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Research Article

Comparative Evaluation of Di-(2-Ethylhexyl) Phthalate and Tri-(2-Ethylhexyl) Trimellitate-Induced Alterations in Serum Albumin in Sprague-Dawley Rats

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ABSTRACT

Background: Plasticizers are widely used in polyvinyl chloride (PVC) products to enhance flexibility and durability. Di-(2-ethylhexyl) phthalate (DEHP), a commonly used phthalate plasticizer, has been associated with toxic effects involving oxidative stress, inflammation, and organ dysfunction. Tri-(2-ethylhexyl) trimellitate (TOTM) has emerged as an alternative plasticizer because of its lower migration potential and reduced systemic availability. However, comparative evidence regarding the renal effects of DEHP and TOTM remains limited.

Objective: The present study aimed to compare the effects of DEHP and TOTM exposure on serum albumin levels in Sprague-Dawley rats.

Methods: Fifty male Sprague-Dawley rats were randomly divided into five groups (n=10/group). Animals received normal saline, DEHP (300 mg/kg/day and 600 mg/kg/day), or TOTM (300 mg/kg/day and 1000 mg/kg/day) through oral gavage for 30 consecutive days. Serum albumin levels were measured at baseline, Day 15, and Day 30. Statistical analysis was performed using repeated-measures ANOVA followed by Tukey's post hoc test.

Results: Baseline serum albumin levels were comparable among all groups. A significant reduction in serum albumin was observed in rats exposed to DEHP 600 mg/kg/day at Day 30 (3.90 ± 0.32 g/dL) compared with control animals (5.40 ± 0.01 g/dL; $p<0.001$). No significant alterations were observed in the DEHP 300 mg/kg/day group or either TOTM-treated group. Repeated-measures ANOVA demonstrated a significant treatment effect ($F=21.26$, $p<0.001$, $\eta^2=0.65$).

Conclusion: High-dose DEHP exposure significantly reduced serum albumin levels, suggesting impaired renal protein handling and possible glomerular dysfunction. In contrast, TOTM did not significantly influence serum albumin concentrations, indicating a comparatively safer renal profile. These findings support the potential replacement of DEHP with TOTM in biomedical and consumer applications.

Keywords: Di-(2-Ethylhexyl) Phthalate, Tri-(2-Ethylhexyl) Trimellitate, Plasticizers, Serum Albumin, Nephrotoxicity, Sprague-Dawley Rats.

INTRODUCTION

Plasticizers are synthetic compounds extensively incorporated into polyvinyl chloride (PVC) products to improve flexibility, durability, and elasticity.[1] Di-(2-ethylhexyl) phthalate (DEHP) is one of the most widely used phthalate plasticizers due to its effective plasticizing properties and compatibility with PVC materials.[2] It is commonly used in food packaging, medical devices, toys, cosmetics, and household products.[3] Because DEHP is physically incorporated rather than chemically bound to the PVC polymer matrix, it can migrate into surrounding environments and biological

systems.[4] Human exposure to DEHP occurs through several routes, including ingestion, inhalation, dermal contact, and intravenous exposure through medical devices.[5] Continuous exposure to DEHP has raised concerns regarding its potential adverse effects on human health, particularly renal toxicity.[6]

Experimental studies have demonstrated that DEHP and its primary metabolite, mono-(2-ethylhexyl) phthalate (MEHP), can induce oxidative stress and cellular injury in renal tissues.[7] DEHP exposure has also been reported to disrupt mitochondrial function, leading to impaired energy metabolism and renal tubular damage.[8] Inflammatory activation represents another important mechanism involved in DEHP-induced nephrotoxicity, with increased expression of inflammatory mediators contributing to renal structural and functional impairment.[9] Furthermore, activation of peroxisome proliferator-activated receptors (PPARs) has been suggested to play a significant role in mediating DEHP-associated toxic responses.[10] Endothelial dysfunction and altered nitric oxide signalling have additionally been implicated in the progression of DEHP-induced renal injury.[11]

Due to increasing concerns regarding the toxicity of DEHP, alternative plasticizers have been developed for applications requiring prolonged human contact, particularly medical and food-related uses.[12] Tri-(2-ethylhexyl) trimellitate (TOTM) has emerged as a potential substitute because of its higher molecular weight, lower migration rate from PVC materials, and reduced biological availability compared with DEHP.[13] Toxicokinetic investigations have demonstrated that TOTM undergoes limited absorption and is primarily eliminated through fecal excretion, suggesting reduced systemic exposure.[14] However, despite its increasing use, evidence regarding the long-term effects of TOTM on renal function remains limited.

