



Renoprotective effect of silymarin on tenofovir-induced nephrotoxicity in Wistar rats

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Abstract

Tenofovir disoproxil fumarate (TDF) enjoys widespread use in HIV therapy but is associated with nephrotoxicity. In resource-constrained settings with limited alternatives, strategies to reduce TDF-related kidney damage are essential. This study investigated the nephroprotective and ameliorative effects of Silymarin (SLM) on TDF-induced nephrotoxic rats. Sixty rats were divided into twelve groups and observed over forty-two days. Control groups received distilled water, TDF (600 mg/kg), or SLM (140 mg/kg) alone. Experimental groups received graded doses of SLM as pre-treatment, co-administration, or post-treatment relative to tenofovir exposure. Renal function was assessed using urine output, serum creatinine, urea levels, faecal output, and renal histopathology. Data were analyzed using one-way ANOVA, and significance was set at $P < 0.05$. Silymarin pre-treatment significantly reduced urine volume compared with the TDF control group ($P < 0.05$). No changes were observed in serum creatinine, urea, or faecal output in the silymarin pre-treated group ($P > 0.05$). Co-administration and post-treatment with silymarin also reduced urine output dose-dependently, while biochemical indices remained unchanged ($P > 0.05$). Histopathological evaluation demonstrated dose-dependent attenuation of renal injury in silymarin-treated rats. Silymarin showed a modest renoprotective effect against TDF-induced renal injury, with pre-treatment appearing more protective than co- or post-treatment. This suggests its potential as a nephroprotective agent in TDF-containing regimens.

Keywords: Antioxidants; Nephrotoxicity; Silymarin; Tenofovir.

INTRODUCTION

Tenofovir disoproxil fumarate (TDF) is a nucleotide reverse transcriptase inhibitor that has been part of first-line HIV treatment for more than two decades. It remains widely used in Sub-Saharan Africa and other low-resource regions because of its good antiviral activity, once-daily dosing and its long-standing inclusion in national ART guidelines [1,2]. Although the drug is generally well tolerated, concerns about kidney toxicity have persisted

over the years. Preclinical and clinical studies have reported TDF-associated renal proximal tubulopathy, and in some cases, leading to acute kidney injury, chronic kidney disease, and Fanconi Syndrome [3–5].

Drug-induced kidney injury contributes substantially to renal problems worldwide. Hospital studies indicate that medications may account for a notable fraction of acute kidney injury episodes, especially in patients exposed to antimicrobials, NSAIDs,

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cancer therapies and antiretrovirals [6]. In Sub-Saharan Africa, where HIV remains common, and laboratory follow-up can be limited, these medication-related kidney problems are frequently encountered in routine care. Several ART cohorts from the region have reported varying degrees of TDF-related renal impairment, with estimates commonly in the range of 2–45% [7,8]. A smaller proportion of patients develop more specific tubular abnormalities, which tend to appear more often in older adults, those on long-term therapy, or those receiving additional nephrotoxic drugs [5,7,9].

These renal effects matter clinically because many national programs still rely heavily on TDF. Safer alternatives, such as tenofovir alafenamide (TAF), are not yet widely available in most clinics, and frequent biochemical monitoring is not always practical. This has created interest in approaches that could reduce TDF-related renal damage and help patients remain on effective therapy without interruption.

Silymarin, a plant extract obtained from *Silybum marianum*, has attracted attention for this reason. It contains several flavonolignans, with silybin being the most active component, and has long been used for its antioxidant and anti-inflammatory properties [10]. Experimental work suggests that silymarin can stabilize cell membranes, reduce oxidative stress and limit lipid peroxidation [11]. In addition to its well-described hepato-protective effects, it has shown protective influence in several models of kidney injury caused by drugs such as cisplatin, gentamicin, vancomycin and paclitaxel [12–15]. These findings have encouraged interest in its possible use as a supportive agent in preventing TDF-related nephrotoxicity.

This study was therefore designed to examine whether silymarin could lessen the kidney damage associated with TDF administration in an experimental rat model.

METHODS

Experimental animals. Sixty (60) male and female albino rats of the Wistar strain, weighing between 100-150 g, were obtained from the Animal House Unit of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Jos, Jos, Nigeria. They were maintained under standard animal laboratory conditions (25 to 30°C for 7 days) and fed standard rat pellets (Grand Cereals Feeds, Nigeria) and water ad libitum. The bedding of the animals was changed every three days. After one week of acclimatization, the rats were divided into twelve (12) groups of five (5) rats for the experiment.

Preparation of tenofovir suspension. The rat model of TDF nephrotoxicity was standardised as described by Abraham et al., with modifications [16]. Each tablet of TDF containing 300 mg was crushed into fine powder using mortar and pestle and suspended in 5 mL of water, giving a 60 mg/mL suspension. A dosage of 600 mg/kg body weight was orally administered daily for twenty-one days to induce nephrotoxicity. Nephrotoxicity was confirmed histopathologically using a pilot study in which two groups of rats ($n = 2$ per group) were treated with either 1 mL of distilled water or tenofovir (600 mg/kg) for 21 days. At the end of the treatment period, the animals were humanely sacrificed, and kidney tissues were harvested and examined histologically for renal damage.

Preparation of silymarin suspension. Each 140 mg silymarin tablet was also crushed into fine powder suspended in 5 mL of water to give a concentration of 28 mg/mL. Both the silymarin control group and all treatment groups across the three experimental designs were treated with a dose of 140 mg/kg, 280 mg/kg or 420 mg/kg of silymarin.

Experimental design. Sixty rats were randomly divided into twelve groups (5 rats

per group). The treatment schedule for each group was as follows:

Group I (Negative Control): Received distilled water orally for 42 days.

Group II (Positive Control – TDF): Received Tenofovir (TDF) 600 mg/kg orally for 42 days.

