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The Protective Role of *Nigella Sativa* Oil-Loaded Polycaprolactone/ Chitosan/ Electrospam Nanofibers against Nephrotoxicity in Rats Exposed to Etbr

Nawras A. Jaleel[1], Haneen N. Hasan[2], Jabbar A. A. Al-Saaidi[3*]

[1]*Department of Physiology, Pharmacology, and Biochemistry, College of Veterinary Medicine, University of Al-Qadisiyah, Iraq. nawras@institution.edu. ORCID: 0000-0003-2022-228*

[2]*Department of Physiology, Pharmacology, and Biochemistry, College of Veterinary Medicine, University of Al-Qadisiyah, Iraq. vet.post22.15@qu.edu.iq. ORCID: 0009-0004-6725-212x.*

[3]**Department of Basic Sciences, College of Dentistry, Al-Ayen Iraqi University, Iraq. Email: jabbar.alsaaidi@alayen.edu.iq. ORCID: 0000-0003-2022-2287*

Abstract

Ethidium bromide's (EtBr) toxic effects have the potential to spread to renal cells, which could lead to cellular dysfunction and death. Numerous potent active compounds with significant therapeutic benefits found in nigella sativa oil (NSO). An efficient technique for producing nanofibers is electrospinning, which stirs polymer solutions using a strong electrical source. The current study aims to fabricate nanofibers from polycaprolactone and chitosan loaded with NSO and examine its nephro-protective effects against EtBr toxicity in male rats. For this purpose, six groups (16 males each) were daily dosed with distilled water (T1), EtBr (T2), PC/NF (T3), a combination of PC/NF and EtBR (T4), PC/NSO/NF (T5), and a combination of PC/NSO/NF and EtBR (T6) for 30 days. Two subgroups were created from each group, and each received treatment for either 15 or 30 days prior to being sacrificed. Following both treatment periods, kidney histology and serum renal function profile were examined. Significant increases in kidney relative weight and renal function markers were noted in the EtBr group. Notably, PCNSONF therapy markedly increased these markers in comparison to the EtBr group. Furthermore, compared to the EtBr group, which exhibited notable cellular necrotic and degenerative changes, the PCNSONF group had structural improvement in kidney histological features and semi-quantitative analysis. In conclusion, manufactured PCNSONF demonstrated improved prophylactic effectiveness against EtBr-induced renotoxicity and an improved serum kidney function profile. Ethidium bromide's impact on renal functioning require more investigation into certain indicators of renal damage.

Keywords: *Nigella sativa* oil, Nanofibers, Polycaprolactone, Ethidium bromide, renal toxicity.

Introduction

Ethidium bromide (EtBr), a synthetic phenanthridium salt, was first synthesized by Adams and used as a fluorescent probe for nucleic acids and as a mitochondrial stain. It is widely used in molecular biology and has relatively low cost [1]. The main disadvantages of EtBr are that it is both toxic and carcinogenic. As a mutagen, EtBr intercalates DNA and inhibits RNA and protein synthesis. EtBr is used as a reagent in research; specifically, it is employed as a visualizing agent in electrophoresis of nucleic acids and also to inhibit the action of DNases in RNA isolation protocols[2]. EtBr is also used to stain cells. In flow cytometry, cells stained with EtBr can be visualized in the FL3 channel after excitation with a helium-neon laser. Because of its high concentration in laser-irradiated cells, EtBr can be used as an indicator of cell death [1]. Although there is sparse research on how EtBr affects renal functioning, what is known about the processes and consequences of nephrotoxic drugs is important. The effects of EtBr on African trypanosome nuclear DNA replication are examined in the first time by **Roy Chowdhury et al.** [3]. Their results imply that EB's disruption of DNA pathways may extend to renal cells, possibly resulting in cellular malfunction and death. This fundamental knowledge poses important queries about the wider effects of EtBr exposure in different biological systems, such as renal tissues.

Many cultures employ medicinal herbs as a reliable source of natural medicines. Traditional medicine uses *Nigella sativa* (NS) to cure a number of illnesses, including gastrointestinal, hepatic, pulmonary, and renal conditions. Previous studies have demonstrated many of its therapeutic properties [4]. Numerous kidney disorders have been successfully treated with NS. Its essential component, thymoquinone (TQ), can guard against kidney damage brought on by many environmental pollutants [5]. It is hypothesized that NS at varying doses for a five-week period has no harmful effects on renal function because all treatment groups showed normal kidney tissue in histology examinations and normal blood levels of urea and creatinine [6]. Development of pharmacokinetics are became possible by advances in nanomedicine, which increase

effectiveness and decrease toxicity. Nanomaterials, which provide targeted medication delivery to specific areas, little side effects, and substantial therapeutic results, have become widely used with the development of nanomedicine [7].

