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Article

Roselle (*Hibiscus sabdariffa* L.) Tea Protects Against Alcohol-Induced Renal Toxicity by Enhancing Endogenous Antioxidant Defence in Wistar Rats

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Abstract. The kidneys are vital organs responsible for maintaining internal homeostasis and excreting metabolic toxins. However, frequent exposure to alcohol, induces severe oxidative stress and subsequent renal dysfunction. Natural antioxidants, particularly the polyphenol-rich roselle (*Hibiscus sabdariffa* L.) tea, offer a promising countermeasure. Despite its recognized benefits, specific interventions leveraging roselle to profoundly upregulate endogenous antioxidant defenses against alcohol-induced nephrotoxicity remain critically underexplored. This study aimed to evaluate the nephroprotective and antioxidative efficacy of roselle tea on renal function and superoxide dismutase (SOD) activity in alcohol-exposed Wistar rats. Thirty male rats were divided into five groups: normal control, alcohol-only (3 g/kg of 30% ethanol), and alcohol with roselle tea (750, 1500, 3000 mg/kg), administered orally for 30 consecutive days. Renal and oxidative biomarkers were subsequently analyzed. Alcohol exposure significantly elevated serum creatinine, urinary protein, and malondialdehyde (MDA), while reducing SOD activity ($p < 0.05$). Conversely, 3000 mg/kg roselle supplementation significantly increased SOD activity by 84.9% and reduced MDA by 51.0%, effectively reversing renal impairment. In conclusion, roselle tea significantly mitigates alcohol-induced nephrotoxicity by enhancing endogenous antioxidant defenses, presenting a viable natural adjunct for renal protection.

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1. Introduction

Alcohol consumption is widely recognized as one of the major public health concerns due to its multisystemic toxic effects, including hepatotoxicity, nephrotoxicity, and oxidative stress [1]. In biomedical and pharmaceutical research, alcohol is also frequently used as a solvent or vehicle in the preparation of chemical and pharmacological compounds because of its ability to dissolve both hydrophilic and lipophilic substances [2–4]. However, the use of alcohol as a solvent in various drug formulations is not without consequences; chronic or repeated exposure may produce systemic side effects, including renal, hepatic, and oxidative disturbances [5]. Therefore, understanding and developing protective strategies against alcohol-induced toxicity is essential, especially for ensuring patient safety in therapeutic and experimental contexts.

The kidneys are vital organs responsible for maintaining internal homeostasis through the regulation of fluid balance, waste excretion, and electrolyte equilibrium. Chronic alcohol intake disrupts these processes by inducing oxidative stress, mitochondrial dysfunction, and inflammatory responses, leading to renal impairment and elevated biochemical markers such as serum creatinine, urinary protein, and malondialdehyde (MDA) [4],[6]. In animal models, alcohol exposure has been shown to cause glomerular and tubular damage, consistent with clinical observations in long-term alcohol users [7].

The primary mechanism of alcohol-induced nephrotoxicity involves oxidative stress. Ethanol metabolism produces excessive reactive oxygen species (ROS), which attack cellular macromolecules, causing lipid peroxidation, protein oxidation, and DNA fragmentation [3]. The imbalance between oxidative and antioxidative systems results in apoptosis, necrosis, and chronic inflammation in renal tissues[4],[6]. Consequently, antioxidant supplementation from natural sources has become an important research focus to counteract these detrimental effects.

Roselle (*Hibiscus sabdariffa* L.), a member of the Malvaceae family, has gained increasing attention for its rich content of bioactive compounds such as flavonoids, phenolics, anthocyanins, and organic acids [5],[8],[9]. Numerous studies have reported its antioxidant, anti-inflammatory, antihypertensive, and hepatoprotective activities [10–12]. The antioxidant potential of roselle, mainly attributed to compounds such as hibiscetin, protocatechuic acid, and anthocyanins, enables it to scavenge free radicals and inhibit lipid peroxidation, thereby preventing oxidative tissue damage [8],[13–16].

Despite the wide acknowledgment of roselle's antioxidant benefits, studies exploring its nephroprotective effects against alcohol-induced renal toxicity remain limited. Moreover, as alcohol is often used as a solvent in pharmaceutical formulations and laboratory studies, understanding natural countermeasures to its side effects is directly relevant to patient safety and pharmacological research [17]. The development of safe, plant-based protective agents such as roselle tea may help mitigate adverse renal effects associated with the medical or experimental use of alcohol-containing formulations, providing a novel preventive approach in both clinical and research settings [2],[6].