Group III (Positive Control – SLM): Received Silymarin (SLM) 140 mg/kg orally for 42 days.

Groups IV–VI (SLM Pre-treatment):

(a) For the first 21 days: received SLM at 140 mg/kg, 280 mg/kg, or 420 mg/kg, respectively.

(b) From day 22 to day 42: continued with the same dose of SLM plus TDF 600 mg/kg.

Groups VII–IX (SLM + TDF Co-administration): Received TDF 600 mg/kg together with SLM at 140 mg/kg, 280 mg/kg, or 420 mg/kg, respectively, throughout the entire 42-day period.

Groups X–XII (SLM Post-treatment):

(a) For the first 21 days: received TDF 600 mg/kg alone.

(b) From day 22 to day 42: continued with TDF 600 mg/kg plus SLM at 140 mg/kg, 280 mg/kg, or 420 mg/kg, respectively.

Measurement of metabolic parameters. To assess the metabolic changes, 12 groups of two rats per group were placed in metabolic cages, and each group was treated as outlined above. The volume of urine for each group was observed and recorded daily. The weight and consistency of the faeces for each group were also observed and recorded daily for the entire experiment period.

Collection of blood sample. After forty-two days (6 weeks) of administration, the rats were humanely sacrificed. A 5 mL sterile syringe with a needle was used to collect blood from the heart via cardiac puncture. The blood sample was transferred into labelled plain bottles for renal function test. The parameters measured in this experiment include serum creatinine and urea.

Laboratory method. Kidney function test was analyzed on the blood sera samples based on standard operating procedures (SOPs) written and maintained in the APIN Public Health Initiative in Nigeria (APIN) supported laboratory ISO 15189 (Vs 2022 SANAS) at the Jos University Teaching Hospital, Jos, Plateau

State Nigeria using Cobas c 311 and Roche 9180 electrode automatic analyzers and their analytical reagents from Roche diagnostics (Germany).

Preparation of internal organs for histopathological examination. The histological method used was according to Makoshi and colleagues [17]. The kidneys were harvested and preserved in 10% buffered formalin. An automatic tissue processor was used for tissue processing, and embedded using the MP3 tissue embedding centre. A rotary microtome machine (Cambridge Microtome, USA) was used for sectioning. Hematoxylin and eosin were used for staining and mounted using dextrin plasticizer xylene (DPX), and slides were observed under low and high-powered field Carl Zeiss microscope (Zeiss, Germany).

Statistical analysis. The data was analyzed using IBM SPSS Statistics for Windows, Version 20 (IBM Corp., Armonk, NY, USA). For data with normal distribution and homogeneity of variance, statistical analysis was performed using a one-way analysis of variance (ANOVA). Where an overall statistically significant difference between groups was observed, further analysis using Tukey's Honestly Significant Differences (HSD) test was performed to determine where the differences lie. The modified one-way ANOVA (Welch ANOVA) was performed for data with unequal variance. Games-Howell post hoc analysis to determine the difference between groups was used if the Welch ANOVA showed an overall statistically significant difference. Data was expressed in mean $\pm$ standard error of mean (S.E.M). The significance of the difference between the mean of the control and the treated groups was considered at $P < 0.05$. For data that violates the assumptions of ANOVA, the non-parametric test Kruskal-Wallis H test was used. And if an overall statistical significance was observed, a post hoc analysis using Dunn's procedure with

a Bonferroni adjustment was performed with such data expressed in median.

RESULTS

Effect of silymarin pre-treatment on serum creatinine and urea levels of tenofovir-treated rats. Pre-treatment with SLM for three weeks before TDF administration decreased the mean serum creatinine concentration relative to control groups (water only, SLM only, and TDF only). However, the differences were not statistically significant ($p > 0.05$). Among the groups treated with graded doses of SLM, the 280 mg/kg group had the lowest creatinine level, followed by 140 mg/kg (30.4 ± 5.3) and 420 mg/kg (33.2 ± 5.2), respectively. Similarly, there was no statistically significant difference in the mean urea levels across the treatment and control groups ($p > 0.05$) (Table 1).

Effect of silymarin and tenofovir co-administration on creatinine and urea levels of rats. The ANOVA test showed no significant differences ($p > 0.05$) in the mean serum creatinine levels between treatment and control groups (Table 2). Creatinine levels increased gradually with graded doses among the SLM+TDF groups (140 mg/kg: 30.4 ± 3.6 ; 280 mg/kg: 30.5 ± 1.2 ; 420 mg/kg: 35.3 ± 4.9), while mean urea levels followed a similar non-significant pattern (140 mg/kg: 45.4 ± 2.5 ; 280 mg/kg: 47.2 ± 4.3 ; 420 mg/kg: 56.6 ± 11.7).

Effect of silymarin on serum creatinine and urea levels of tenofovir pre-treated rats. The mean serum creatinine levels between the treatment and control groups showed no significant difference ($p > 0.05$) following the One-way ANOVA test (Table 3). With increasing SLM dose, creatinine levels reduced slightly (140 mg/kg: $40.0 \pm 0.5 \rightarrow 420$ mg/kg: 36.0 ± 4.0), while urea levels rose modestly (140 mg/kg/kg/kg: $44.0 \pm 6.1 \rightarrow 280$ mg/kg: $50.2 \pm 4.9 \rightarrow 420$ mg/kg: 51.3 ± 0.6). However, these changes were not statistically significant.

Effect of silymarin pre-treatment on urine and faecal output on tenofovir-treated rats. Kruskal-Wallis's test showed a significant difference in median urine output across groups ($p < 0.05$). Dunn's post hoc analysis revealed that the median urine output of the TDF control group was significantly higher than that of the water, SLM control, and all SLM-treated groups (140 mg/kg, 280 mg/kg, and 420 mg/kg, each $P < 0.05$). On the contrary, there was no significant difference in the median faecal output between control and treatment groups ($p < 0.05$) (Table 4).