As a vital organ for waste disposal and preserving the equilibrium of the internal environment, the kidney filters different metabolic wastes. Excess water and other toxins may build up in the body without being expelled due to kidney failure. Preclinical and clinical data now point to the capability of tailored treatment for renal disorders with nanomaterial-based medication delivery. Using carriers with high biocompatibility, such as nanoparticles, is a requirement for using nanomedicine to treat renal disorders [8]. Using carriers with high biocompatibility, such as nanoparticles, is a requirement for using nanomedicine to treat renal disorders. Nanoparticles can be employed to treat kidney illness because of their physical and chemical characteristics, which allow them to cross through biological membranes and reach kidney cells [9].

By adjusting the electrospinning (ES) manufacturing conditions, the technology has demonstrated its effectiveness in creating finely regulated, bead-free, narrow-diameter nanofibrous matrices with modified dimensions in an economical and environmentally friendly features. In biomedical applications, nanofibres (NFs) have been widely exploited as functional nanomaterials in wound healing, regenerative medicine, drug delivery, and medical textiles because of their special qualities [10]. Numerous characteristics of ESNFs, including their vast surface area, transparency, stretchability, one-dimensional nanostructure, flexibility, conductivity, and variable fiber structure, rendered them appropriate for use in medical applications [11]. different routes, including oral, nasal, transdermal, buccal/sublingual, rectal, vaginal, and ocular, have been used to experience ES drug-loaded NFs [12].

While black cumin and its products have shown promising kidney protective effects, information on nanoparticle-guided targeted delivery into kidney is still lacking. Therefore the current study is an attempt to manufacture a composite by loading NSO with synthetic (PCL) and natural (CS) polymers that qualify it to be medically effective and evaluate its effectiveness in protecting the urinary system against exposure to ethidium bromide in male rats.

Materials and methods

Experimental chemicals and solutions

Ethidium bromide (Sigma Company, USA; CAS No. 1239-45-8), Nigella sativa seed oil (100% natural *Nigella sativa* Seed Oil, Origo, Turkey), Polycaprolactone (Sigma Company, USA; CAS No. 24980-41-4), and Chitosan (Sigma Company, USA; CAS No. 9012-76-4) were utilized in this study. Ten grams of EtBr powder were dissolved in one liter of distilled water to create an EtBr solution (1%; w/v). The male rats were supplemented with a single oral dose of EtBr (10 mg/mL/kg of body weight) daily for four weeks [13].

Electrospining solution for preparation of PCNF and SPCNSONF fabrication

A number of solutions were made in order to fabricate the PCNSONF mat. NSO solution was prepared by mixing 1 mL of NSO with 5 µL of Tween 80. The chitosan (CS) solution (0.2%) was made by mixing 0.2% acetic acid (2M NaOH was added to bring the pH down to 5) and stirring constantly until the CS was completely dissolved. Twelve weight percent of PCL was dissolved in a mixture of DCM:DMF (7:3 v/v) while being vigorously stirred for an hour to create a pure polycabrolactone (PCL) electrospinning solution. After an hour of vigorous stirring, CS was sonicated for half an hour. A sufficient quantity of PCL was supplied following even dispersion. The hybrid solution's PCL/CS mixing solution was established at 10 weight percent. The PCNSONF mat was then made in accordance with **Kahdim** et al. [14] and **Reshmi** et al. [15]. In order to synthesize the PCL/CS/NSO mixture, a 7:3 v/v mixture of DCM, DMF, and CS was mixed with 7 weight percent NSO. After adding the appropriate amount of PCL, the mixture was agitated for three hours. The flow rate for electrospinning was then set at 0.5% per hour, the applied voltage was set at 21 kilovolts, the tip-collector distance was set at 12 cm, and the drum collector's rotation speed was fixed at 700 rpm.

Physical characterization of PCNSONF

The physical properties of the manufactured PCNSONF were characterized by means of scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy [16, 17].

