Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 9 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

PROTECTIVE EFFECTS OF AQUEOUS *OENOTHERA* FLOWER EXTRACT AGAINST ARTEMISNIN INDUCED HEPATO-RENAL INJURY

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ABSTRACT

Background: The liver and kidneys are primary targets of xenobiotic-induced toxicity due to their central role in metabolism and detoxification. Artemisinin is a widely used antimalarial drug with demonstrated efficacy against Plasmodium species. Still, it is increasingly associated with hepatotoxic and nephrototoxic side effects at high doses, mediated through oxidative stress and inflammation. Oenothrera is rich in Gamma-linolenic acid, polyphenols, and flavonoids, with antioxidant and anti-inflammatory properties. However, the potential effect of aqueous *Oenothera* flower extracts remains unexplored.

Objective: This study investigated the protective effects of aqueous Oenothera flower extract against artemisinin-induced hepato-renal injury.

Methods: Ten male rabbits were divided into two groups: a control group (n=5) and an experimental group (n=5). The experimental group received artemisinin (80 mg/kg) for 3 days, followed by aqueous Oenothera flower extract (250 mg/kg/day, suspended in 15 mL of distilled water) for 11 days. Biochemical markers were measured, and histological analysis of liver and kidney tissues was performed.

Results: Artemisinin significantly elevated serum creatinine, urea, ALT, AST, and bilirubin levels and caused histological degeneration in hepatic and renal cells. Post-treatment with Oenothera flower extract restored biochemical markers to baseline and improved histological alterations by day 14

Conclusion: Aqueous Oenothera flower extract showed hepatoprotective and nephroprotective effects against artemisinin-induced injury.

Keywords: Toxicity, Detoxification, Liver, In Vivo Study, Oenothera

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Introduction

The liver and kidneys are central to metabolic regulation, detoxification, and maintenance of homeostasis, making them primary targets of xenobiotic-induced toxicity Artemisinin, a front-line antimalarial compound, has proven efficacy against Plasmodium species but is increasingly reported to exert hepatotoxic and nephrotoxic side effects under high dose exposure primarily mediated through oxidative stress, mitochondrial dysfunction, and inflammatory responses [1]. Given these adverse outcomes, the exploration of natural compounds with antioxidant and anti-inflammatory properties to counteract such damage has become an important area of research. Various medicinal plants have been recognized as valuable sources of bioactive compounds with hepatoprotective and nephroprotective potential. Among them, Oenothera biennis L. (evening primrose) has garnered considerable attention due to its rich content of polyunsaturated fatty acids, particularly gamma-linolenic acid (GLA), as well as a diverse array of polyphenols and flavonoids with strong antioxidant properties [2, 3]. Clinical and experimental evidence demonstrates that evening primrose oil exerts protective effects in inflammatory and oxidative stress-related disorders by modulating biochemical markers and restoring tissue architecture [4, 5].
Recent studies highlight its role in mitigating toxicant-induced hepatic and renal injury. For example, Evening primrose oil has reversed histopathological changes and improved biochemical parameters in animal models of drug-induced hepatotoxicity [1]. Likewise, extracts of Oenothera bennis showed a significant decrease in oxidative stress, fibrosis, and apoptosis in hepatic and renal tissues affected by toxicity [6]. These findings underscore the therapeutic promise of Oenothera in protecting vital organs from xenobiotic damage. Despite substantial data on evening primrose oil and seed extracts, aqueous extracts of Oenothera flowers remain underexplored [7]. Moreover, to date, no study has specifically evaluated their potential to counteract artemisinin-induced hepato-renal injury. Addressing this gap, the present study investigates the ameliorative effects of aqueous Oenothera flower extracts on histological and biochemical alterations in the liver and kidney of rabbits treated with artemisinin

Methodology

Experimental Animals and Housing

Ten healthy male Oryctolagus cuniculus rabbits (600–1400 g) were procured from the local market (Lahore, Pakistan). Animals were acclimated for 5 days in the animal house at the Government P.G. Islamia College (W), Lahore, under controlled environmental conditions (temperature 27 ± 2 °C; 12–14 h light/dark cycle). Rabbits were housed in clean metallic cages with ad libitum access to water, standard chow, and Trifolium alexandrinum fodder. The Ethical Committee approved all animal procedures

Experimental Design

Animals were randomly divided into two groups (n = 5 each):
Control group: Received standard diet and water only.
Experimental group: Received oral **artemisinin (80 mg/kg)** for three consecutive

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days, followed by **aqueous** *Oenothera* **flower extract (250 mg/kg/day, suspended in 15 mL distilled water)** for **11 days (Figure 1)**.