Serum albumin is an important biochemical indicator of renal integrity and protein homeostasis.[15] Although albumin is synthesized in the liver, reduced circulating albumin levels may reflect increased urinary albumin loss resulting from disruption of the glomerular filtration barrier.[16] Previous epidemiological studies have demonstrated associations between phthalate exposure and altered renal biomarkers, including increased urinary albumin excretion.[2] However, experimental evidence directly comparing the effects of DEHP and TOTM on serum albumin alterations remains scarce.

Although several studies have investigated the toxicological effects of DEHP or evaluated the safety profile of TOTM independently, direct comparative studies examining their effects on renal biomarkers under identical experimental conditions are limited. Therefore, the present study was conducted to compare the effects of DEHP and TOTM exposure on serum albumin levels in Sprague-Dawley rats. The findings of this investigation may provide further insight into the relative renal safety of TOTM as an alternative plasticizer for biomedical and consumer applications

MATERIALS AND METHODS

Study Design: This experimental randomized controlled animal study was conducted to compare the effects of Di-(2-ethylhexyl) phthalate (DEHP) and Tri-(2-ethylhexyl) trimellitate (TOTM) on serum albumin levels in Sprague-Dawley rats. The study protocol was approved by the Institutional Animal Ethics Committee, and all experimental procedures were performed according to institutional guidelines for the care and use of laboratory animals.

Experimental Animals: Fifty healthy male Sprague-Dawley rats aged 6–8 weeks and weighing 250–300 g were obtained from the National Institute of Health (NIH), Islamabad, Pakistan, for the duration of one year, from September 2020 to September 2021. Animals were acclimatized for one week before the initiation of the experiment. Rats were maintained under controlled laboratory conditions with a temperature of 22±2°C, relative humidity of 50–60%, and a 12-hour light / dark cycle. Standard laboratory chow and water were provided ad libitum throughout the experimental period.

Sample Size Calculation: The sample size was calculated using the resource equation method for animal experiments as described by Arifin and Zahiruddin, and a total of ten animals per group was considered appropriate for the present study.[1]

Experimental Groups: Following acclimatization, animals were randomly divided into five groups (n=10/group). Group I served as the control group and received normal saline. Group II received DEHP at a dose of 300

mg/kg/day, whereas Group III received DEHP at 600 mg/kg/day. Group IV received TOTM at 300 mg/kg/day, while Group V received TOTM at 1000 mg/kg/day.

Chemicals and Treatment Protocol: DEHP (99% purity) was obtained from Science Centre, Rawalpindi, Pakistan, while TOTM (95% purity) was procured from ATS Synthetic Pvt. Ltd., Lahore, Pakistan. Dose selection was based on previously published toxicological studies evaluating the effects of DEHP and TOTM in experimental animals.[2] DEHP was administered orally at doses of 300 mg/kg/day and 600 mg/kg/day, while TOTM was administered at doses of 300 mg/kg/day and 1000 mg/kg/day. All treatments were administered once daily through oral gavage for 30 consecutive days. The control group received an equivalent volume of normal saline throughout the experimental period.

Blood Collection and Serum Preparation: Blood samples were collected at three different time points: baseline (Day 0), mid-exposure (Day 15), and completion of the experiment (Day 30). Baseline and terminal blood samples were collected following euthanasia, whereas Day 15 samples were obtained through intracardiac puncture under appropriate anaesthesia. Blood samples were allowed to clot and were centrifuged at 3000 rpm for 10 minutes. The separated serum samples were stored at −20°C until biochemical analysis.

Measurement of Serum Albumin: Serum albumin concentration was measured using commercially available enzymatic assay kits (Apex International Suppliers, Lahore, Pakistan) according to the manufacturer's instructions. Results were expressed as grams per decilitre (g/dL).

