RESEARCH ARTICLE

Impact of Docetaxel and Dutasteride-Loaded Nanostructured Lipid Carriers on Male Rats: A Preclinical Evaluation

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ABSTRACT
In this current investigation, we explored the impacts of Nanostructured Lipid Carriers (NLC) containing docetaxel (Dxt) and dutasteride (Dst) on male rats. Nanostructured lipid carriers (NLC) offer a promising drug delivery platform engineered to augment the efficacy of medications such as docetaxel (Dxt) and docetaxel (Dst) through a tailored production method. However, the potential acute toxicity administered Dxt-Dst NLC remains uncertain. Dxt-Dst-NLC was prepared by melt-emulsification and ultra-sonication techniques Male rats were administered DTX-DST NLC intravenously for 14 days. Throughout the treatment period, diligent observations were made regarding the rats' body weight and food consumption once the treatment concluded. On the 15th day, the rats were euthanized, and their hematological, biochemical, and histopathological profiles were evaluated. Dxt-Dst-NLC administered at 25 mg/kg caused liver damage observed in histopathological observations. Also, the elevation of SGOT and SGPT was observed. We observed inflammation at the injection site on the tail of these findings providing valuable insights that can serve as a foundation for further developing Dxt-Dst NLC as a potential therapeutic medication
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INTRODUCTION

Nanostructured Lipid Carriers (NLC) represent an advanced class of lipid nanoparticles renowned for their Improved drug-loading efficiency and prolonged release characteristics.[1] These nanoparticles demonstrate excellent biocompatibility and bioavailability. Typically, NLC is composed of a mixture of liquid and solid lipids, with the drug substances encapsulated within the solid lipid matrix. Ensuring the solid lipids' biocompatibility and biological relevance is crucial for their applications. NLCs typically possess a particle size smaller than 1000 nanometers and may be administered via oral, intravenous, pulmonary, and transdermal administration routes.[2-7] Docetaxel (Dxt) has become a standard treatment option in both first-line and second-line therapies for different types of cancers, such as breast, lung, and prostate cancers.[8] Docetaxel (Dxt) exerts its cytotoxic effects by facilitating the assembly of tubulin into stable microtubules and preventing their disassembly.[9] However, its clinical application is hindered by poor water solubility and side effects.[10] Additionally, multidrug resistance (MDR) can develop through complex mechanisms, such as NF-κB overexpression, DNA repair activation, and increased drug efflux, further limiting DTX's effectiveness.[11,12] To address multidrug resistance (MDR), researchers have investigated the use of synergistic drug combinations instead of single-drug therapies to improve treatment outcomes [13,14] Dutasteride is recognized as a dual inhibitor of SRD5A types I and II, valued for its efficacy in addressing benign prostatic hyperplasia and diminishing the risk of prostate cancer. Achieving a synergistic anticancer effect necessitates the co-delivery of Dxt and Dst to the same target in an ideal ratio.[15] Nevertheless, this presents challenges due to variations in physical properties, metabolic rates, pharmacokinetics, and drug distribution throughout the body.[16] In light of combination therapy, Considerable attention has been directed toward developing alternative delivery systems utilizing nanotechnology to maintain optimized synergistic drug ratios. This nanotechnology-based approach holds promise in enhancing the therapeutic effectiveness of Dxt and Dst combination therapy. Dxt and Dst nanostructured lipid carriers were fabricated to overcome MDR and to explore combinations of drugs with synergistic effects rather than using a single drug administration to improve therapeutic outcomes. In this research, we have fabricated Dxt-Dst-NLC by Melt-emulsification and ultra-sonication techniques. Its Effects on male rats were performed.

METHODS

Animal and Experimental Design

Male Wister rats 180 to 220 grams were used for the study. Rats underwent a one-week acclimatization period. The animals were accommodated in pairs within

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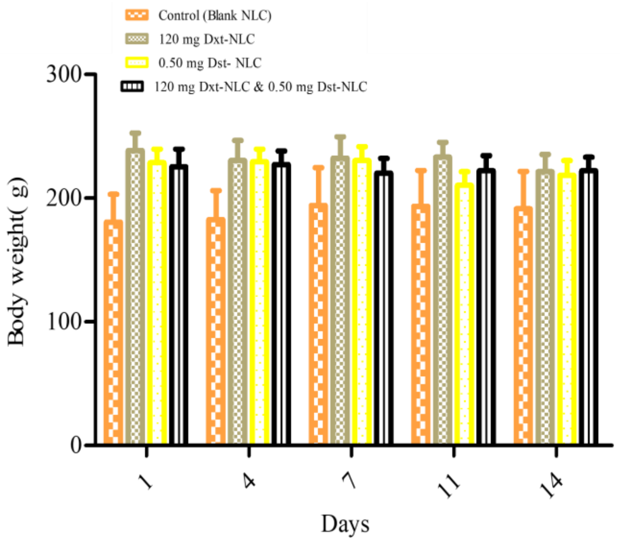

Figure 1: Effect of administration of NLC on Body weight of Control and treatment animals. The results are presented as mean values ± S.E.M., with 6 animals in each group (n=6).