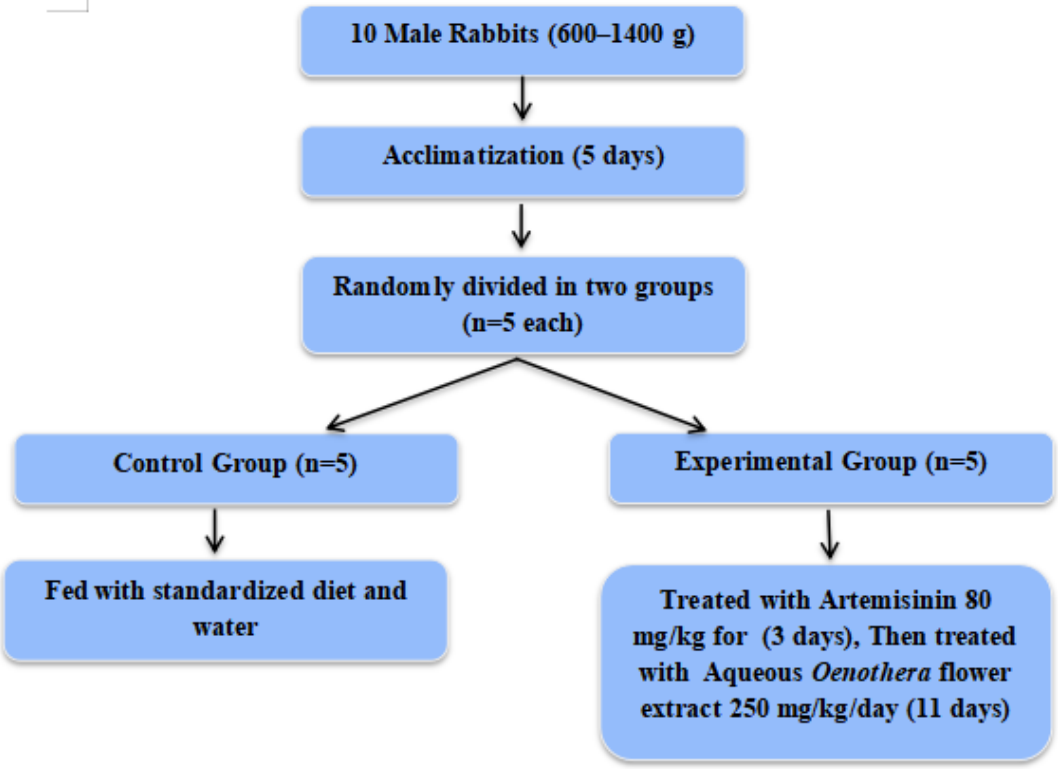

Figure 1 Experimental Design showing grouping and treatment of animals

Plant Material and Extract Preparation

Fresh Oenothera flowers were obtained from a local market, air-dried at room temperature, powdered, and stored in an airtight container. The extract was prepared fresh daily (250 mg/15 mL water).

Blood sampling schedule*:*

Blood sampling was done on different days. On day 1, blood sampling for treatment was performed. After administration of Artemisinin, blood sampling was done to evaluate its effect on day 4. After that, on Days 7, 10, and 14, sampling was done to assess the impact of Oenothera**.** On Day 14, animals were sacrificed; the liver and kidneys were collected for histo-pathological analysis.

Biochemical Analysis and Histopathology

In Biochemical analysis, different parameters of the liver and kidney were assessed. For evaluating kidney function, creatinine and urea concentrations were measured, whereas liver function was assessed by determining levels of Alanine aminotransferase, Aspartate aminotransferase, and total bilirubin using commercial kits and spectrophotometry. Hepatic and Renal tissues were fixed in 10% buffered

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formalin, embedded in paraffin, sectioned at 5 µm, stained with H&E, and examined by light microscopy.