Effect of silymarin and tenofovir co-administration on urine and faecal output of rats. A Kruskal-Wallis test indicated a significant difference in median urine output among the experimental groups ($P < 0.05$; Table 5). Dunn's post hoc analysis showed that the tenofovir control group had significantly higher urine output ($P < 0.05$) compared with the distilled water control, SLM control, and SLM 140 mg/kg + TDF 600 mg/kg, SLM 280 mg/kg + TDF 600 mg/kg, and SLM 420 mg/kg + TDF 600 mg/kg groups. However, no significant differences ($P > 0.05$) were observed in median faecal output between the control and treatment groups (Table 5).

Effect of silymarin on urine and faecal output of tenofovir pre-treated rats. A Kruskal-Wallis test indicated a significant difference in median urine output among the experimental groups ($P < 0.05$; Table 6). Post hoc analysis with Dunn's test revealed that the TDF control group had significantly higher urine output compared to the water control ($P < 0.05$), silymarin 140 mg/kg ($P < 0.05$), TDF 600 mg/kg + silymarin 280 mg/kg ($P < 0.05$), and TDF 600 mg/kg + silymarin 420 mg/kg ($P < 0.05$) groups. Conversely, there were no significant differences ($P > 0.05$) in median faecal output between control and treatment groups (Table 6).

Histopathological evaluation of kidney tissue. Histopathological assessment of renal tissues demonstrated marked tenofovir-induced kidney damage, as illustrated in Plates 1–9. Kidneys from distilled water-treated rats showed normal glomerular and tubular architecture, whereas tenofovir-treated controls exhibited tubular degeneration, loss of epithelial lining, and severe vascular congestion (Plates 1–3). Silymarin pre-treatment at 140–420 mg/kg preserved renal

architecture with only mild histological alterations, indicating a protective effect. In the co-administration groups (Plates 4–6), renal injury persisted, with moderate to severe tubular degeneration, necrosis, and vascular congestion observed across doses. Post-treatment with silymarin following tenofovir exposure (Plates 7–9) showed dose-dependent improvement, with partial restoration of glomerular and tubular structures at higher doses.

Table 1: Effect of silymarin pre-treatment on serum creatinine and urea levels of tenofovir treated rats

Treatment groups	Serum creatinine ($\mu\text{mol/L}$)	Urea (g/dL)
	Mean $\pm$ SEM	Mean $\pm$ SEM
Distilled Water	43.8 $\pm$ 5.5	36.8 $\pm$ 6.1
Tenofovir 600 mg/kg	41.0 $\pm$ 4.8	47.7 $\pm$ 3.2
Silymarin 140mg/kg	33.7 $\pm$ 2.6	43.0 $\pm$ 1.0
SLM 140 mg/kg + TDF 600 mg/kg	30.5 $\pm$ 5.3	57.5 $\pm$ 5.4
SLM 280 mg/kg + TDF 600 mg/kg	27.7 $\pm$ 5.5	48.5 $\pm$ 2.7
SLM 420 mg/kg + TDF 600 mg/kg	33.2 $\pm$ 5.2	49.4 $\pm$ 9.3

Table 2: Effect of silymarin and tenofovir co-administration on creatinine and urea levels of rats

Treatment Groups	Serum Creatinine ($\mu\text{mol/L}$)	Urea (g/dL)
	Mean $\pm$ SEM	Mean $\pm$ SEM
Distilled Water	43.8 $\pm$ 3.4	36.8 $\pm$ 6.1
Tenofovir 600 mg/kg	41.0 $\pm$ 4.8	47.7 $\pm$ 3.2
Silymarin 140 mg/kg	33.7 $\pm$ 2.6	43.0 $\pm$ 1.0
Silymarin 140 mg/kg & TDF 600 mg/kg	30.4 $\pm$ 3.6	45.4 $\pm$ 2.5
Silymarin 280 mg/kg & TDF 600 mg/kg	30.5 $\pm$ 1.2	47.2 $\pm$ 4.3
Silymarin 420 mg/kg & TDF 600 mg/kg	35.3 $\pm$ 1.6	54.3 $\pm$ 12.8

Table 3: Effect of silymarin on serum creatinine and urea levels of tenofovir pre-treated rats

Treatment Groups	Serum Creatinine ($\mu\text{mol/L}$)	Urea (g/dL)
	Mean $\pm$ SEM	Mean $\pm$ SEM
Distilled Water 1 ml/kg	43.8 $\pm$ 3.4	36.8 $\pm$ 6.1
Tenofovir 600 mg/kg	41.0 $\pm$ 4.8	47.7 $\pm$ 3.2
Silymarin 140 mg/kg	33.7 $\pm$ 2.6	43.0 $\pm$ 1.0
TDF 600 mg/kg + Silymarin 140 mg/kg	40.0 $\pm$ 0.5	44.0 $\pm$ 6.1
TDF 600 mg/kg + Silymarin 280 mg/kg	42.5 $\pm$ 6.1	50.2 $\pm$ 4.9
TDF 600 mg/kg + Silymarin 420 mg/kg	36.0 $\pm$ 4.0	51.3 $\pm$ 0.6

Table 4: Effect of silymarin pre-treatment on urine and faecal output on tenofovir treated rats

Treatment Groups	Urine (mL/day)	Faeces (g/day)
	Median	Median
Distilled Water 1 ml/kg	5.7*	17.1
Tenofovir 600 mg/kg	11.3	17.8
Silymarin 140 mg/kg	5.5*	18.5
Silymarin140 mg/kg + TDF 600 mg/kg	7.4*	16.3
Silymarin 280 mg/kg + TDF 600 mg/kg	5.3*	16.6
Silymarin 420 mg/kg +TDF 600 mg/kg	5.4*	16.6

Note: * $p < 0.05$ compared to Tenofovir 600mg/kg

Table 5: Effect of silymarin and tenofovir co-administration on urine and faecal output of rats