Although the antioxidant advantages of roselle are widely recognised, research investigating its nephroprotective properties against alcohol-induced kidney damage is scarce. Prior research has shown that roselle exhibits protective benefits against nephrotoxicity caused by many chemical agents, including isoniazid, rifampicin, and aluminium chloride, mostly via generalised antioxidant pathways. Nevertheless, targeted research on its effectiveness against alcohol-induced oxidative damage, especially its ability to significantly enhance endogenous superoxide dismutase (SOD), is limited, highlighting a crucial void that this work intends to address [5],[18].

The chosen doses of roselle tea (750, 1500, and 3000 mg/kg body weight) were methodically determined based on prior acute toxicity and efficacy research. Research demonstrates that aqueous extracts of H. sabdariffa have a substantial safety margin (with an LD50 above 5000 mg/kg) and display dose-dependent organ-protective properties that generally become pronounced at 500 mg/kg, remaining well-tolerated up to 3000 mg/kg in rodent models [19].

The present study was designed to evaluate the protective and antioxidative effects of roselle tea on renal function in male Wistar rats exposed to alcohol. We hypothesized that daily administration

of roselle tea could attenuate oxidative stress and improve renal biomarkers — serum creatinine, urinary creatinine, urinary protein, and MDA levels. The findings are expected to contribute to the development of safe herbal interventions for patients or models exposed to alcohol-containing pharmaceutical preparations.

2. Experimental Section

The initial stage of this research involved the preparation of the botanical extract and the acclimatization of the experimental animals. Dried calyces of *Hibiscus sabdariffa* L. were verified by a trained botanist, ground, and infused in distilled water at 90–95 °C for 15 minutes before being filtered. Although direct phytochemical screening was omitted, this standardized aqueous infusion methodology is extensively established in pharmacognosy literature for extracting polar bioactive compounds like anthocyanins, flavonoids, and polyphenols [8],[14]. Concurrently, thirty healthy male Wistar rats (aged 2–3 months, 250–300 g) were housed under standard laboratory conditions with free access to a standard pellet diet and water ad libitum. All experimental procedures were conducted in accordance with institutional guidelines for animal care and were approved by the Institutional Ethics Committee (Approval No. 42/UN.16.10.D.KEPK-FF/2023).

Following the acclimatization phase, the implementation stage commenced by randomly dividing the rats into five distinct groups consisting of six animals each. The normal control group received an equivalent volume of distilled water via intragastric gavage to ensure equal handling stress across all subjects. The disease-model group received an oral administration of 30% ethanol at a dose of 3 g/kg body weight, which is a dose widely used to induce chronic alcohol-related oxidative injury. The three remaining treatment groups received the same alcohol dose along with the freshly prepared roselle tea extract at respective doses of 750, 1500, and 3000 mg/kg body weight. All experimental treatments were administered orally via intragastric gavage once daily for a duration of 30 consecutive days.

The evaluation stage began at the end of the experimental period, wherein rats were placed in individual metabolic cages for a 24-hour urine collection before being anesthetized for cardiac blood sampling. The collected blood was centrifuged at 3000 rpm for 10 minutes to isolate the serum, which was then stored at −20 °C until further biochemical analysis. Renal function was assessed by measuring serum creatinine via the colorimetric Jaffé reaction, while urinary creatinine and protein were determined spectrophotometrically using commercial diagnostic kits. Furthermore, oxidative stress was evaluated by measuring malondialdehyde (MDA) levels using the TBARS method, and endogenous antioxidant capacity was quantified by spectrophotometrically measuring superoxide dismutase (SOD) activity. Finally, all collected quantitative data were statistically analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test, with a p-value of <0.05 considered statistically significant.

