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Protective potential of *Thaumatococcus daniellii* in copper sulphate-induced nephrotoxicity in adult Wistar rats

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Abstract
Copper (Cu) is essential for enzymatic functions but poses health risks at high levels, causing oxidative stress and damaging the kidneys. *Thaumatococcus daniellii*, rich in bioactive compounds, has antioxidant and protective properties. Recent studies suggest its potential in mitigating heavy metal toxicity, particularly in the kidneys. Accordingly, this study was aimed at investigating the effect of *T. daniellii* on copper sulphate-induced nephrotoxicity in adult Wistar rats. Thirty (30) adult Wistar rats were randomly divided into six (6) groups of five (5) rats each. Group A served as control; Group B received 200 mg/Kg body weight of copper sulphate; Group C received 250 mg/Kg body weight of *Thaumatococus danielli*; Group D received 1000 mg/Kg body weight of *Thaumatococus danielli*; Group E received 200 mg/Kg body weight of copper sulphate + 250 mg/Kg body weight of *Thaumatococus danielli*; Group F received 200 mg/Kg body weight of copper sulphate + 1000 mg/Kg body weight of *Thaumatococus danielli*. All administration lasted for 28 days and was done orally using an oral gavage. Following the sacrifice of the experimental rats, the kidneys were collected for antioxidant enzymes activity, lipid peroxidation, renal function and histological assessments. Findings from this study revealed that copper sulphate-exposed rats demonstrated significant (p<0.05) dysregulated antioxidant enzymes activity, increased lipid peroxidation, urea and creatinine concentrations as well as compression of the glomerulus, collapse of the tubular lumen and interstitial congestion. However, administration with *T. daniellii* showed significant improvements as evidenced by significant antioxidant enzymes activity, reduced MDA, Urea and creatinine concentrations, and normal kidney histology. In conclusion, findings from this study showed that *T. daniellii* possesses nephroprotective properties against copper sulphate-induced toxicity in Wistar rats.

Keywords: Copper sulphate, Nephrotoxicity, *T. daniellii*, kidney, Wistar rat

Introduction
A crucial trace element that is widely distributed in both human and animal tissues, copper (Cu) is essential for a number of enzyme functions [1]. It is an essential part of metalloenzymes such as cytochrome oxidase, peroxidases, and catalase [1, 2]. However, there are serious health and environmental hazards associated with high copper levels, especially in drinking water [3, 4, 5]. Materials such as building components, sheets, strips, and alloy products are among the sources of copper contamination [3, 5]. Excessive copper induces oxidative stress by promoting the overproduction of reactive oxygen species (ROS), leading to lipid peroxidation and subsequent cellular damage [6, 7].

Copper can be absorbed through the skin, lungs, and gastrointestinal tract, subsequently entering the bloodstream [1, 2, 8, 9]. Once in circulation, it binds to plasma proteins such as albumin and amino acids and is transported to the liver [8, 9]. In the liver, copper is incorporated into ceruloplasmin and released back into the bloodstream [7, 9]. The kidneys and liver are the primary targets of copper toxicity, particularly in cases involving copper sulphate ($CuSO_4$) [10]. Excessive copper accumulation in these organs can result in severe damage, including hepatotoxicity and renal failure [10, 11, 12]. Given the critical role of the kidneys in maintaining homeostasis, understanding the mechanisms and impacts of copper toxicity is essential for evaluating its potential health risks.

Thaumatococcus daniellii, commonly known as the African sweet prayer plant, is gaining recognition for its potential as a safe and beneficial natural product [13, 14]. The plant is widely known for producing thaumatin, a naturally sweet protein, but it also contains bioactive compounds with diverse biological activities [13, 14, 15]. These include antioxidant, anti-inflammatory, and anti-mutagenic properties, making it valuable for both traditional medicine and modern applications [13, 14, 15].

Additionally, it is commonly used for food wrapping and as a source of natural fibers [14, 15]. In traditional medicine, *Thaumatococcus daniellii* is valued for its health-promoting properties, including its use in managing conditions such as respiratory issues, appetite loss, liver and kidney ailments, and other systemic disorders [13, 15, 16, 17]. The plant's bioactive components have shown promise in cleansing the body and enhancing overall well-being

Given its broad-spectrum biological functions, *Thaumatococcus daniellii* is implicated as a potential protective and therapeutic agent in mitigating the toxic effects of heavy metals. Accordingly, this study aimed to assess the nephroprotective potential of *Thaumatococcus daniellii* against copper-induced nephrotoxicity in Wistar rats.