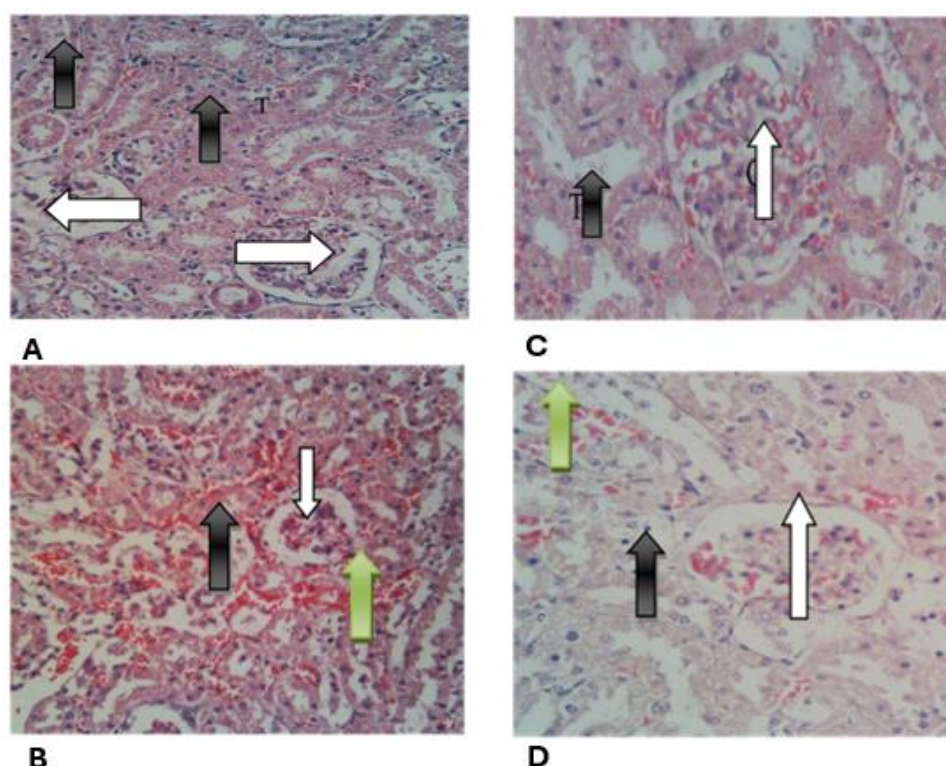
Treatment Groups	Urine (mL/day)	Faeces (g/day)
	Median	Median
Distilled Water 1ml/kg	5.7*	17.1
Tenofovir 600 mg/kg	11.3	17.8
Silymarin 140 mg/kg	5.5*	18.5
Silymarin 140 mg/kg and TDF 600 mg/kg	5.4*	16.8
Silymarin 280 mg/kg and TDF 600 mg/kg	5.0*	16.5
Silymarin 420 mg/kg and TDF 600 mg/kg	5.5*	18.5

Note: * $P < 0.05$ when compared with tenofovir 600 mg/kg control group

Table 6: Effect of silymarin on urine and faecal output of tenofovir pre-treated rats

Treatment Groups	Urine (mL/day)	Faeces (g/day)
	Median	Median
Distilled Water 1 ml/kg	5.7*	17.1
Tenofovir 600 mg/kg	11.3	17.8
Silymarin 140 mg/kg	5.5*	18.5
TDF 600 mg/kg + Silymarin 140 mg/kg	9.1	16.8
TDF 600 mg/kg + Silymarin 280 mg/kg	7.4*	17.1
TDF 600 mg/kg + Silymarin 420 mg/kg	6.5*	18.2

* $P < 0.05$ when compared with tenofovir 600 mg/kg control group

**Plate1:** Effect of Silymarin (140 mg/kg) Pre-treatment on the Kidney of Tenofovir-Treated Rats (H&E x 400)

- A- Water: Kidney showing normal glomerulus (White arrow) and tubules (black arrow)
- B- Tenofovir (Control): Kidney showing moderately normal glomerulus (White arrow), tubular degeneration and loss of epithelial lining (Black arrow) with severe vascular congestion (Green arrow)
- C- Silymarin (Control): white arrow showing moderately normal glomerulus and tubule (Black arrow)
- D- Silymarin (Pre-treatment) at 140 mg/kg: white arrow showing moderately normal glomerulus and tubule (Black arrow) with mild haemorrhage (Green arrow)

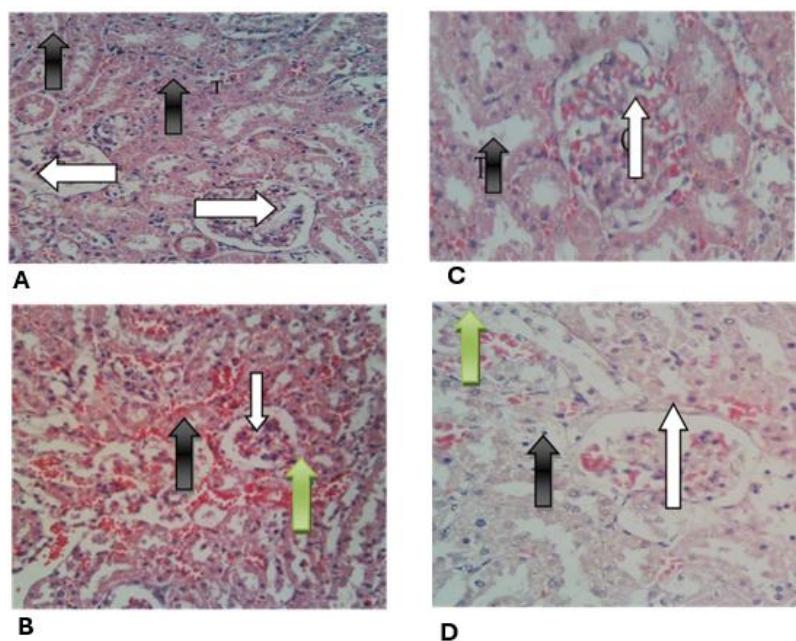


Plate 2: Effect of silymarin (280 mg/kg) pre-treatment on the kidney of tenofovir-treated rats (H&E x 400)