Animal experimentation and experimental protocol

For the current investigation, 96 mature male rats weighing 152±12 g at 92 days of age were split into six groups (16 per group): T1 (intact control group not receiving any treatment), T2 (a group receiving 10 mg of EtBr/Kg BW/day), T3 (PCNF-supplemented group receiving 0.207 mg of PCNF/ Kg BW/day), T4 (a group supplemented with a combination of

EtBr and PCNF), T5 (PCNSONF-supplemented group receiving 0.207 mg of PCNSONF /Kg BW/day), and T6 (a group supplemented with a combination of EtBr and PCNSONf). Two treatment sessions (15 and 30 days) were part of the trial. At the conclusion of each session, 8 animals from each group were anaesthetized (by intraperitoneal injection of 0.3 ml ketamine + 0.1 ml xylazine/kg BW). Following their sacrifice and dissection, non-heparinized tubes were used to draw blood from the abdominal vein for assessment of kidney function markers. To calculate the relative weight, the kidney of each male was disconnected and weighed. The histological alteration was examined by dislocation the kidneys and preserving them in a 10% formalin solution.

Evaluation of kidney function markers

The Spectrum-Egyptian Co. for Biotechnology kit's instructions were followed in order to measure the serum levels of bilirubin, urea nitrogen, creatinine, and the BUN: creatinine ratio.

Histological sections preparation and staining

The kidneys were displaced and subsequently submerged in 10% formalin. Hematoxylin and Eosin stains were applied after the histological sections were prepared **[18]**

Semi-quantitative analysis

Semi-quantitative analysis procedure was utilized to analyze the kidney's pathological changes in the histological sections [19].

Statistical Analysis:

The present data was analyzed using Graph Pad Prism, Version 5. The average plus the standard deviation were used to present the results. The degree of mean difference between groups was evaluated using one-way ANOVA and Newman-Keuls. To distinguish between the periods, the student t-test was employed. $P<0.05$ was considered a significant difference [20].

Results

Relative kidney's weight

Compared with control, the kidneys' relative organ weights (g/100 g of body weight) were significantly greater ($p<0.05$) in male rats exposed to EtBr (T2 group) and those treated with a combination of EtBr and PCNF (T4 group) during both experiment periods, as illustrated in **Figure (1)**. However, there was no discernible change in male rats treated with a combination of PCNSONF and EtBr (T6 group). However, there were insignificant differences ($p>0.05$) between intact males treated with PCNSONF (T5 group), PCNF (T3 group), and the control (T1 group).

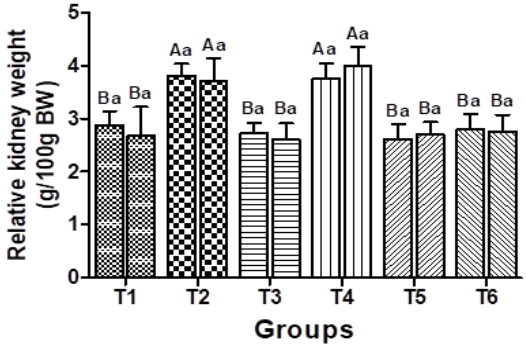

Figure 1: Kidney relative's weight (g/ 100 g BW) of male rats treated for 15 and 30 days with distilled water (T1), Ethedium Bromide; EtBr (T2), Polycaprolactone/ Chitosan nanofiber; PCNF (T3), EtBr and PCNF (T4), Polycaprolactone/ Chitosan nanofiber/ Nigella sativa seed oil; PCNSONF (T5), EtBr and PCNSONF (T6).

Renal function test

The rats that were either untreated (T1 group), treated with PCNF (T3 group), or treated with PCNSONF (T5 group) had serum levels of bilirubin, urea nitrogen, and creatinine that were significantly lower ($p<0.05$) than those of their corresponding EtBr-exposed rats (**Figure 2**). When comparing the EtBr-exposed groups with each other, untreated (T2

group) and PCNF-treated (T4 group) males revealed insignificant differences between their means, but they were significantly higher ($p<0.05$) than PCNSONF-treated males (T5 group). The urea nitrogen: creatinine ratio of all groups that unexposed to EtBr (T1, T3, and T5 groups) and those that were treated with a combination of EtBr and PCNSONF (T6 group) showed insignificant differences between each other, however, they were significantly greater ($p<0.05$) than that of the untreated EtBr-exposed males (T2 group) and the EtBr-exposed PCNF-treated males (T4 group), which were not significantly different from each other **(Figure 2)**.