Materials and Methods

Plant Extract: Fresh *Thaumatococcus daniellii* was procured from a farm in Igue-Iheya community, Edo State, Nigeria. The sample was identified and authenticated in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. The plant material was then air-dried at room temperature for two weeks to prevent the degradation of bioactive compounds. The dried sample was then pulverized into a fine powder using a blender. A portion of the powdered sample was weighed and placed in an airtight container with 100 g of water at room temperature. The mixture was occasionally shaken to enhance extraction. Filtration was performed using filter paper, a conical flask, and a funnel to separate the filtrate from the residue. The resulting extract was stored in a sample bottle and refrigerated for preservation.

Experimental Animals: Wistar rats for this study were procured and housed in the Animal Holding, Department of Anatomy, University of Benin, Benin City, Nigeria. They were fed Top feeds grower mash and clean water *ad libitum*. The animals were weighed at the commencement and weekly afterwards. The rats were allowed to acclimatize for two-weeks period before the commencement of administration.

Experimental Design: Thirty (30) Wistar rats weighing between 160g and 180g were used for this study. They were randomly assigned into six (6) groups (A, B, C, D, E, and F) of five (5) rats each. Group A served as control; Group B received 200 mg/Kg body weight of copper sulphate; Group C received 250 mg/Kg body weight of *Thaumatococus danielli*; Group D received 1000 mg/Kg body weight of *Thaumatococus danielli*; Group E received 200 mg/Kg body weight of copper sulphate + 250 mg/Kg body weight of *Thaumatococus danielli*; Group F received 200 mg/Kg body weight of copper sulphate + 1000 mg/Kg body weight of *Thaumatococus danielli*. All administration lasted for 28 days and was done orally using an oral gavage.

Biochemical analysis: The antioxidant activity was evaluated following established protocols: malondialdehyde [MDA] [18]; glutathione [GSH] [19]; superoxide dismutase [SOD] [20]; and catalase [CAT] [21]. Additionally, creatinine and urea concentrations were measured established methods [22].

Histological Assessments: The harvested kidney tissues were processed and routinely stained using hematoxylin and eosin, according to the method previously reported [23].

Results

Effect of Treatment on Oxidative Stress: In copper sulfate-only treated rats, there was a significant decrease (p<0.05) in the levels of SOD, CAT, and GPx, accompanied by a corresponding significant increase (p<0.05) in MDA concentration compared to the control group. Conversely, in copper sulfate-exposed rats treated with *Thaumatococcus daniellii*, a significant increase (p<0.05) in SOD, CAT, and GPx levels was observed, along with a corresponding significant decrease (p<0.05) in MDA concentration.

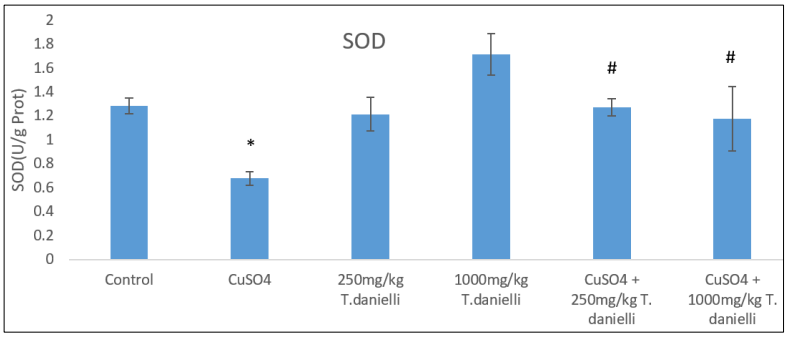

Fig 1: Superoxide dismutase enzyme activity across the experimental groups. Values are given as mean ± SEM. * p<0.05 compared to control; # p<0.05 compared copper sulphate only.