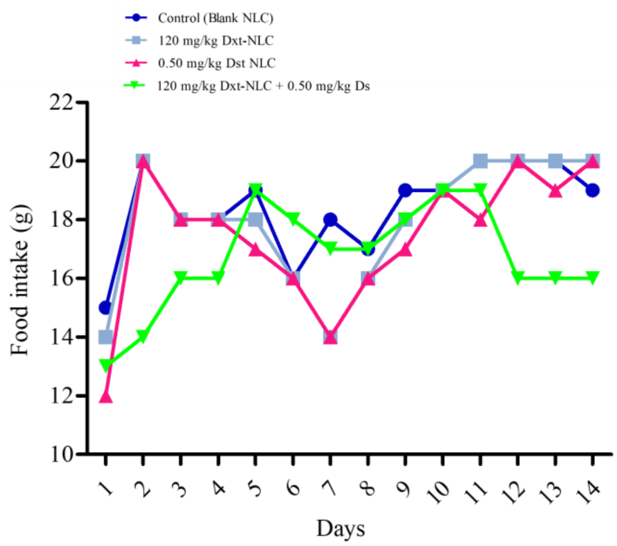

Figure 2: Effects of administration of NLC on food intake. The results are presented as mean values ± S.E.M., with 6 animals in each group (n=6).

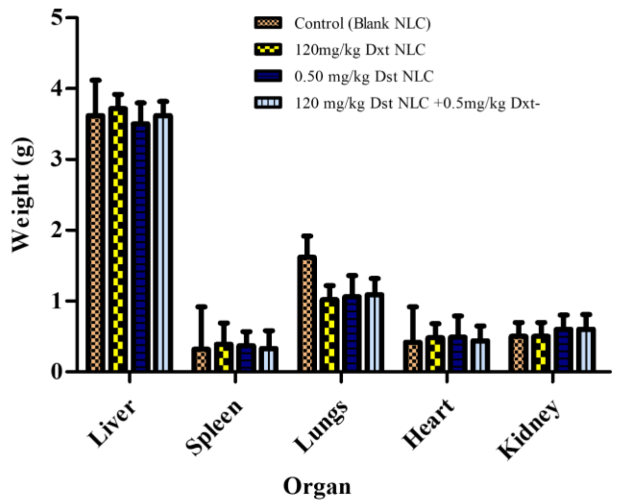

Figure 3: Effects of administration of NLC on the organ-to-body weight ratio. The results are presented as mean values ± S.E.M., with 6 animals in each group (n=6).

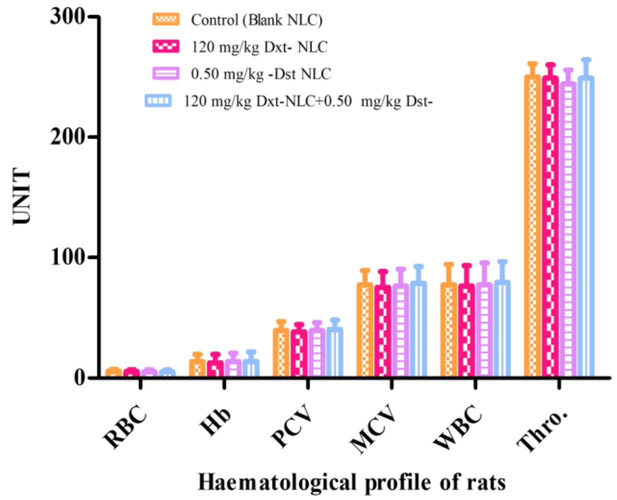

Figure 4: - Effect of administration of NLC on the hematological profile of Rats. The results are presented as mean values ± S.E.M., with 6 animals in each group (n=6).