- A- Water: Kidney showing normal glomerulus (White arrow) and tubules (black arrow)
- B- Tenofovir (Control): Kidney showing moderately normal glomerulus (White arrow), tubular degeneration and loss of epithelial lining (Black arrow) with severe vascular congestion (Green arrow)
- C- Silymarin (Control): white arrow showing moderately normal glomerulus and tubule (Black arrow)
- D- Silymarin (Pre-treatment) at 280mg/kg: white arrow showing moderately normal glomerulus and tubule (Black arrow) with mild haemorrhage (Green arrow)

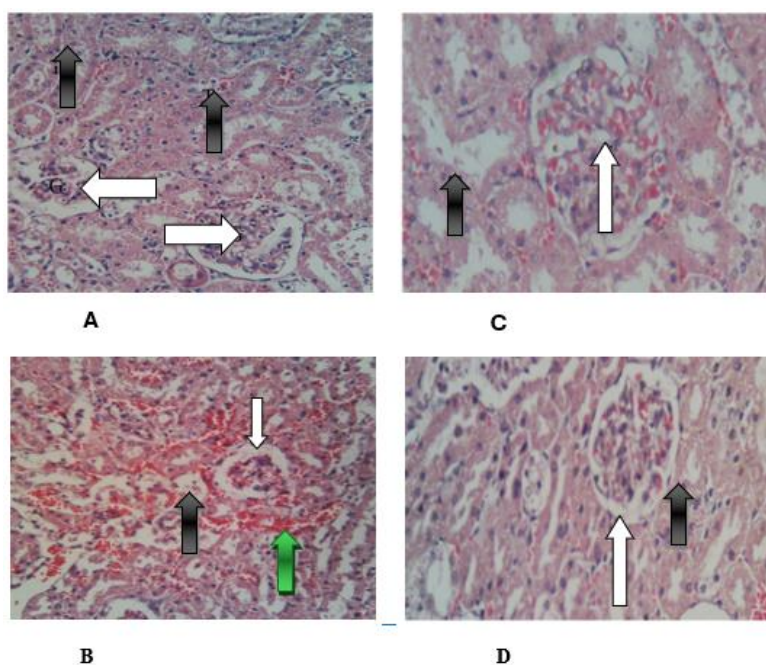


Plate 3: Effect of silymarin (420 mg/kg) pre-treatment on the kidney of tenofovir-treated rats (H&E x 400)

- A- Water: Kidney showing normal glomerulus (White arrow) and tubules (black arrow)
- B- Tenofovir (Control): Kidney showing moderately normal glomerulus (White arrow), tubular degeneration and loss of epithelial lining (Black arrow) with severe vascular congestion (Green arrow)
- C- Silymarin (Control): white arrow showing moderately normal glomerulus and tubule (Black arrow)
- D- Silymarin (Pre-treatment) at 420 mg/kg: white arrow showing moderately normal glomerulus and tubule (Black arrow)

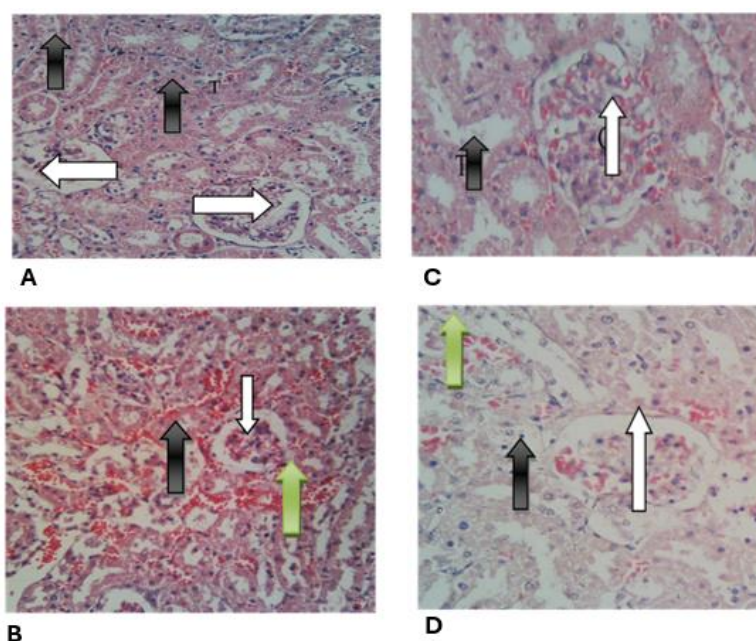


Plate 4: Effect of silymarin (140 mg/kg) and tenofovir co-administration on the kidney of rats (H&E x 400)

A- Water: Kidney showing normal glomerulus (White arrow) and tubules (black arrow)

B- Tenofovir (Control): Kidney showing moderately normal glomerulus (White arrow), tubular degeneration and loss of epithelial lining (Black arrow) with severe vascular congestion (Green arrow)

C- Silymarin (Control): white arrow showing moderately normal glomerulus and tubule (Black arrow)

D- Silymarin (Co-administration) at 140 mg/kg: Green arrow showing severe vascular congestion, tubular degeneration and necrosis (Black arrow)