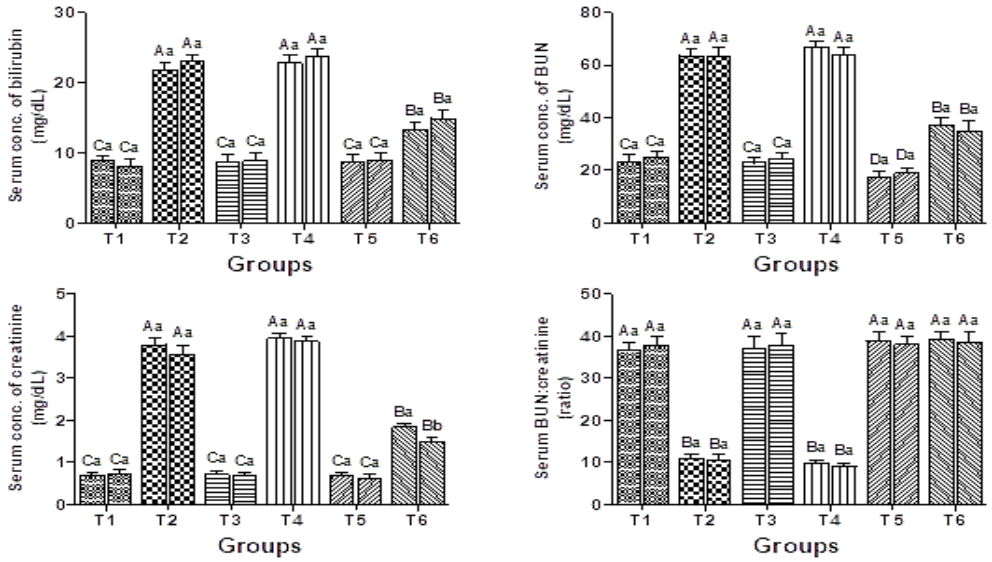

Figure 2: Kidney function tests (serum concentration of bilirubin, blood ura nitrogen, creatinine, and BUN:creatinine ratio)ALT; IU/L, AST; IU/L, and ALP; IU/L) of male rats treated for 15 and 30 days with distilled water (T1), Ethedium Bromide; EtBr (T2), Polycaprolactone/ Chitosan nanofiber; PCNF (T3), EtBr and PCNF (T4), Polycaprolactone/ Chitosan nanofiber/ Nigella sativa seed oil; PCNSONF (T5), EtBr and PCNSONF (T6).

Semi-quantitative analysis of renal pathological alterations

In a semi-quantitative study of the kidneys' histological sections, significant variations ($p<0.05$) were observed between the experimental group's scores for each of the histopathological characteristics, including necrosis, degeneration, inflammatory response, and the presence of cellular debris. There are insignificant variations between the kidney features (**Table 1**) of male rats treated with PCNSONF (T6 group) and non-EtBr-exposed male rats (T1, T3, and T5 groups). They were, however, significantly lower ($p>0.05$) than the ETBr-exposed PCNF-treated rats (T4 group) and untreated EtBr-exposed rats (T2 group), which did not differ significantly from one another.

Table 4-2: Semi-quantitative score (analysis) of kidney's pathological characteristics

Lesion	**Experimental Groups**					
	T1	**T2**	**T3**	**T4**	**T5**	**T6**
Necrosis	0.52±0.02B	4.13±0.23 A	0.59±0.03 B	4.07±0.29 A	0.62±0.03 B	0.66±0.08 B
Degeneration	0.36±0.06B	3.85±0.27 A	0.35±0.07 B	3.73±0.09 A	0.38±0.03 B	0.37±0.09 B
Inflammatory reaction	0.43±0.06B	4.62±0.37 A	0.45±0.05 B	4.56±0.28 A	0.49±0.06 B	0.46±0.09 B
Cellular debris	0.55±0.05B	3.17±0.18 A	0.62±0.07 B	3.08±0.21 A	0.49±0.05 B	0.61±0.08 B

Male rats treated for 15 and 30 days with distilled water (T1), Ethedium Bromide; EtBr (T2), Polycaprolactone/ Chitosan nanofiber; PCNF (T3), EtBr and PCNF (T4), Polycaprolactone/ Chitosan nanofiber/ Nigella sativa seed oil; PCNSONF (T5), EtBr and PCNSONF (T6).

Histopathological changes of kidney sections

Figures (3), (5), and (7) illustrate normal histological features of renal cortex tissue taken from rats in the control group and the two groups treated with PC/NF and PCNSO/NF, respectively. These sections show the glomeruli and proximal and distal convoluted tubules, which are representative of the regular components of the nephrons.