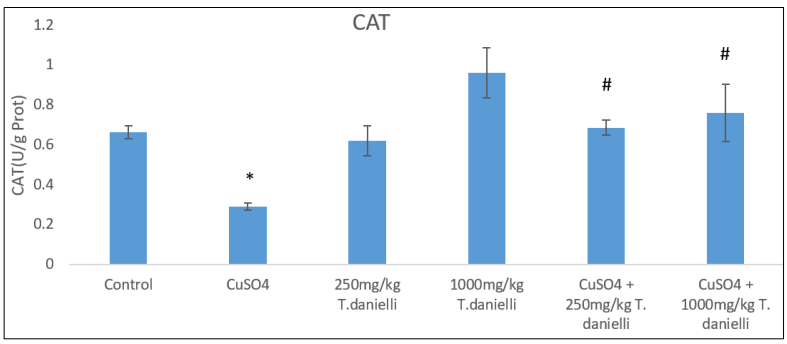

Fig 2: Catalase enzyme activity across the experimental groups. Values are given as mean ± SEM. * p<0.05 compared to control; # p<0.05 compared copper sulphate only.

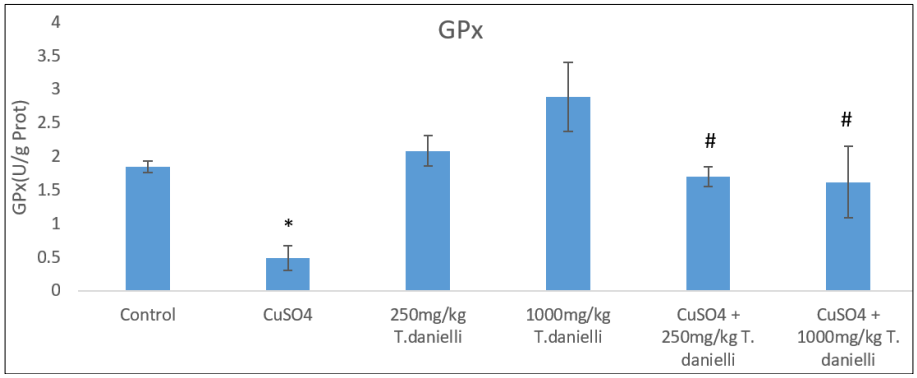

Fig 3: Glutathione peroxidase enzyme activity across the experimental groups. Values are given as mean ± SEM. * p<0.05 compared to control; # p<0.05 compared copper sulphate only.

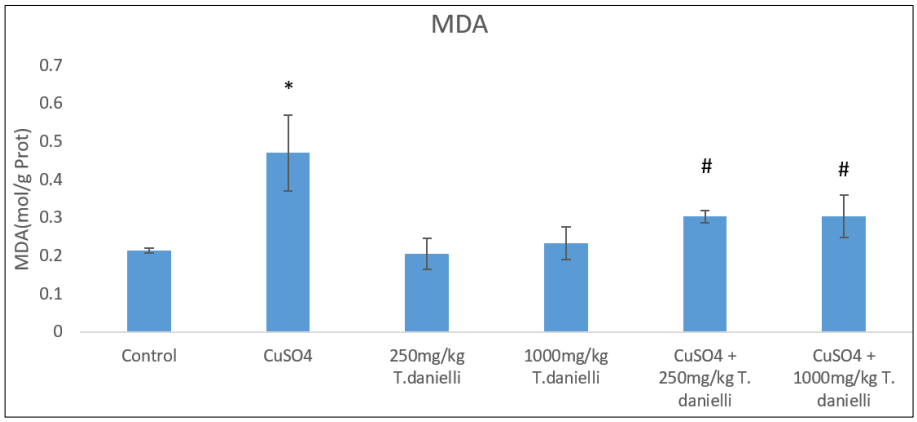

Fig 4: Malondialdehyde concentrations across the experimental groups. Values are given as mean ± SEM. * p<0.05 compared to control; # p<0.05 compared copper sulphate only.

Effect of Treatment on Renal Function: In rats treated with copper sulfate alone, urea and creatinine levels showed a significant increase (p<0.05) compared to the control group. However, in copper sulfate-exposed rats treated with *Thaumatococcus daniellii*, a significant decrease (p<0.05) in urea and creatinine levels was observed.