Statistical Analysis: Statistical analysis was performed using IBM SPSS Statistics software (Version XX; IBM Corp., Armonk, NY, USA). Continuous variables were presented as mean ± standard deviation (SD). Changes in serum albumin concentrations over time among different experimental groups were analysed using repeated-measures analysis of variance (ANOVA). Tukey's post hoc test was applied for multiple comparisons. Effect size was calculated using partial eta squared (η^2). A p-value <0.05 was considered statistically significant.

RESULTS

Baseline Characteristics: A total of 50 male Sprague-Dawley rats completed the experimental protocol and were included in the final analysis. Animals were randomly allocated into five groups with ten rats in each group. The mean age of animals was 7.8 ± 0.32 weeks, while the mean baseline body weight was 293 ± 9.02 g. No statistically significant differences were observed among groups at baseline, indicating successful randomization and comparable initial characteristics.

Effect of DEHP and TOTM Exposure on Serum Albumin Levels: The changes in serum albumin concentrations following exposure to DEHP and TOTM are presented in Table 1. Baseline serum albumin levels were comparable among all experimental groups, demonstrating similar starting conditions before treatment administration.

During the experimental period, no significant alterations in serum albumin concentration were observed in the control group, DEHP 300 mg/kg/day group, TOTM 300 mg/kg/day group, or TOTM 1000 mg/kg/day group. However, rats exposed to DEHP at 600 mg/kg/day demonstrated a progressive decline in serum albumin concentration, with a marked reduction observed at Day 30.

Repeated-measures ANOVA demonstrated a statistically significant treatment effect on serum albumin levels ($F=21.26$, $p<0.001$), with a large effect size ($\eta^2=0.65$), indicating that treatment exposure accounted for approximately 65% of the observed variation in serum albumin concentration.

At Day 30, serum albumin levels decreased significantly in the DEHP 600 mg/kg/day group (3.90 ± 0.32 g/dL) compared with the control group (5.40 ± 0.01 g/dL). In contrast, serum albumin levels remained stable in the DEHP 300 mg/kg/day group (5.34 ± 0.32 g/dL), TOTM 300 mg/kg/day group (5.31 ± 0.32 g/dL), and TOTM 1000 mg/kg/day group (5.35 ± 0.18 g/dL).

Table 1. Serum albumin concentrations (g/dL) following DEHP and TOTM exposure

Group	Day 0	Day 15	Day 30
Control	5.30 ± 0.06	5.39 ± 0.03	5.40 ± 0.01
DEHP 300 mg/kg/day	5.40 ± 0.01	5.40 ± 0.01	5.34 ± 0.32

DEHP 600 mg/kg/day	5.41 ± 0.02	5.30 ± 0.31	3.90 ± 0.32
TOTM 300 mg/kg/day	5.20 ± 0.31	5.20 ± 0.62	5.31 ± 0.32
TOTM 1000 mg/kg/day	5.40 ± 0.16	5.40 ± 0.01	5.35 ± 0.18

Repeated-measures ANOVA: $F=21.26$, $\eta^2=0.65$, $p<0.001$

Dose-Dependent Response

A dose-dependent reduction in serum albumin concentration was observed following DEHP exposure. The lower dose of DEHP (300 mg/kg/day) did not produce significant changes during the 30-day exposure period; however, administration of 600 mg/kg/day resulted in a substantial decline in serum albumin levels. In contrast, both doses of TOTM maintained serum albumin concentrations comparable with control animals, suggesting minimal effects on protein homeostasis under the present experimental conditions.