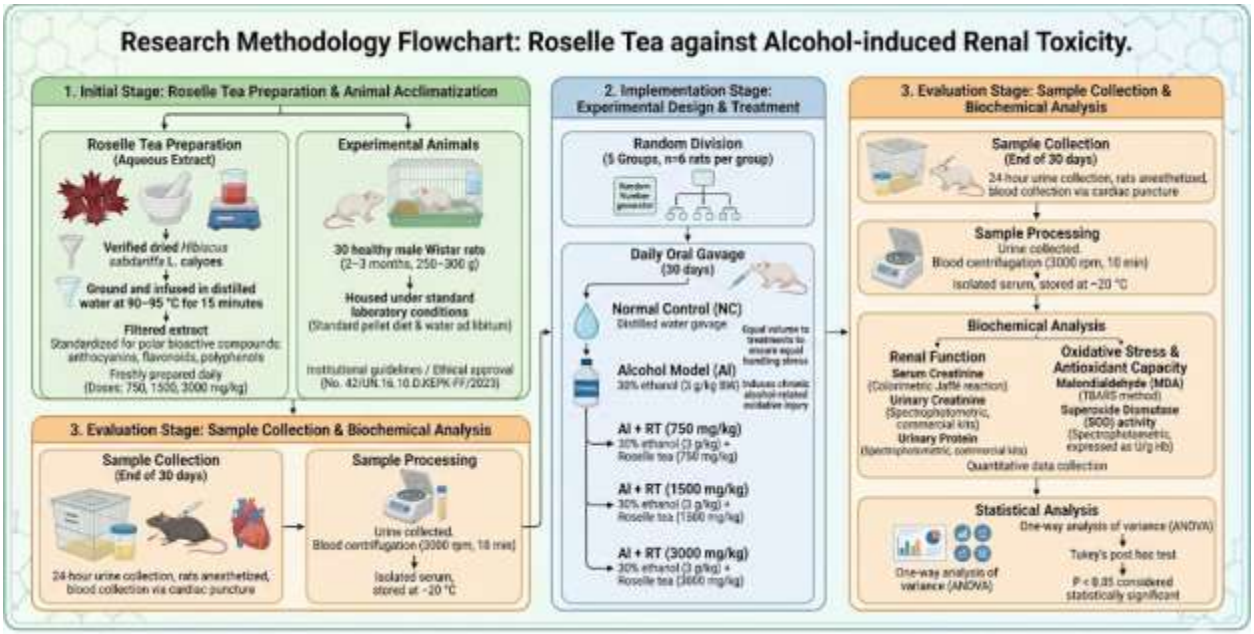

Figure 1. Research Flowchart.

3. Results and Discussion

The administration of alcohol for 30 days induced significant renal dysfunction and oxidative imbalance in Wistar rats, which was evident from the elevated levels of serum creatinine, urinary protein, and malondialdehyde (MDA), alongside reduced urinary creatinine and superoxide dismutase (SOD) activity ($p < 0.05$). However, supplementation with roselle (*Hibiscus sabdariffa* L.) tea demonstrated a dose-dependent reversal of these detrimental alterations. The complete biochemical parameters are presented in Table 1. Additionally, the qualitative phytochemical screening of the roselle tea extract positively confirmed the abundant presence of flavonoids, phenolics, and anthocyanins, consistent with the expected bioactive profile of *H. sabdariffa* calyces.

Table 1. Effect of Roselle Tea on Renal Function, Oxidative Stress, and Antioxidant Activity in Alcohol-Exposed Wistar Rats

Parameter	Control (A)	Alcohol (B)	Roselle 750 mg/kg (C)	Roselle 1500 mg/kg (D)	Roselle 3000 mg/kg (E)	*p*-value
Serum creatinine (mg/dL)	0.68 ± 0.05	1.12 ± 0.10[a]	0.95 ± 0.08[ab]	0.79 ± 0.06[b]	0.70 ± 0.05[b]	< 0.001
Urinary creatinine (mg/dL)	24.5 ± 2.4	15.8 ± 1.7[a]	18.9 ± 2.1[a]	21.7 ± 1.9[b]	23.8 ± 2.0[b]	< 0.001
Urinary protein (mg/dL)	5.3 ± 0.9	12.6 ± 1.5[a]	9.8 ± 1.2[ab]	7.2 ± 0.8[b]	5.8 ± 0.9[b]	< 0.001
MDA (nmol/mL)	2.1 ± 0.3	4.7 ± 0.6[a]	3.9 ± 0.5[ab]	2.8 ± 0.4[b]	2.3 ± 0.3[b]	< 0.001
SOD (U/g Hb)	437.86 ± 28.69	402.54 ± 28.74[a]	562.15 ± 18.31[b]	676.29 ± 38.47[b]	744.35 ± 24.80[b]	< 0.001

Data are expressed as mean ± SD (n = 6). Superscripts indicate statistical significance compared with: [a]=control group (A); [b]=alcohol group (B). Statistical analysis: one-way ANOVA with Tukey post hoc test.

Rats in the alcohol-only group exhibited a significant increase in MDA levels and a reduction in SOD activity compared to the normal control, indicating elevated oxidative stress and an impaired endogenous antioxidant defense system. Alcohol metabolism generates excessive reactive oxygen species (ROS), primarily through the cytochrome P450 2E1 pathway, which attack cellular macromolecules, cause lipid peroxidation, and deplete antioxidant enzymes [3],[20].