Figures (4) and (6) show the kidney tissues taken from male rats that were given an EtBr and a combination of EtBr and PC/NF, respectively. In the cortical nephrons, there was a noticeable deviation from normal morphology and glomerular atrophy. The kidney of male rats given a combination of EtBr and PCNSO/NF (Figure 8) showed no abnormalities, with the exception of a few shrunken glomeruli and dilated capsular gaps. Both the distal and proximal renal tubules were in normal condition.

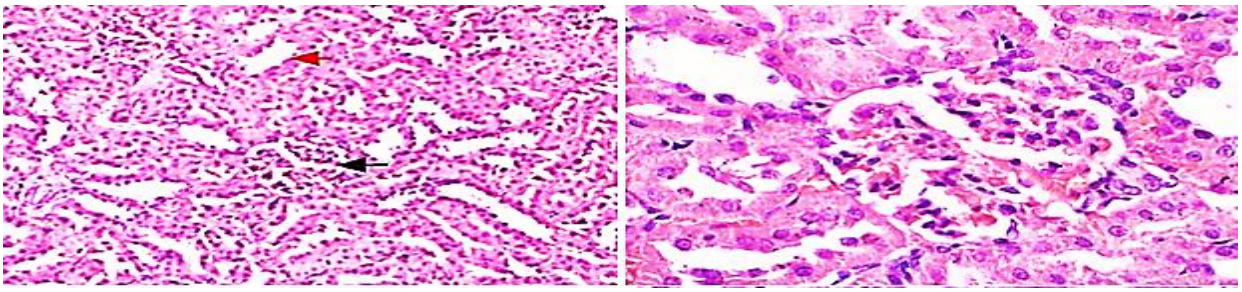

Figure (3a and b): Micrographs (H&E, x10 and x40, respectively) from the Kidney of T1 group (control male rats: daily supplemented with distilled water for 4 weeks). The micrographs show normal glomeruli and renal convoluted tubules (proximal and distal) of cortical nephrons.

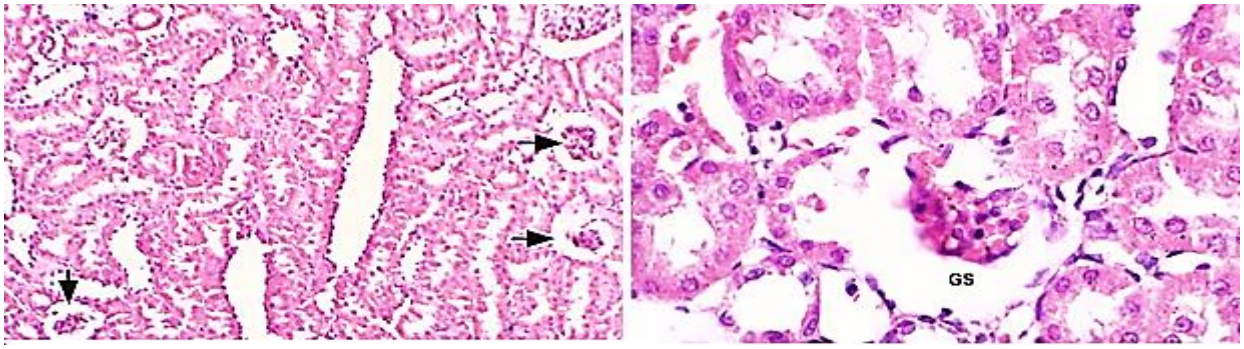

Figure (4a and b): Micrographs (H&E, x10 and x40, respectively) from the Kidney of T2 group (EtBr-treated male rats: daily supplemented with 10 mg/kg of ethidium bromide for 4 weeks). The micrographs show shows necrosis of the glomeruli (yellow arrows), sloughing of the epithelial layer of the renal tubules (black arrow), thickening of the wall of the renal tubules and deposition of eosinophil exudate (blue arrow).