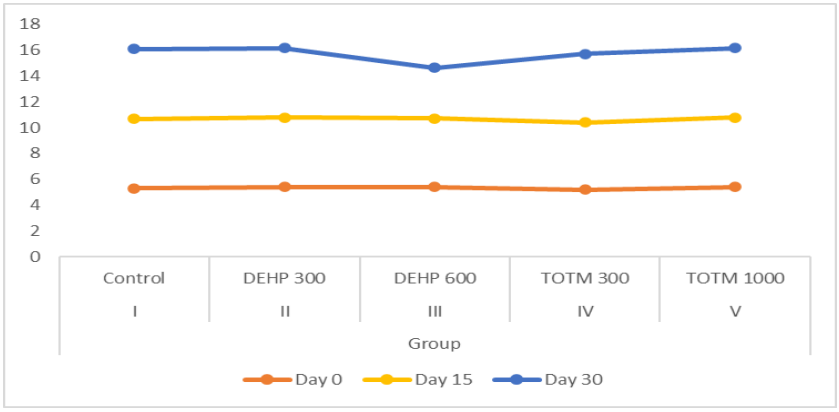

Figure 1: Dose-Dependent Changes in Serum Endothelial Nitric Oxide Synthase (G/Dl) Following Administration of Different Concentrations of DEHP (Di-2-Ethylhexyl Phthalate) and TOTM (Tri-2-Ethylhexyl Trimellitate) at Days,0,15 and 30 in Sprague Dawley Rats.
**P< 0.01 Is Considered Significant

DISCUSSION

The present study investigated and compared the effects of Di-(2-ethylhexyl) phthalate (DEHP) and Tri-(2-ethylhexyl) trimellitate (TOTM) on serum albumin levels in Sprague-Dawley rats following 30 days of repeated oral exposure. The major finding of this study was that high-dose DEHP exposure (600 mg/kg/day) significantly reduced serum albumin concentration, whereas low-dose DEHP (300 mg/kg/day) and both tested doses of TOTM did not produce significant alterations. These findings indicate that DEHP exhibits a dose-dependent adverse effect on renal protein homeostasis, while TOTM demonstrates a comparatively safer renal profile under similar experimental conditions.

Serum albumin is an important biochemical marker used for assessing renal integrity and glomerular function.[15] although albumin is synthesized primarily by hepatocytes, reduction in circulating albumin levels may occur due to excessive urinary albumin loss caused by disruption of the glomerular filtration barrier.[16] Therefore, decreased serum albumin may represent an early indicator of renal dysfunction and altered protein handling.

In the present study, the significant decline in serum albumin observed in rats exposed to DEHP at 600 mg/kg/day suggests impairment of glomerular permeability and renal functional integrity. Previous epidemiological investigations have reported that phthalate exposure is associated with increased urinary albumin excretion, supporting the potential role of phthalates in inducing early renal injury.[15] similarly, urinary phthalate metabolites have been associated with altered renal function biomarkers in adult populations, indicating that chronic exposure may adversely affect kidney health.[16]

The nephrotoxic effects of DEHP are largely attributed to its metabolism into mono-(2-ethylhexyl) phthalate (MEHP), which possesses greater biological activity than the parent compound.[5] MEHP has been reported to induce oxidative stress through excessive generation of reactive oxygen species, resulting in cellular damage and impaired renal function.[7] Increased oxidative stress promotes lipid peroxidation, mitochondrial injury, and disruption of cellular homeostasis within renal tubular epithelial cells.[8]

Oxidative stress is considered one of the primary mechanisms responsible for DEHP-induced nephrotoxicity. Experimental studies have demonstrated that DEHP exposure increases reactive oxygen species production and reduces antioxidant defence mechanisms in renal tissues.[7] Ma et al. reported that phthalate exposure caused significant oxidative damage accompanied by renal structural alterations in experimental animals.[7] Similarly, Ashari et al. demonstrated that mitochondrial dysfunction and oxidative injury contribute significantly to DEHP-mediated renal impairment.[8]

Inflammatory pathways also contribute to DEHP-associated renal toxicity. Exposure to DEHP has been shown to increase the expression of inflammatory mediators, including tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), which promote renal tissue inflammation and cellular injury.[10] Chronic inflammatory activation may damage podocytes and glomerular endothelial cells, increasing permeability of the filtration barrier and promoting albumin leakage.[10]

Endothelial dysfunction represents another mechanism involved in DEHP-induced renal injury. Reduced nitric oxide availability and impaired vascular regulation following phthalate exposure may contribute to renal microvascular damage and deterioration of glomerular function.[11] These mechanisms provide a possible explanation for the reduction in serum albumin observed in the high-dose DEHP group of the present study.