Treatment with roselle tea significantly and dose-dependently reduced MDA levels and increased SOD activity, with the moderate (1500 mg/kg) and high (3000 mg/kg) doses being particularly effective. Roselle is rich in polyphenols [21], flavonoids [10], and anthocyanins [22], which exert direct antioxidant effects by scavenging free radicals such as superoxide anions and hydroxyl radicals, thereby terminating lipid peroxidation chain reactions [4],[6]. Furthermore, the marked increase in SOD activity demonstrates that roselle activates endogenous antioxidant systems—likely through the Nrf2–Keap1 signaling pathway—rather than acting solely via direct radical scavenging [23–25]. The chelation of transition metals by polyphenolic compounds also minimizes hydroxyl radical generation via Fenton reactions. This dual mechanism effectively minimizes membrane lipid oxidation and oxidative damage in renal tissues [4],[16],[26].

The oxidative and inflammatory injury caused by alcohol administration significantly impaired renal filtration and structural integrity, reflected by increased serum creatinine and urinary protein, alongside decreased urinary creatinine excretion [27-28]. Increased serum creatinine is a sensitive marker of diminished glomerular filtration rate (GFR), which functionally indicates damage to glomerular endothelium and mesangial cells. Concurrently, proteinuria signifies early structural injury to the glomerular basement membrane and podocytes. Furthermore, alcohol-induced oxidative stress damages tubular epithelial cells, reducing creatinine secretion and promoting back-leak [26],[29].

The Roselle treatment markedly improved these clinical signs, indicating enhanced functional indicators that signify the maintenance of renal structural integrity [10],[22],[30]. The reduction in serum creatinine suggests a preserved or restored GFR, attributable to roselle's ability to stabilize podocyte cytoskeletons, inhibit glomerular cell apoptosis, and maintain renal blood flow through enhanced nitric oxide bioavailability. The subsequent increase in urinary creatinine excretion in treated rats reflects the restoration of tubular secretory and reabsorptive capacities, driven by the antioxidant and antiapoptotic actions of roselle's phenolic constituents [28]. Additionally, roselle markedly reduced urinary protein leakage, indicating the stabilization of glomerular filtration selectivity. The polyphenols and anthocyanins in roselle inhibit TGF-β–induced fibrosis and oxidative membrane injury, thereby maintaining the glomerular structure. By suppressing pro-inflammatory mediators, roselle reduces proteolytic damage, which complements its antioxidative protection [15],[21],[31].

By mitigating oxidative stress and restoring antioxidant balance, roselle suppresses NF-κB activation and downstream pro-inflammatory cytokine release, thereby reducing renal inflammation [32-33]. The enhanced antioxidant capacity, particularly increased SOD activity, preserves endothelial nitric oxide (NO) bioavailability and attenuates vasoconstriction, contributing to improved renal perfusion. Collectively, these converging antioxidative, anti-inflammatory, vasoprotective, and antifibrotic mechanisms preserve nephron structure, enhance renal clearance, and prevent the biochemical manifestations of alcohol-induced renal toxicity [13],[26].

A disadvantage of this study is the lack of direct histological assessment (e.g., Hematoxylin and Eosin staining). The identified nephroprotective benefits are mostly derived from strong biochemical and functional indicators rather than direct microscopic tissue examinations. Moreover, the absence of detailed phytochemical characterisation, including total phenolic or flavonoid content, of the extract batch constitutes an additional constraint. Nonetheless, stringent botanical verification and compliance with a standardised preparation methodology were instituted to guarantee pharmacological consistency. Future research using comprehensive kidney histology and quantitative extract analysis is essential to thoroughly clarify these pathways.

4. Conclusion

This study demonstrates that *Hibiscus sabdariffa* (roselle) tea offers significant protection against alcohol-induced nephrotoxicity by counteracting oxidative stress, inflammation, and structural renal damage. The administration of roselle effectively reduced lipid peroxidation (MDA) and upregulated endogenous antioxidant enzyme activity (SOD), leading to the normalization of renal biomarkers and the preservation of both glomerular and tubular functions. Mediated by its polyphenolic and anthocyanin constituents, roselle emerges as a promising phytotherapeutic agent to mitigate renal dysfunction associated with alcohol-based solvent exposure, highlighting the need for future clinical trials to confirm its translational potential in human applications.

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