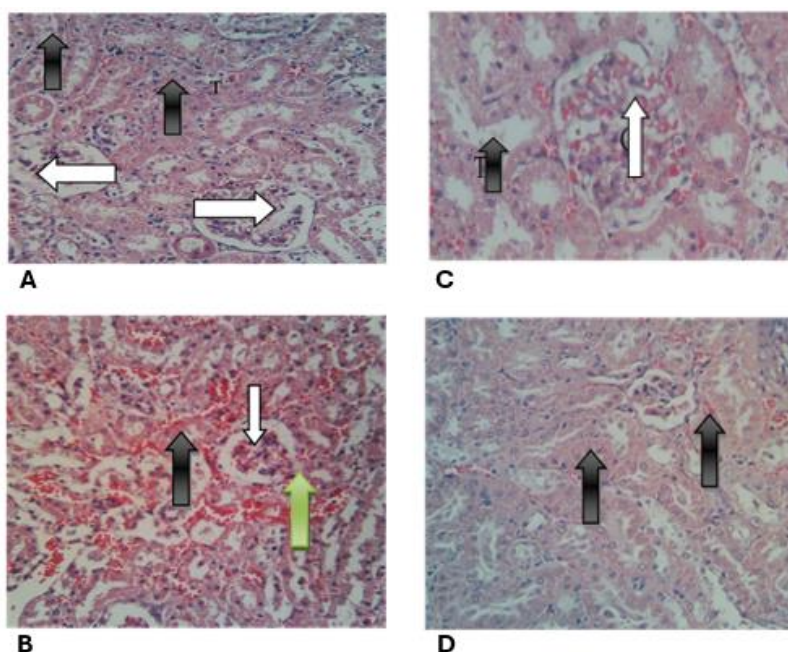


Plate 5: Effect of silymarin (280 mg/kg) and tenofovir co-administration on the kidney of rats (H&E x 400)

A- Water: Kidney showing normal glomerulus (White arrow) and tubules (black arrow)

B- Tenofovir (Control): Kidney showing moderately normal glomerulus (White arrow), tubular degeneration and loss of epithelial lining (Black arrow) with severe vascular congestion (Green arrow)

C- Silymarin (Control): white arrow showing moderately normal glomerulus and tubule (Black arrow)

D- Silymarin (Co-administration) at 280 mg/kg: Black arrows showing, moderate tubular degeneration and necrosis

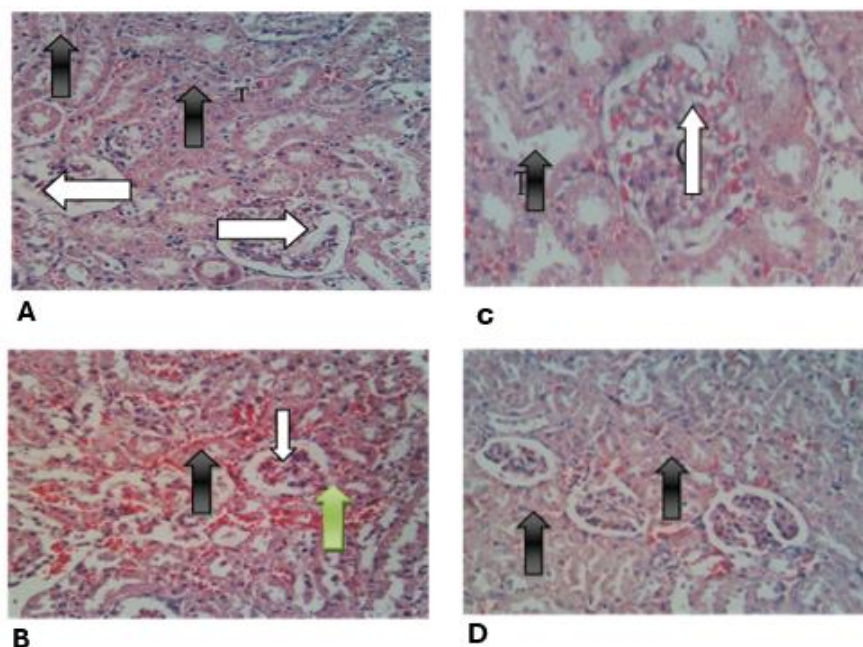


Plate 6: Effect of silymarin (420 mg/kg) and tenofovir co-administration on the kidney of rats (H&E x 400)

- A- Water: Kidney showing normal glomerulus (White arrow) and tubules (black arrow)
- B- Tenofovir (Control): Kidney showing moderately normal glomerulus (White arrow), tubular degeneration and loss of epithelial lining (Black arrow) with severe vascular congestion (Green arrow)
- C- Silymarin (Control): white arrow showing moderately normal glomerulus and tubule (Black arrow)
- D- Silymarin (co-administration) at 420 mg/kg: Black arrows showing, moderate to severe tubular degeneration and necrosis

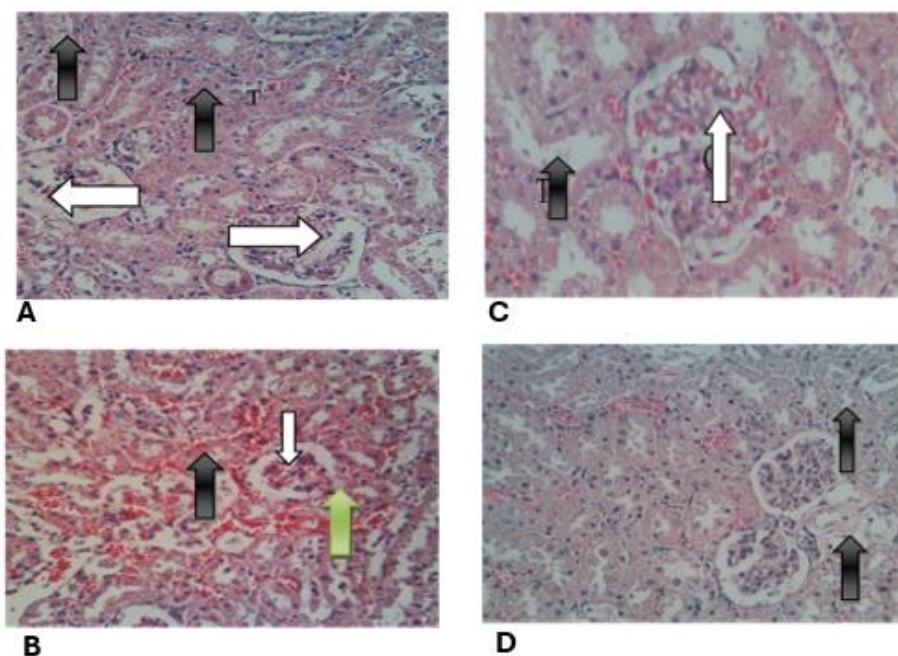


Plate 7: Effect of silymarin (140 mg/kg) on the kidney of tenofovir pre-treated rats (H&E x 400)