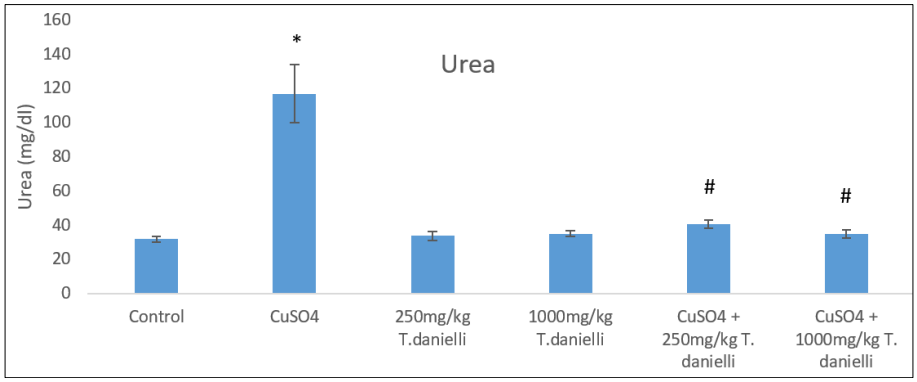

Fig 5: Serum Urea levels across the experimental groups. Values are given as mean ± SEM. * p<0.05 compared to control; # p<0.05 compared copper sulphate only.

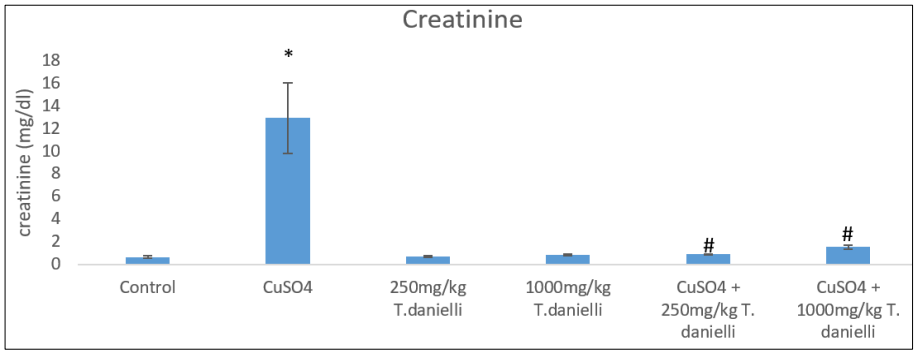

Fig 6: Serum Creatinine levels across the experimental groups. Values are given as mean ± SEM. * p<0.05 compared to control; # p<0.05 compared copper sulphate only.

Effect of Treatment on Histology

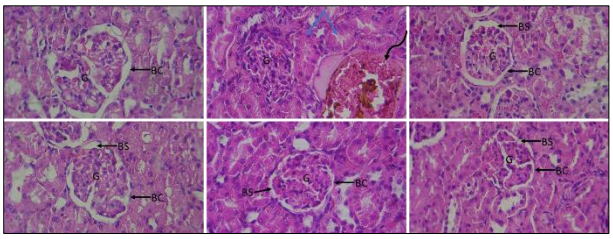

Plate 1: Representative photomicrograph of renal cortex of the experimental groups: A (Control group), B (100 mg/kg of copper sulphate), C (250 mg/kg BW of T. daniellii), D (1000 mg/kg BW of T. daniellii), E (100 mg/kg of copper sulphate + 250 mg/kg BW of T. daniellii), F (100 mg/kg of copper sulphate + 1000 mg/kg BW of T. daniellii) (H&E; 400x); A, C, D, E and F are normal with renal corpuscles made up of glomerulus (G), Bowman's capsule (BC) and Bowman's space (BS) as well as patent renal tubules (RT). In B, there is compression of the glomerulus (Gc), collapse of the tubular lumen (blue arrow) and interstitial congestion (coiled arrow).