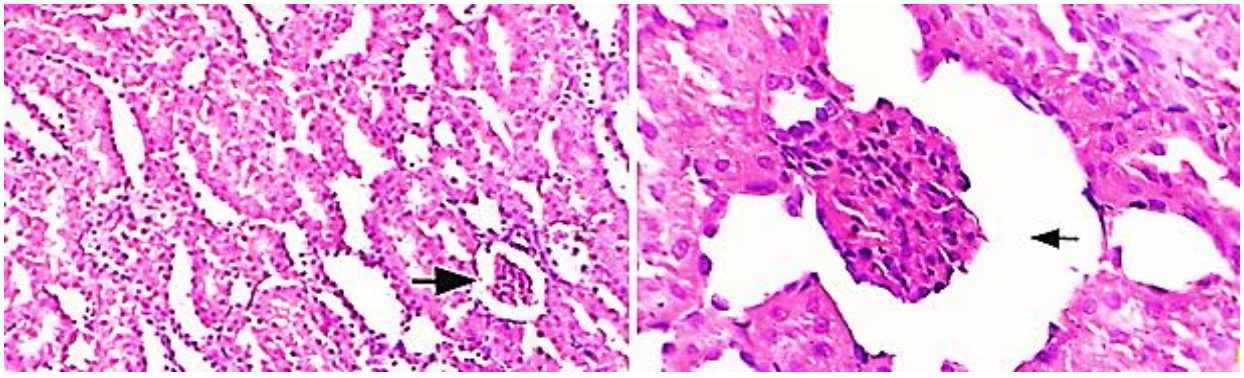

Figure (5a and b): Micrographs (H&E, x10 and x40, respectively) from the Kidney of T3 group (Polycaprolactone- Chitosan/ Nanofibrils-treated male rats: daily supplemented with 0.207 mg/kg of PCN/Nf for 4 weeks). The micrographs show normal, but dilated, glomeruli (black arrow) and renal convoluted tubules (proximal and distal) of the nephrons.

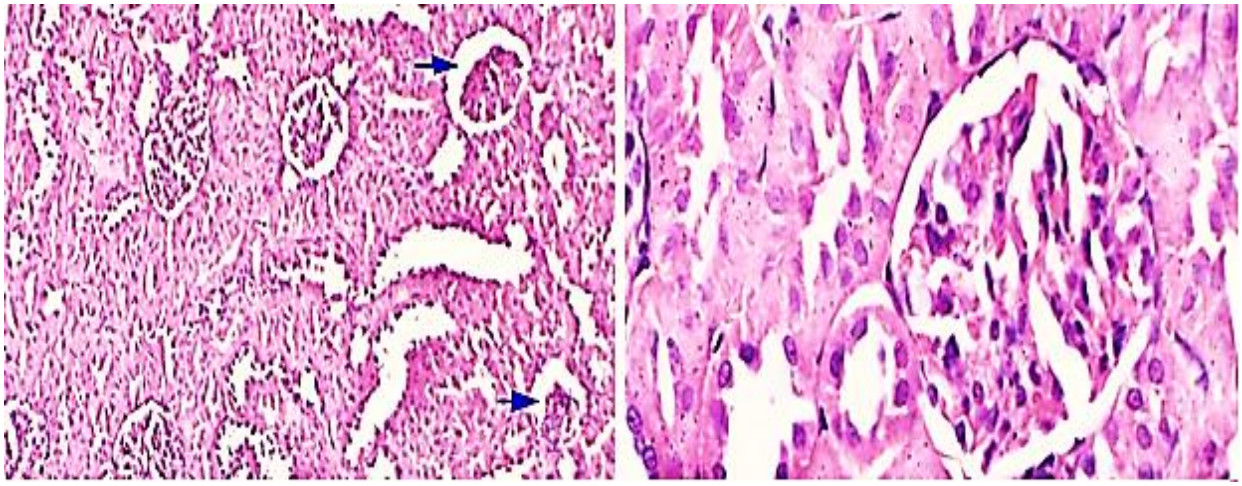

Figure (6a and b): Micrographs (H&E, x10 and x40, respectively) from the kidney of T4 group (male rats treated with a combination of 10 mg/kg of EtBr and 0.207 mg/kg of Polycaprolactone- Chitosan/ Nanofibrils for 4 weeks). The micrographs show glomerular shrinkage and little deviation from normal anatomy of the cortical nephrons.