cages under carefully regulated environmental conditions. The ambient temperature was upheld between 29-32°C, humidity levels were maintained at 70-80%, and a 12-hour light-dark cycle was implemented, as per OECD guidelines in 2008. Dxt and Dst NLC were administered intravenously (i.v) to male Wistar rats via the tail at doses of daily separately and in combination for 14 days. For the control group, six rats received normal saline. The animals were closely monitored within the initial 24-hour period and subsequently daily for a total of 14 days. Daily records were maintained for rat feeding, water intake, and body weights. On the fifteenth day of the trial, Ketamine was used to anesthetize the rats (25 mg/kg; intraperitoneal) and xylazine (10 mg/kg; intraperitoneal). We obtained fresh blood samples for hematological and biochemical analyses. Additionally, we weighed the liver, spleen, lung, heart, and kidneys of the rats. To examine any potential macroscopic morphological alterations, liver and kidney organs were positioned on square grid lines. Furthermore, we computed each rat's organ weight ratio to body weight. To ensure the organs were adequately preserved for subsequent analyses, they were immersed in a 10% formalin fixative solution.

Biochemical and Hematological Evaluation

An automated hematology analyzer was utilized to assess various hematological parameters, including red blood cell count, hemoglobin concentration, hematocrit, packed cell volume, mean corpuscular volume, white blood cell count, and platelet count. A chemical analyzer was employed for the biochemical analysis.

To evaluate liver function, serum levels of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), SGPT, SGOT, creatinine, urea, albumin, total protein, and total bilirubin were

Table 1: Effects of NLC Administration on Serum Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), and Alanine Aminotransferase (ALT).

Group	(ALP) (U/L)	(AST) (U/L)	(ALT) (U/L)
Control (Blank NLC)	150.07 ± 11.50	180.26 ± 13.50	145.6± 12.35
120 mg/kg Dxt- NLC	90.08 ± 11.60	160.36± 13.40	136.6± 12.06
0.50 mg/kg -Dst NLC	89.23± 12.90	150.23± 12.35	137.4± 12.63
120mg/kg Dxt-NL + 0.50 mg/kg Dst-NLC	95.26 ± 12.60	165.23 ± 11.45	156.3± 13.69

The results are presented as mean values ± S.E.M., with 6 animals in each group (n=6). *No significant difference

Table 2: Effects of administration NLC on serum Creatinine, Urea, albumin, total protein, total bilirubin.

Group	Creat(mmol/L)	Urea(mmol/L)	Albumin (mmol/L)	Tot. Prot (mmol/L)	Tot Bil. (mmol/L)
Control (Blank NLC)	51.89 ± 11.56	12.63 ± 11.50	45.32± 12.90	47.63± 17.12	21.52±18.63
120 mg/kg Dxt- NLC	52.36± 10.58	11.36 ± 12.60	42.23± 12.06	48.53 ± 16.12	22.51±17.23
0.50 mg/kg -Dst NLC	49.39 ±12.57	10 .63± 12.35	43.36± 12.60	50.63± 17.23	23.45± 8.23
120mg/kg Dxt-NL +0.50 mg/kg Dst-NLC	47.69± 13.52	11.23 ± 13.69	44.37± 11.60	51.36± 17.56	24.56± 2.36

The results are presented as mean values ± S.E.M., with 6 animals in each group (n=6). per group. *No significant difference

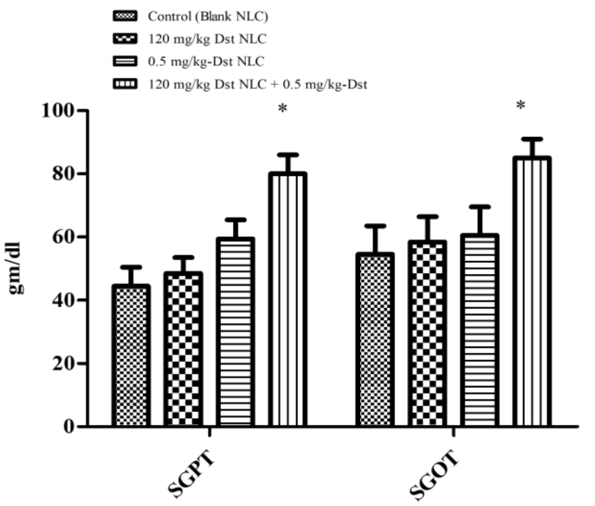

Figure 5: Effects of administration of NLC on the biochemical profile of rats. The results are presented as mean values ± S.E.M., with 6 animals in each group (n=6).

measured. Renal function was assessed by analyzing blood urea nitrogen and serum creatinine levels. A kit was used for the biochemical analysis, and the procedures were carried out according to the manufacturer's instructions.