Statistical Analysis

Data expressed as mean ± SD (n = 5). Effects of treatment and time were analyzed by repeated-measures ANOVA, with **$p < 0.05$** considered significant

Results

Kidney Function Tests

Serum creatinine and urea concentrations revealed significant changes following artemisinin intoxication and subsequent Oenothera treatment. Baseline creatinine was 1.45 ± 0.07 mg/dL, which increased markedly to 2.90 ± 0.04 mg/dL after artemisinin administration on day 4. With Oenothera extract treatment, creatinine levels steadily declined to 1.16 ± 0.01 mg/dL on day 7, 1.20 ± 0.16 mg/dL on day 10, and approximately 1.10-1.15 mg/dL by day 14, approaching baseline values. Similarly, urea concentration rose from 42.5 ± 1.63 mg/dL at baseline to 69 + 1.22 mg/dL after artemisinin exposure. Treatment with Oenothera extract reduced urea levels to 57 ± 1.84 mg/dL on day 7, 54 ± 1.63 mg/dL on day 10, and approximately 45-50 mg/dL on day 14, reflecting near normalization (Table 1).

Table 1: Renal Creatinine and Urea Levels in Rabbits

Day	Creatinine (mg/dL)	Urea (mg/dL)
1 (Baseline)	1.45 ± 0.07	42.5 ± 1.63
4 (Artemisinin)	2.90 ± 0.04*	69 ± 1.22*
7 (*Oenothera*)	1.16 ± 0.01*	57 ± 1.84*
10 (*Oenothera*)	1.20 ± 0.16*	54 ± 1.63*
14 (*Oenothera*)	1.12±0.03	45–50

P<0.05

Liver Function Tests

Liver biomarkers also demonstrated significant amelioration by Oenothera extract. Baseline bilirubin concentration was 0.25 ± 0.11 mg/dL, which doubled to 0.51 0.01 mg/dL after artemisinin intoxication. Treatment with Oenothera markedly reduced bilirubin to 0.27 0.09 mg/dL on day 7, 0.10 ± 0.08 mg/dL on day 10, and stabilized at 0.10 mg/dL by day 14. Likewise, ALT increased from 62.5 ± 1.01 U/L at baseline to 88 ± 0.61 U/L following intoxication, but significantly declined to 40 ± 1.03 U/L on day 7, 46 ± 0.07 U/L on day 10, and 40 U/L by day 14 after treatment. A similar trend was observed in AST, which rose from 82.5 ± 0.61 U/L to 95 ± 1.01 U/L after artemisinin exposure, then decreased to 40 ± 2.04 U/L on day 7, 32 ± 1.63 U/L on day 10, and 32 ± 2.1 U/L by day 14 (Table 2).

Table 2: Hepatic Function Parameters

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Day	Bilirubin (mg/dL)	ALT (U/L)	AST (U/L)
1 (Baseline)	0.25 ± 0.11	62.5 ± 1.01	82.5 ± 0.61
4 (Artemisinin)	0.51 ± 0.01*	88 ± 0.61*	95 ± 1.01*
7 (*Oenothera*)	0.27 ± 0.09*	40 ± 1.03*	40 ± 2.04*
10 (*Oenothera*)	0.10 ± 0.08*	46 ± 0.07*	32 ± 1.63*
14 (*Oenothera*)	0.10	40	32± 2.1

P<0.05

Histological Findings

Histopathological examination supported the biochemical findings. In the kidney, control sections showed normal glomeruli and intact tubular structures, while artemisinin treatment caused tubular necrosis, haemorrhages, fibrosis, and glomerulonephritis. After Oenothera extract administration, renal tissues demonstrated reduced degeneration, preserved glomerular morphology, and improved tubular integrity. In the liver, standard hepatocyte architecture and central vein structure were observed in controls, whereas artemisinin intoxication induced hydropic degeneration, sinusoidal dilations, vacuolation, necrosis, and inflammatory infiltrates. Treatment with Oenothera extract alleviated these alterations, showing reduced vacuolation, fewer degenerative changes, and comparatively preserved hepatocytes (Table 3; Figure 2).