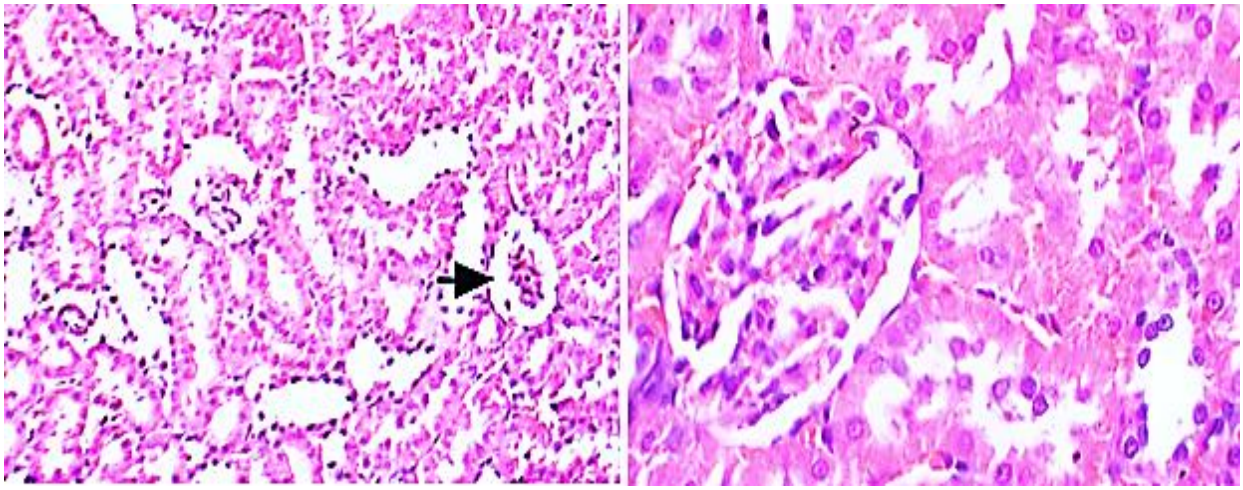

Figure (7a and b): Micrographs (H&E, x10 and x40, respectively) from the kidney of T5 group (Polycaprolactone-Chitosan-*Nigella sativa* oil/ Nanofibrils-treated male rats: daily supplemented with 0.207 mg/kg of PCNSO/Nf for 4 weeks). The micrographs show normal glomeruli and renal convoluted tubules (proximal and distal) of cortical nephrons (black arrow).

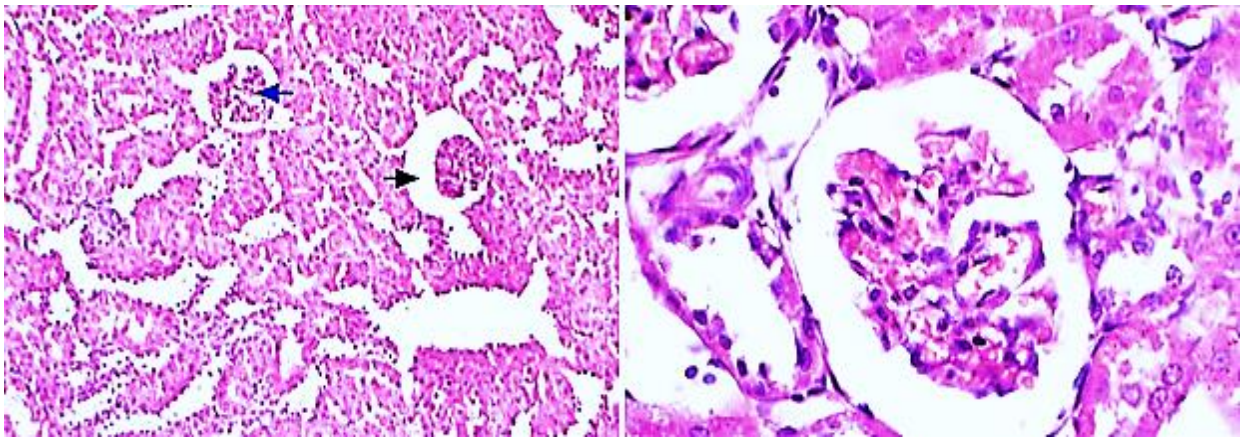

Figure (8a and b): Micrographs (H&E, x10 and x40, respectively) from the kidney of T4 group (male rats treated with a combination of 10 mg/kg of EtBr and 0.207 mg/kg of Polycaprolactone- Chitosan- *Nigella sativa* oil/ Nanofibrils for 4 weeks). The micrographs show structurally normal nephrons. The renal proximal and distal convoluted tubules were normal and unaffected.

Discussion

In order to lessen the associated negative effects of toxicants, this study intends to augment the preventive benefits of antioxidants by transporting them into cells using NSO-loaded encapsulated nanofibers (PCNSONF). Numerous research have examined the effects of natural herbs and their constituents, including NSO, as alternative therapies for a range of disorders caused by exposure to different toxicants [21]. To demonstrate the bioefficacy of any natural or synthetic substance and to get the potent pharmacological effects, the biomolecules' bioavailability needs to be controlled [22]. The bioefficacy of bioactive compounds may be increased by improving the bioavailability of a natural dietary ingredient [23].

