-α and IL-6. [51-53] Remarkably, lutein therapy dramatically decreased IL-6 and TNF-α in renal tissues. This demonstrated that lutein had anti-inflammatory effects by preventing or reducing the release of TNF-α and IL-6 mediated by doxorubicin.

Programed cell death, or apoptosis, is characterised by distinct morphological characteristics and energy-dependent metabolic pathways.[54] This innate cell-suicide program protects the organism and ensures healthy growth by maintaining tissue homeostasis and removing defective or damaged cells that can impair normal function.[55] Apoptotic death is essential for several processes, including hormone-dependent atrophy, normal cell turnover, immune system development and function, chemically induced cell death, and embryonic development. Many diseases, including autoimmune disorders, neurological diseases, and numerous malignancies, are influenced by dysregulation of apoptotic signalling and excessive or inadequate apoptosis. Caspases play a major role in the apoptotic process.Since they really cleave cellular components during apoptosis, the term "caspases" refers to cysteine-dependent aspartate-specific proteases, which are essential to the initiation and completion of the process. When compared to the control group, the study found that rats exposed to doxorubicin exhibited a significant decrease in Bcl-2 and a large rise in renal caspase-3 activity. Doxorubicin-mediated free radical generation must have altered the DNA sequence by significantly causing uncoupling and DNA damage, which in turn damaged the structure of the renal cells. It is known that this pathology leads to the production of cytochrome-C by bax, which in turn leads to the formation of the procaspase/Apaf complex. This complex triggers the activation of caspase-9, an initiator caspase that cleaves multiple times to produce the cell executional caspase, caspase-3, and caspase-7. Nonetheless, Bcl-2 is essential for this intrinsic apoptotic pathway because it inhibits the release of cytochrome-C by bax, which regulates caspase activation.[16] The significant decrease in Bcl-2 and increase in renal caspase-3 levels suggest that doxorubicin caused apoptosis. Remarkably, lutein therapy raised Bcl-2 activities and markedly decreased kidney caspase-3 activities. In line with earlier research, the reduced caspase-3 and increased Bcl-2 activities indicate that lutein has anti-apoptotic properties.[15, 56]

Autophagy is the process by which cytoplasmic components are broken down within lysosomes.[57, 58] Autophagy is mediated by a unique organelle called the autophagosome, which absorbs a fragment of the cytoplasm. Recent studies have clearly demonstrated that autophagy has a greater range of physiological and pathological roles than previously believed. These roles include microorganism killing, development, anti-aging, tumour suppression, antigen presentation, starvation adaptation, intracellular protein and organelle clearance, and cell death.[59, 60] Maintaining the balance between cellular catabolism and anabolism requires the protein known as the mammalian target of rapamycin, or mTOR. mTOR complex 1 (mTORC1) was identified as a master regulator of autophagy because the autophagy process required mTORC1 to be suppressed.

Although lutein restored mTOR expression, which indicates homeostatic maintenance, cell proliferation, and cell survival, doxorubicin reduced mTOR expression, which is in charge of cell growth, homeostasis, and autophagy inhibition.[61] In line with the findings of Oyovwi et al.[15], the group given doxorubicin showed a large drop in mTOR expression and a considerable increase in beclin-1 when compared to the control group. However, Beclin-1, an evolutionarily conserved protein, is a well-known positive regulator of this process due to its critical role in the formation of autophagosomes, the double-membrane vesicles that hold degradation targets and merge with lysosomes. However, lutein therapy corrected the negative effects of doxorubicin by restoring autophagic marker expression, which normalised the autophagic process. The rise in beclin-1 in doxorubicin-treated rats indicates an over-increasing autophagic flux.

Significant kidney tissue distortion, including glomerular deterioration, tubular necrosis, and inflammatory cell infiltration, was brought on by doxorubicin treatment, which is consistent with previous studies.[62, 63] However, these histoarchitectural abnormalities were significantly lessened by lutein therapy. Furthermore, the doxorubicin group's increased collagen depositionwhich leads to the development of fibrotic plague was noticeable more significant than that of the control group. Interestingly, fibrosis was significantly reduced during lutein administration, as seen by the normal appearance of the renal tissues.

Conclusion

Finally, this investigation linked doxorubicin to renal impairment, which was demonstrated by elevated creatinine, urea, and sodium levels accompanied by a proportional decrease in potassium, bicarbonate, Na/K ATPase, and H/K ATPase. Additionally, the DOX group was linked to oxidative stress, which showed up as increased MDA and nitrite and decreased renal antioxidants like catalase, SOD, GST, and GSH. This was accompanied by an increase in caspase-3 activities, beclin-1, and fibrosis alongside a decrease in Bcl-2 and mTOR. Significant modifications in histoarchitecture were also seen. Interestingly, these pathological alterations were considerably reduced by lutein therapy. These results therefore showed that lutein may be a therapeutic drug that can be used to cure or prevent nephrotoxicity linked to doxorubicin. The attenuative effects of lutein may be linked to apoptosis inhibition, mTOR/beclin-1 activity modification, and a decreased oxido-inflammatory stress.

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