- A- Water: Kidney showing normal glomerulus (White arrow) and tubules (black arrow)
- B- Tenofovir (Control): Kidney showing moderately normal glomerulus (White arrow), severe tubular degeneration and loss of epithelial lining (Black arrow) with severe vascular congestion and hemorrhage (Green arrow)
- C- Silymarin (Control): white arrow showing moderately normal glomerulus and tubule (Black arrow)
- D- Silymarin (140 mg/kg): Black arrows showing, severe tubular degeneration and loss of epithelial lining.

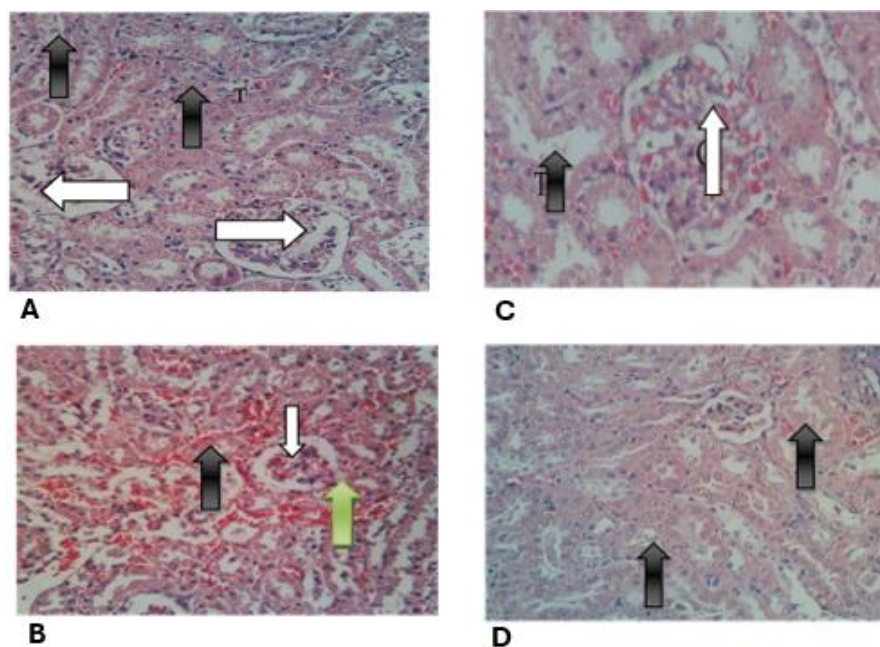


Plate 8: Effect of silymarin (280 mg/kg) on the kidney of tenofovir pre-treated rats (H&E x 400)

- A- Water: Kidney showing normal glomerulus (White arrow) and tubules (black arrow)
- B- Tenofovir (Control): Kidney showing moderately normal glomerulus (White arrow), severe tubular degeneration and loss of epithelial lining (Black arrow) with severe vascular congestion and hemorrhage (Green arrow)
- C- Silymarin (Control): White arrow showing moderately normal glomerulus and tubule (Black arrow)
- D- Silymarin at 280 mg/kg: Black arrows showing, severe tubular degeneration and loss of epithelial lining.

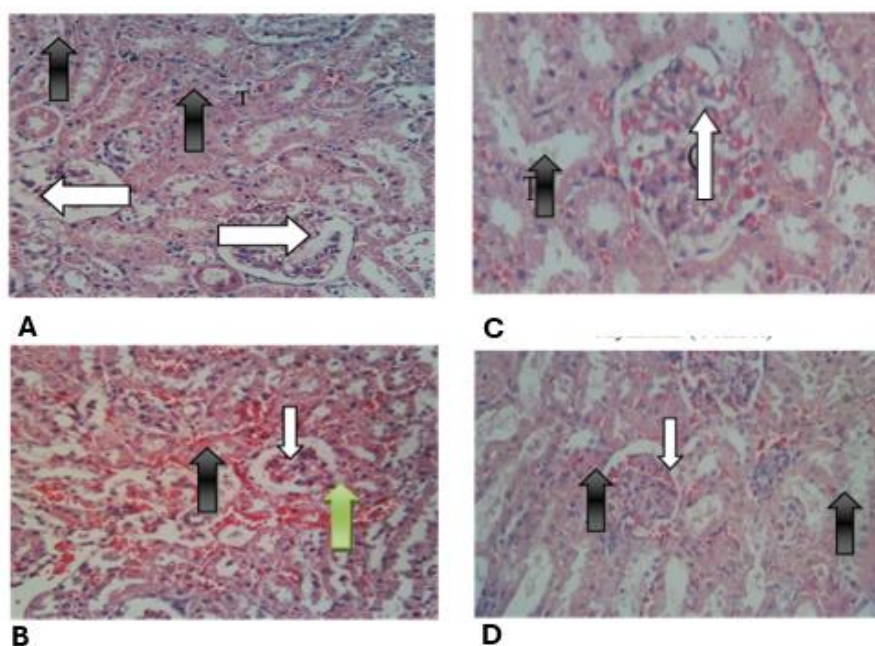


Plate 9: Effect of silymarin (420 mg/kg) on the kidney of tenofovir pre-treated rats (H&E x 400)