Histopathological analysis

The liver and kidneys were carefully dissected, separated from the surrounding fascia, and then preserved in Bouin's solution. Stained with hematoxylin and eosin and examined using a 40X microscope.

Statistical Analysis

The data are expressed as mean ± standard error of the mean (SEM) values. Statistical analysis was conducted using GraphPad Prism version 5.

RESULTS

The investigation into the action of Dxt-DST-NLC on various parameters in male Wistar rats yielded several significant findings.

Body Weight

Throughout the study, no notable changes in body weight were detected in either the control group or the group receiving 0.50 mg/kg Dxt-DST-NLC, as shown by the statistical analysis ($p > 0.05$) (Figure 1). This suggests that the treatment did not negatively impact the overall body weight of the rats.

Food Intake

An analysis of food consumption among the groups showed no significant differences between the control and treatment groups (Figure 2). This finding suggests that the administration of Dxt-DST-NLC did not affect the rats' eating habits.

Ratios of Organ Weight to Body Weight

The evaluation of organ-to-body weight ratios showed no significant differences ($p > 0.05$) between the groups for the liver, spleen, lungs, heart, and kidneys (Figure 3). This absence of variation reinforces the conclusion that the treatment did not adversely affect organ development or function.

Hematological Profile

The hematological assessment of rats receiving 0.50 mg/kg i.v. NLC showed no notable differences in red blood cell count (RBC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), white blood cell count (WBC), or platelet counts compared to the control group. This suggests that the treatment had no significant impact on the hematological parameters of the rats

Biochemical Profile

The biochemical analysis revealed higher levels of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) in rats that received intravenous NLC compared to the control group (Figure 4 and 5). However, no statistically significant alterations were observed in the serum levels of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, urea, albumin, total protein, and total bilirubin (Tables 1 and 2). These findings indicate that although there was an increase in

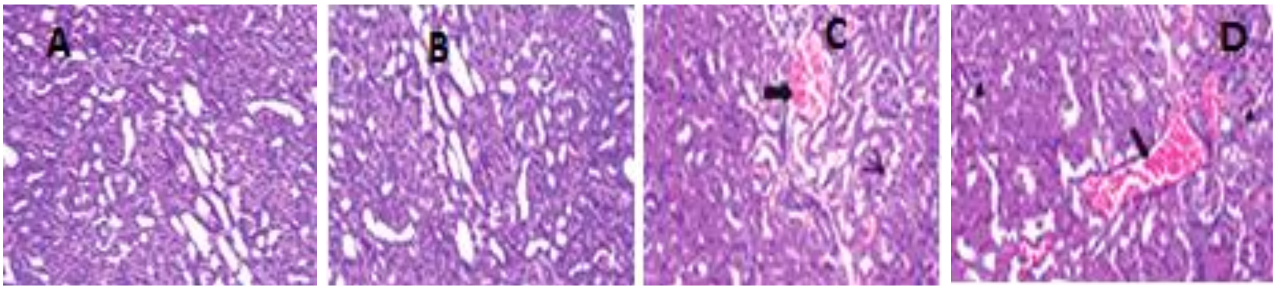

Figure 6: Histology of the liver after treatment with Dxt-Dst-NLC

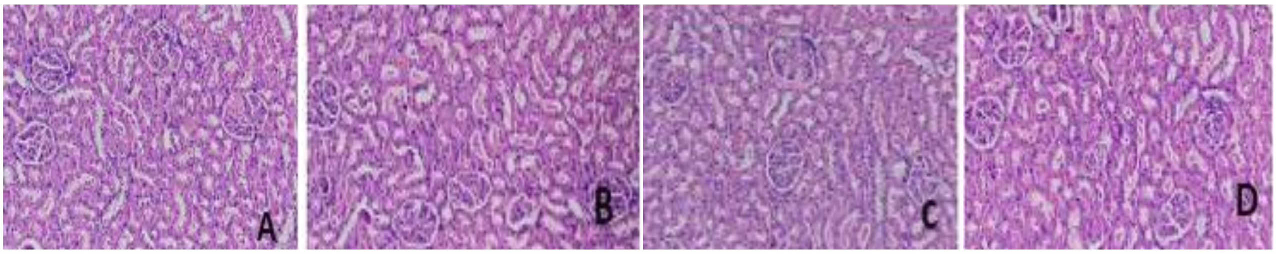

Figure 7: Histology of the kidney after treatment with Dxt-Dst-NLC

Figure 8: Effect of NLC at the injection site

hepatic enzymes, other biochemical markers remained unchanged.