In contrast to DEHP, TOTM exposure did not produce significant changes in serum albumin levels at either tested dose. The stability of serum albumin concentrations in TOTM-treated animals indicates that TOTM has limited effects on renal protein handling under the current experimental conditions. The comparatively favourable safety profile of TOTM may be related to its physicochemical characteristics, including higher molecular weight and reduced migration from PVC materials.[3]

Toxicokinetic studies have demonstrated that TOTM is poorly absorbed following oral exposure and undergoes limited systemic distribution compared with DEHP.[12] Höllerer et al. reported that TOTM is predominantly eliminated unchanged, resulting in lower tissue exposure and reduced toxicity compared with phthalate plasticizers.[12]

The reduced biological availability of TOTM may explain the absence of significant alterations in serum albumin observed in the present study. Eckert et al. demonstrated that TOTM exhibits lower migration from medical-grade PVC tubing compared with DEHP, suggesting reduced exposure during medical applications.[3]

Previous comparative toxicological studies have also supported the lower toxicity profile of TOTM. Renaut and Whiteley reported that TOTM produced fewer systemic toxic effects compared with DEHP in experimental animal models.[13] Similarly, regulatory assessments have indicated that TOTM has a favourable safety profile for use in food-contact materials due to its limited migration and low systemic absorption.[4]

The dose-dependent effect observed with DEHP exposure further supports the biological plausibility of the findings. The absence of significant changes following 300 mg/kg/day DEHP administration suggests that lower exposure levels may be compensated by endogenous antioxidant mechanisms and renal repair processes. However, prolonged exposure to higher concentrations may exceed these protective mechanisms, resulting in measurable renal dysfunction.

The major strength of the present study is the direct comparison of DEHP and TOTM under identical experimental conditions. Serial measurement of serum albumin at baseline, Day 15, and Day 30 allowed evaluation of progressive changes over time rather than relying on a single endpoint measurement.

Despite these strengths, some limitations should be considered. Only serum albumin was assessed in the current investigation, whereas additional renal parameters such as urinary albumin excretion, serum creatinine, blood urea nitrogen, renal histopathology, oxidative stress markers, and inflammatory cytokines were not evaluated. Future studies incorporating these parameters would provide a more comprehensive understanding of the mechanisms responsible for DEHP-induced renal injury. Furthermore, extrapolation of animal findings to humans should be performed cautiously due to differences in metabolism and toxicokinetic responses between species.

CONCLUSION

The present study demonstrates that repeated exposure to high-dose DEHP significantly reduces serum albumin levels in Sprague-Dawley rats, suggesting impaired renal protein handling and possible disruption of glomerular integrity. The observed dose-dependent response indicates that prolonged exposure to higher concentrations of DEHP may overwhelm

protective antioxidant and repair mechanisms, resulting in renal dysfunction.

In contrast, neither low-dose DEHP nor TOTM exposure at 300 mg/kg/day and 1000 mg/kg/day significantly affected serum albumin concentrations. The maintenance of normal albumin levels in TOTM-treated animals suggests a lower nephrotoxic potential compared with DEHP.

These findings provide experimental evidence supporting the consideration of TOTM as a safer alternative plasticizer for biomedical and consumer applications. However, further investigations incorporating molecular, biochemical, and histopathological assessments are required to establish the long-term renal safety profile of TOTM.

Limitations

The present study evaluated only serum albumin as an indicator of renal injury. Additional renal biomarkers, including urinary albumin excretion, creatinine, urea, cystatin-C, oxidative stress parameters, inflammatory mediators, and renal histopathology, were not included. Future studies incorporating these parameters may provide deeper insight into the mechanisms underlying DEHP-induced nephrotoxicity. Moreover, because this investigation was conducted in an animal model, direct translation of findings to human exposure scenarios requires further clinical evaluation.

Declarations

Ethical Approval: The study was conducted after approval from the Institutional Animal Ethics Committee, and all procedures complied with institutional guidelines for animal care and experimentation.

Funding: No external funding was received for this study.

Conflict of Interest: The authors declare no conflict of interest.

Data Availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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