Discussion

The kidney is particularly susceptible to copper toxicity due to its critical roles in filtration and excretion [10]. Excessive intracellular copper accumulation can disrupt redox homeostasis, triggering a cascade of harmful effects such as inflammation, degeneration, apoptosis, and necrosis [10, 11, 12]. This study demonstrated that exposure to $CuSO_4$ at a dose of 200 mg/kg resulted in significant renal injury, evidenced by elevated serum levels of creatinine and urea. Creatinine and urea are reliable markers for assessing kidney impairment, as they are nitrogenous waste products eliminated primarily by the kidneys. The findings align with those of Dai *et al*. [24], who reported that administering $CuSO_4$ at 200 mg/kg for 28 days in mice caused increased serum creatinine and urea levels. Furthermore, pronounced pathological changes were observed in the renal tissues of rats exposed to $CuSO_4$, including glomerular compression, tubular lumen collapse, and interstitial congestion. These structural abnormalities corroborated the alterations in serum renal function markers and mirrored the observations of Dai *et al*. [24], who documented tubular degeneration, cast formation, and glomerular degeneration in $CuSO_4$-treated mice. Similarly, Wang *et al*. [25] reported that exposing chickens to $CuSO_4$ at a dietary dose of 300 mg/kg for 12 weeks led to significant alterations in renal histoarchitecture, including tubular cell degeneration and necrosis. The presence of atrophied glomeruli and tubular casts indicates impaired glomerular filtration, providing a plausible explanation for the observed decline in kidney function.

These renal injuries are likely attributed to oxidative stress, which disrupts cell membrane integrity and functionality, leading to tissue damage. Numerous studies have highlighted that heavy metals can disrupt the oxidant–antioxidant equilibrium, resulting in the generation and accumulation of free radicals and, ultimately, oxidative stress [26, 27, 28, 29]. Oxidative stress arises from an imbalance between the production of free radicals and the body's antioxidant defense mechanisms [30]. SOD and CAT are integral components of the first line of antioxidant defense, acting as scavengers for free radicals [31, 32]. SOD catalyzes the conversion of superoxide radicals into hydrogen peroxide, while CAT, located in peroxisomes, facilitates the breakdown of hydrogen peroxide into water and oxygen [31, 32]. GPx, an essential enzymatic antioxidant, plays a critical role in reducing hydrogen peroxide and lipid hydroperoxides by using glutathione as a substrate [31]. Consequently, SOD, CAT, and GPx are reliable indicators for assessing antioxidant capacity. Additionally, MDA serves as a crucial marker of oxidative damage and free radical activity, as it is a byproduct of lipid peroxidation [33].

In this study, the significant increase in MDA levels within renal tissues following Cu exposure suggests an intensification of lipid peroxidation, highlighting the detrimental oxidative effects of copper. Concurrently, the marked reduction in SOD, CAT, and GPx activities in the kidneys of $CuSO_4$-exposed rats compared to the control group reflects the depletion of these critical antioxidants in combating ROS. These findings indicate that excessive Cu disrupts the antioxidant defense system, thereby inducing oxidative stress. Our results align with previously reported studies [24, 34, 35, 36, 37], further corroborating the oxidative damage associated with copper toxicity.

The findings of this study revealed the protective potential of *T. daniellii* extract against $CuSO_4$-induced nephrotoxicity. This protective effect was evident through the normalization of serum renal function parameters, restoration of antioxidant marker levels as well as reduction in lipid peroxidation in renal tissues, and preservation of renal histological structure in $CuSO_4$-exposed rats treated with *T. daniellii*. These findings align with previous studies that highlighted the protective role of *T. daniellii* in mitigating nephrotoxicity caused by agents such as potassium bromate, streptozotocin, and monosodium glutamate [38, 39, 40]. The protective action of *T. daniellii* against renal oxidative injury may be attributed to its enhancing effect on the antioxidant enzymes and inhibitory action on ROS synthesis. Similarly, previous studies have documented the antioxidant potential of *T. daniellii* [41, 42, 43]. The antioxidant activity of *T. daniellii* is likely attributed to its rich content of phenolic and flavonoid compounds, which act as free radical scavengers, redox-active transition metal chelators, and enzyme modulators [41, 43].

Conclusion

The study demonstrated that copper sulfate ($CuSO_4$) exposure significantly induced oxidative stress and nephrotoxicity in Wistar rats, as evidenced by elevated serum urea and creatinine levels, along with renal histological damage. However, treatment with *Thaumatococcus daniellii* extract effectively mitigated these adverse effects. The extract restored antioxidant enzyme activities, reduced lipid peroxidation (measured by MDA levels), and improved renal function markers. Histological examination revealed preservation of renal tissue structure in rats treated with the extract, further confirming its protective role. These findings suggest that *Thaumatococcus daniellii* possesses nephroprotective properties, likely due to its antioxidant and free radical scavenging capabilities, offering a potential therapeutic approach to combat copper-induced nephrotoxicity.

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