Table 3: Histopathological Changes

Group	Liver findings	Kidney findings
Control	Normal hepatocytes, intact sinusoids	Normal glomeruli, intact tubules
Day 4 (Artemisinin)	Hydropic degeneration, vacuolation, sinusoidal dilation, necrosis	Tubular degeneration, haemorrhage, focal dilations
Day 7 (*Oenothera*)	Partial improvement, reduced vacuolation	Reduced tubular damage, partial recovery
Day 10 (*Oenothera*)	Improved hepatocytes, with less sinusoidal dilation	Reduced haemorrhage, improved tubules
Day 14 (*Oenothera*)	Near-normal histology	Near-normal renal architecture

Kidney Histology Liver Histology

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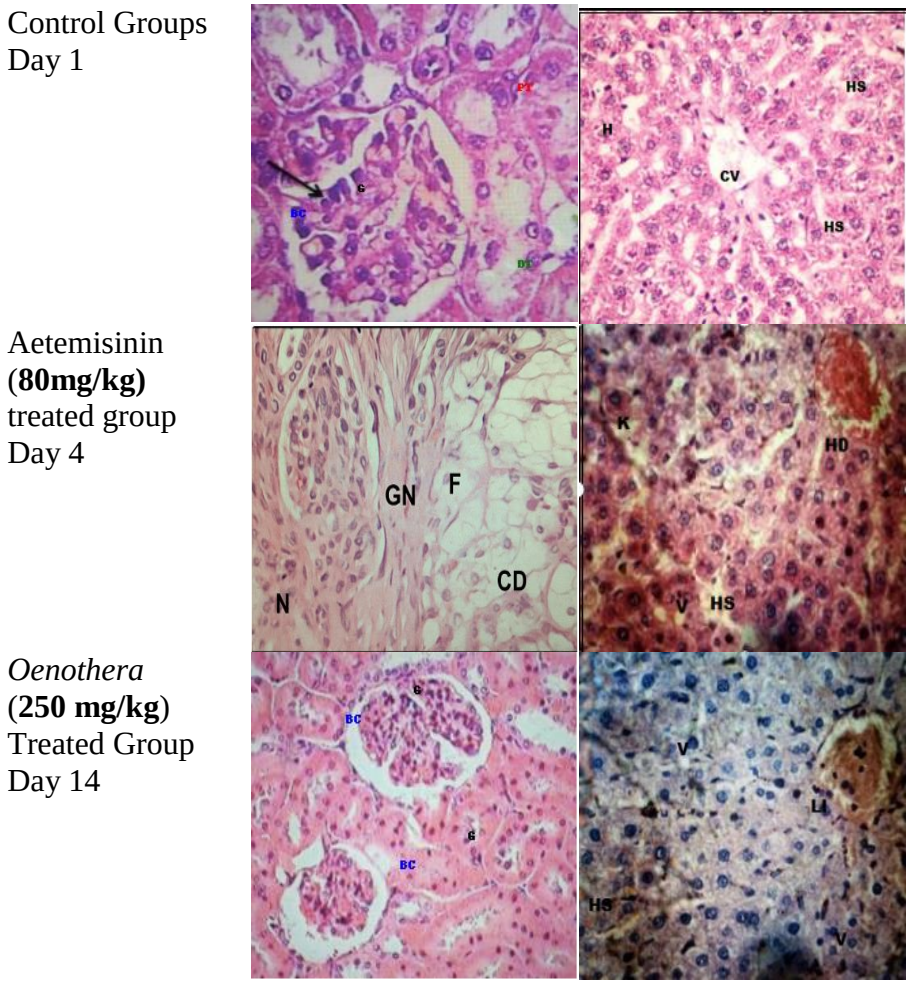

Figure 2 Comparison of histological analysis of the control, Artemisinin, and Oenothera-treated groups, showing typical and recovered architecture

Discussion

Artemisinin used in antimalarials are effective but can cause organ damage with repeated or high-dose exposure. Studies in rodents have shown that artemisinin derivatives can elevate liver enzymes and impair renal function, indicating hepato-renal toxicity. For example, mice treated with standard ACT regimens (artemether/lumefantrine or artesunate/amodiaquine) exhibited significantly increased plasma levels of AST, ALT, ALP, and bilirubin, along with histopathological evidence of hepatocyte degeneration [8]. Similarly, in vivo experiments on rats have reported that artemisinin combination therapies lead to elevated serum liver enzymes (ALT, AST, ALP), consistent with hepatic injury [9]. Elevated creatinine and urea levels are often observed in such models, reflecting impaired glomerular filtration and kidney function [10]. These findings establish a baseline: artemisinin exposure can induce oxidative stress and tissue damage in the liver and kidney, which our study also noted as initial alterations in biochemical markers and histology before Oenothera treatment.