The biologically active ingredients of NSO could be protected from degradation by the development of the present NSO-loaded nanocomposites. The circulation, target specificity, bioavailability, stability, and water solubility of NSO bioactive chemicals could also be enhanced by employing this technique. The current bioactive compound's principal pharmacological function, such as its antioxidant qualities, could therefore be carried out more effectively. For this, a variety of techniques have been proposed, such as improved stability and dispersibility [24] and bioavailability [25,26].

It can be inferred that PCNSONF has distinct characteristics that make it appropriate for a variety of biomedical applications due to its unique physicochemical properties, which are primarily caused by quantum phenomena occurring at the nanoscale (less than 100 nm) and its high surface area to volume ratio. Drug carriers are becoming a popular NP in medical applications because of their concentrated and prolonged release of the potent medicinal components they contain [27, 28]. For the NSO compounds to work effectively in some applications, they need to be enclosed in a carrier due to their hydrophobic nature. High stability and steady biological activity are displayed by the NSO-based NPs [29, 30].

Generally, NSO's ability to counteract the effects of oxidative stress and free superoxide radicals generated by EtBr administration may be responsible for the positive benefits of PCNSONF that were stated in this study. Oxidative stress is brought on by the massive production of free superoxide radicals, which are brought on by lipid peroxidation in cell membranes, stimuli of hepatic cell metabolism, activation of RNA synthesis, activation of protein biosynthesis, and competitive inhibition by changing the external cell membrane of the hepatocyte [31].

Black seed was found to significantly ameliorate the structural and functional damage to the body's organs in patients who had been adversely affected by the toxicant [32, 33]. Evidence of the reduced negative impact of EtBr on vital organ functions in rats treated with PCNSONF was demonstrated in the current study by the sharp decline in kidney function marker’s levels after PCNSONF therapy. According to these improvements, it is possible to speculate that NSO's beneficial effects on metabolism resulted from its ability to prevent lipoperoxidation and protect against free radicals [31,34,35].

Since EtBr poisoning overloads the cellular antioxidant defense capacity, the augmented oxidative stress in EtBr-exposed rats may be the cause of the lower kidney weight, but the huge decrease of body weight led to increased kidney’s relative weight. Reactive oxygen species then begin to target the cell's amino acid stores as well as macromolecules such proteins, lipids, and DNA [36–38]. Therefore, the change in oxidative status will significantly oxidize the pool of amino acids, while cytotoxicity will have a significant influence on lipids and proteins simultaneously. In contrast, in a group undergoing combination therapy, PCNSONF effectively providing protection against EtBr toxicity. This might be because both enzymatic and non-enzymatic antioxidant sets have long-lasting antioxidant properties.

Importantly, we revealed that rats exposed to EtBr had considerably higher serum levels of kidney function markers, but all were improved by using nanocomposites loaded with NSO. This could be attributed to the action of thymoquinone, an active element found in NSO that is thought to be a substantial component with high therapeutic capabilities due to its pharmacological activities [33]. In order to improve the imbalance that already existed, PCNSONF was used as an alternative treatment. This may have to do with the mutual influence of NSO on the production of antioxidants, the suppression of free radicals production, or both. Compounds in NSO, specifically thymoquinone, may attempt to activate the body's endogenous antioxidants by supplying the iso-enzymes, such as Mn, Cu, and Zn [39].

According to data from toxico-kinetic studies, EtBr was distributed primarily in the liver, kidneys, lung, brain, testes, and blood [40]. The pathological effect observed in male rats exposed to EtBr (T2 group) was in line with the results of other researchers who noted that the organs most likely to be affected by EtBr toxicity appear to be the liver, kidney, and central and peripheral nerve systems. They stated that EtBr inserts into DNA and affects DNA metabolic events that are essential to nerve cell function after being absorbed by specific carriers or undergoing the proper biochemical adaptation [41]. EtBr is considered a DNA-damaging agent, and in the current work, PCNSONF supplementation mimics its mode of action.

Conclusion

In conclusion, renal histopathology damage, including degeneration, necrosis, and structural disarray, was generated by exposure to EtBr for 15 and 30 days at a dose of 10 mg/kg of body weight. However, the combination of EtBr and PCNSONF treatment showed the most promising results demonstrated the most encouraging outcomes, since nearly normal histological structures and no degradation was shown, suggesting the possible restorative properties of PCNSONF. Out of all the groups, the PCNSONF-treated group showed the best regeneration overall. It is necessary to do additional study on certain renal injury markers in order to better understand the mechanism of renal toxicity caused by EtBr exposure and the therapeutic effect of PCNSONF on renal functioning.

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