Histological Examination of Liver

Histological analysis conducted after 14 days of treatment revealed that liver tissue from the control group of rats displayed a typical and healthy morphology (Figure 6). In contrast, the liver tissue from the Dxt-DST-NLC treated rats exhibited distinct pathological spots, as indicated by arrows A and B. Despite these findings, the control groups continued to demonstrate normal liver architecture.

Histological Examination of Kidneys

The kidneys of the rats also underwent histological examination, showing normal histoarchitecture (Figure 7). However, in the group receiving 0.50 mg/kg Dxt-DST-NLC, the appearance of spots (arrows C and D) was noted, suggesting possible alterations due to the treatment.

Injection Site Inflammation

Additionally, Inflammation was noted at the injection sites on the tails. of the rats following the administration of Dxt-DST-NLC, with signs of irritation noted on both day 1 and day 14 (Figure 8). This inflammation points to a potential local reaction to the treatment that could warrant further investigation. Overall, the results suggest that although some biochemical and histological changes were observed, the overall health and well-being of the rats remained largely unaffected by the treatment with Dxt-DST-NLC.

DISCUSSION

This study assessed the effects of intravenously administered Dxt-Dst-NLC on overall health, vital organs, and blood parameters. During the 14-day study period, there were no reported mortalities at doses of 0.50 mg/kg, indicating that Dxt-Dst-NLC was not lethal at these levels. Furthermore, none of the animals showed body weight fluctuations greater than 15% of their initial weight, indicating that no adverse reactions were impacting the rats' well-being. Changes in body weight are considered as indicators of potential adverse effects caused by drugs and chemicals.

Furthermore, rats treated with Dxt and Dst-NLC exhibited no variation in their food consumption. Adverse reactions to the treatment may induce stress in the animals, potentially affecting their eating patterns.[17] Administering DTX and DST NLC at a dose of 25 mg/kg did not lead to any fatalities. Furthermore, there were no significant differences in the organ-to-body weight ratios that were compared between the control and treatment groups, indicating that DTX and DST NLC did not induce any organ toxicity. The evaluation of organ-to-body weight ratios is a crucial Outcome in toxicological studies, as it helps determine possible adverse effects of chemical substances.[18,19] In addition, it functions as a reliable marker for detecting any chemical-induced changes in organ function. Following the intravenous administration of 0.50 mg/kg of Dxt and Dst NLC, The blood profile results stayed within the normal limits. The hematopoietic system is highly susceptible to toxic substances, and many toxicants can cause damage to it. Therefore, evaluating chemicals for hematoxicity can be a crucial step in Anticipating potential risks linked to the tested chemical or drug.[20] Furthermore, it's essential to highlight that the substance was administered directly into the bloodstream in this experiment. Consequently, we can confidently conclude that Dxt-Dst NLC Does not cause any adverse effects on the circulatory system. The 120 mg/kg + 0.50 mg/kg i.v Dxt and Dst NLC groups exhibited elevated levels of SGOT and SGPT, which may indicate liver tissue damage. Histological examinations of the liver in the treated groups revealed abnormalities and alterations in the normal structures, suggesting that Dxt and Dst NLC caused structural and functional changes in the kidneys. Harmful substances, especially heavy metals, have the potential to accumulate in critical organs, leading to damage and potentially fatal consequences. In addition, these organs are the main location for metabolizing and synthesizing a variety of vital compounds. They play a crucial role in detoxifying harmful substances, making them ideal organs for evaluating the toxic effects of a drug. In the case of orally administered Dxt and Dst NLC, both biochemical and hematological parameters demonstrated no signs of toxicity at doses up to 25 mg/kg. However, with prolonged consumption of Dxt-Dst NLC, histological assessments suggested potential kidney toxicity, while the functions of other organs remained intact. Rats administered intravenously with a dose of 0.50 mg per kilogram Dxt-Dst NLC exhibited the advancement of spots in their kidneys. Additionally, inflammation was noted in the rats' tails, which is hypothesized to be attributed to the application of tween 80 in the formulation of Dxt and Dst NLC. Including tween 80 in the synthesis of Dxt and Dst NLC was intended to enhance particle stability.[21] Similar incidents of skin inflammation at the injection site have been documented in previous studies where the substance was administered.[22,23] To avoid potential undesirable effects when developing Dxt and Dst NLC.

CONCLUSION

The treatment of Docetaxel and Dutasteride NLC showed liver toxicity, leading to no observed mortality. Additionally, inflammation occurred at the injection site. These findings offer valuable data that can be leveraged for further use. Dxt and Dst NLC are safe. Further studies will develop Dxt and Dst NLC as potential therapeutic drugs.

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