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Given the role of oxidative stress in artemisinin-induced organ damage, antioxidant-rich plant extracts are a logical intervention to test. Many medicinal plants have demonstrated hepatoprotective and nephroprotective effects by scavenging free radicals, reducing inflammation, and enhancing endogenous antioxidant defences. Evening primrose (Oenothera biennis), known for its high content of gamma-linolenic acid and phenolic compounds, is one such plant with potential therapeutic benefit [10]. The extract or oil of Oenothera has been traditionally used for its anti-inflammatory properties and is reported to influence eicosanoid synthesis, which could modulate injury pathways in tissues [11].

Jiang, Huang [12] examined organophosphate pesticide (fenitrothion) toxicity in rats. They found marked hepatic distress, as evidenced by significant elevations in serum ALT, AST, ALP, GGT, and bilirubin, among other markers, after exposure to fenitrothion. In contrast, Evening Primrose Oil was found to reduce these effects. Rats administered evening primrose oil and toxin combination demonstrated nearly normal levels of biochemical and physical parameters. In a study by Hormozi, Ahmadvand [13], gentamicin-induced nephrotoxicity showed significant signs of renal injury, tubular damage, glomerular deterioration, and elevated levels of creatinine and blood urea nitrogen in rats. After treating with evening primrose oil, a significant decrease in these parameters was observed.

Onyekere Peculiar, Odoh Uchenna [14] also proved the ameliorative effects on kidney and liver injuries caused by diofelanec with the use of Artemisia absinthium. Similarly, Saoudi, Badraoui [15] investigated the use of Artemisia campestris oil in mitigating the toxicity induced by chlorpyrifos in rats. In both cases, the bioactive compounds induced a considerable improvement in the physical and biochemical parameters. Co-treatment with Artemisia oil mitigated these biochemical alterations and histopathological changes.

In coincidence, the aforementioned studies have common results that plant extracts with antioxidant and anti-inflammatory activities promote recovery from biochemical and physical tissue damage resulting from toxicity. In our experimental rabbits, it was observed that artemisinin elevated certain liver enzymes (ALT, AST, and ALP) and renal parameters (creatinine and urea), along with alterations in liver and kidney function. Later, post-treatment with the aqueous Oenothera flower extract showed an improvement in these parameters [16].

Protective agents decrease ALT and AST levels by inhibiting the damage to the hepatocyte membranes, which contain these enzymes. This is similar to the decreases with different extracts of Artemisia in the case of diclofenac toxicity and with EPO in rats exposed to pesticides [17]. The lower levels of the kidney function parameters exhibited the ability of Oenothera to do renal restoration. This finding aligns with the study using gentamicin, where evening primrose oil was shown to reduce creatinine and blood urea nitrogen levels [10].

Considering histology, in the case of untreated groups with artemisinin toxicity, the liver sections would exhibit necrosis, inflammation, or vacuolation, and the kidney sections would reveal tubular necrosis [18].

With Oenothera extract therapy, lesions were notably less severe. Comparable outcomes have been documented in the literature [2].

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Mechanistically, the protective role of Oenothera flower extract against artemisinin toxicity can be attributed to its bioactive components, which combat oxidative stress. Artemisinin's toxicity is partly mediated by reactive oxygen species during its metabolism [8]. Oenothera extract probably contains polyphenols (e.g. flavonoids) that enhance antioxidant enzyme activity and directly scavenge free radicals. Medineli, Mert [10] showed that co-administration of EPO with gentamicin preserved antioxidant enzyme activities in kidney tissue.

Gamma-linolenic acid (GLA) from Oenothera is a precursor to anti-inflammatory prostaglandins (PGE1 series). The gentamicin study observed an increase in PGE2 and calcium levels in the EPO group, indicating modulation of pathways that enhance renal blood flow and mitigate injury [19].

The trends seen in our experiment align well with the above-cited studies. However, future research should explore compound isolation and clinical relevance.

Declarations

Author contribution: All authors contributed equally to the manuscript and study.

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

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