- A- Water: Kidney showing normal glomerulus (White arrow) and tubules (black arrow)
- B- Tenofovir (Control): Kidney showing moderately normal glomerulus (White arrow), severe tubular degeneration and loss of epithelial lining (Black arrow) with severe vascular congestion and hemorrhage (Green arrow)
- C- Silymarin (Control): white arrow showing moderately normal glomerulus and tubule (Black arrow)
- D- Silymarin (420 mg/kg): white arrow showing moderately normal glomerulus and tubule (Black arrow)

DISCUSSION

This study investigated the potential of silymarin to reduce renal injury associated with tenofovir disoproxil fumarate (TDF) exposure in rats. Tenofovir remains widely used in HIV treatment and, despite its clinical benefits, has long been linked to nephrotoxicity, primarily targeting the proximal tubules of the kidney [18,19]. As a pro-drug, TDF is converted to tenofovir after absorption, and the active drug is primarily eliminated unchanged via the kidneys through glomerular filtration and active tubular secretion, resulting in high intracellular accumulation in proximal tubular cells, which contributes to mitochondrial dysfunction and renal injury.

In this work, silymarin was administered before, during, or after TDF exposure, and its effects were assessed using biochemical indices, urine and faecal outputs, and histopathological findings. Although many of the observed differences did not reach statistical significance, several consistent patterns emerged that are worth considering in relation to existing evidence.

Across the treatment groups, silymarin pre-treatment was associated with lower mean serum creatinine values compared with the TDF-only group, with the most noticeable trend occurring at the 280 mg/kg dose. While these changes were not statistically significant, they are in line with earlier reports describing the antioxidant and anti-inflammatory actions of silymarin in drug-induced renal injury models [11]. The absence of statistically significant differences may be related to the duration of exposure or the degree of renal injury induced in this model. Urea levels also did not differ significantly between groups. This contrasts with findings from some nephrotoxicity studies in which silymarin reduced serum urea levels [20], but it does not exclude the presence of renal stress, particularly at the tubular level.

Serum creatinine remains a widely accepted and routinely used indicator of renal function. While tenofovir-induced renal injury often involves early proximal tubular dysfunction, elevations in serum creatinine typically reflect more established impairment of renal function [19,21]. In the present study, serum creatinine was therefore interpreted alongside histopathological findings to provide an integrated assessment of renal injury.

Urine output was significantly increased in animals that received TDF alone, consistent with impaired tubular reabsorption and previously described tubular toxicity of tenofovir [18]. In contrast, animals treated with silymarin showed urine output values closer to those of the control groups, suggesting partial preservation of tubular function. Similar effects have been reported in experimental studies where silymarin reduced tubular dysfunction in drug-induced nephrotoxicity models [22]. No meaningful differences were observed in faecal output, indicating that gastrointestinal motility was not appreciably affected by either tenofovir or silymarin in this model.

Histological examination provided further insight into the pattern of injury. Rats exposed to TDF demonstrated tubular degeneration and vascular congestion, features commonly reported in tenofovir-associated renal injury [19]. These changes were less pronounced in animals that received silymarin before TDF exposure, whereas the co-administration and post-treatment groups continued to show evidence of tubular damage. This pattern suggests that the renoprotective effect of silymarin was more evident when given before tenofovir exposure than when introduced after injury had already begun. Similar timing-dependent protective effects have been observed in other nephrotoxicity models involving cisplatin and gentamicin [12,13].

One possible reason for this timing-dependent effect relates to the mechanism of

tenofovir toxicity. Tenofovir accumulates within proximal tubular cells through organic anion transporters, leading to mitochondrial dysfunction, oxidative stress, and subsequent cellular injury [19,23]. Once mitochondrial damage and structural degeneration are established, recovery may be limited. Administering silymarin before tenofovir exposure may enhance antioxidant defenses, stabilize cellular and mitochondrial membranes, and reduce susceptibility to oxidative injury [11,23]. In contrast, later administration may be insufficient to reverse ongoing cellular damage.

Overall, the findings suggest that silymarin may attenuate early tenofovir-induced renal injury, particularly when administered before drug exposure. This observation may be relevant in settings where tenofovir remains a cornerstone of HIV treatment and where routine renal monitoring or access to alternative agents such as tenofovir alafenamide is limited [9]. While the protective effects observed in this study were modest and largely non-significant, they point toward a potential role for adjunctive antioxidant strategies aimed at reducing renal risk rather than reversing established damage.

Several limitations should be acknowledged. As with all animal studies, extrapolation to human disease is constrained by species differences in renal physiology and drug handling. In addition, the use of healthy rats does not fully capture the renal effects of HIV infection itself, which may independently contribute to kidney dysfunction [6]. Future studies incorporating longer exposure periods, optimized dosing, and molecular markers of oxidative stress and mitochondrial injury may help clarify the extent of silymarin's renoprotective potential in the context of tenofovir therapy.

The findings from this study also raise several questions that would benefit from further investigation. The duration of exposure used here may not have been sufficient to fully

demonstrate the extent of renal protection that silymarin could offer, particularly in the context of chronic tenofovir use. Longer-term studies, as well as experiments using a broader range of doses, may help clarify whether the trends observed in this model can become more pronounced over time. It would also be useful to include more sensitive markers of tubular injury and oxidative stress, since reliance on serum creatinine alone may underestimate early renal damage. Finally, studies that better reflect the clinical setting, including HIV-infected animal models or carefully designed clinical studies, would be important for determining how these findings translate to patients receiving tenofovir-based therapy.

Conclusion. In summary, this study demonstrates a consistent, though largely non-significant, trend toward renal protection with silymarin in tenofovir-induced nephrotoxicity. The effect appears more pronounced when silymarin is administered before tenofovir exposure, supporting its possible role as a preventive adjunct rather than a treatment for established renal injury.

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