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In-vitro study: *Murraya koenigii* Leaf (Curry leaf) extract's anti-oxidant effect on NaF-induced sheep RBC oxidative stress

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Abstract
In this study, the antioxidant properties of *Murraya koenigii* were investigated in the context of oxidative stress induced by sodium fluoride in sheep red blood cells. Erythrocytes are particularly susceptible to oxidative damage due to their high content of polyunsaturated fatty acids. The experiment involved a 24-hour incubation of red blood cells with sodium fluoride, with ascorbic acid and *Murraya koenigii* were investigated in the context of oxidative stress induced by sodium fluoride in sheep used as protective agents against oxidative stress. Assessment of oxidative stress was based on changes in SOD, GSH, and MDA levels in the RBCs. Treatment with both ascorbic acid and *Murraya koenigii* extract successfully restored these levels to normal, indicating their potential to mitigate oxidative stress induced by sodium fluoride in erythrocytes.

Keywords: *Murraya koenigii* Leaf, anti-oxidant, NaF, RBC

Introduction
Under typical circumstances, the body has several defenses in place to neutralize reactive oxygen species (ROS), which are natural by products of metabolism. Oxidative stress arises when there's an imbalance between ROS and antioxidants [1]. As defined by Sies, oxidative damage occurs when this balance tilts in favor of ROS, potentially causing harm [2]. Fluoride is known to disrupt antioxidant enzymes, leading to increased ROS formation. Fluoride exists naturally in various forms and is commonly used in compounds, but it's not universally present in nature. When fluoride is in drinking water, it's completely ionic, rapidly and thoroughly crossing the intestinal lining and interfering with vital energy processes in living organisms [3]. While fluoride has a significant preventive effect in reducing tooth cavities at low doses, higher levels can result in dental and skeletal fluorosis. Some studies suggest that fluoride may boost the generation of reactive oxygen species and decrease the activity of antioxidant enzymes like glutathione peroxidase, superoxide dismutase and catalase [4]. However, the precise mechanism behind these systemic effects remains unknown.
NaF has been found to impact ROS levels and antioxidant properties in mouse kidneys. Fluoride exposure has been demonstrated to induce oxidative damage in various organs such as the liver, kidney, testicles, spleen, brain, heart and cecal tonsil, while also reducing the expression of key antioxidant enzymes in different animals [5]. A 70-day administration of fluoride in female mice revealed oxidative damage in the liver [6]. Despite existing research on the link between fluoride and oxidative damage, comprehensive investigations into the molecular mechanisms of NaF-induced hepatic oxidative damage in mice are lacking. This damage is associated with increased levels of reactive oxygen species and malondialdehyde activity, leading to reduced mRNA expression and activity of antioxidant enzymes [6].
Murraya koenigii, commonly known as curry leaf, is a plant native to South Asia in the Rutaceae family. Its leaves are prized for their aromatic flavour and are a vital ingredient in Indian and South Asian cuisine, especially in curries [7]. Beyond its culinary role, curry leaf is believed to have medicinal potential, including antioxidant, antimicrobial, anti-inflammatory, and anti-diabetic properties. Traditional medicine has utilized it to address various issues like digestion, diabetes, and skin conditions [8]. Packed with essential oils, flavonoids, polyphenols, and other bioactive compounds, curry leaf adds both taste and potential health benefits.

This versatile herb enjoys popularity in both traditional and modern cooking as well as herbal medicine [9].

Materials and Methods

Leaf extract preparation

Freshly harvested *Murraya koenigii* leaves were air-dried, powdered, and stored in an airtight container. To extract their compounds, 10 grams of the powder were dissolved in 100 ml of ethanol and shaken on an orbital shaker for 48 hours. The extract was then filtered using Whatman Filter Paper and subsequently evaporated. Finally, the resulting extract was dissolved in millipore water at a concentration of 100 mg/ml. (Fig-1).

Preparation of 10% RBC suspension

2 ml of sheep blood was collected from the jugular vein and placed in an EDTA vial. After that, it was centrifuged at 4 °C for 10 minutes at 1000 rpm. The resulting plasma was washed in PBS three to four times until a clear supernatant was obtained. To create a 10% RBC solution, 200 µl of RBC pellet was mixed with 1800 µl of cold PBS and the solution was stored at -20 °C for future use. (Fig-1).

Fig 1: Pictorial representation of leaf extract and RBC suspension preparation.

Induction of oxidative stress

The 10% RBC suspension was divided into distinct sets and exposed to various concentrations of Sodium Fluoride, Ascorbic acid and *Murraya koenigii* leaf extract. These sets were then incubated on an orbital shaker for 24 hours, following the experimental protocol.

Table 1: Experimental Design

Group	Treatment
I	RBC control (no treatment)
II	RBC + NaF (50 µM)
III	RBC + NaF (50 µM) + ascorbic acid (100 µg)
IV	RBC + NaF (50 µM) + *Murraya koenigii* (100 µg)

Antioxidant enzymes

Protein concentration was determined employing the Lowry method. The assessment of tissue lipid peroxidation, SOD and GSH is followed a previously documented method [10].

Statistics: The experimental results were presented as mean ±SE values. Statistical analysis was conducted using Graph Pad Prism, involving one-way analysis of variance followed by Tukey's multiple comparison test. Significance was determined at $p<0.05$.

Results

In Group II (toxic), total protein decreased from 9.67±1.5 (control) to 5.4±0.58. Groups III and IV also showed significant total protein reductions (7.2±0.42 and 6.5±0.51, respectively). SOD and GSH activities in Group 2 (NaF-treated) were markedly lower ($p<0.01$) than in Group 1 (control). GSH levels were 77.53±3.71 µg GSH/mg of protein in NaF-treated RBCs, increased to 116.5±3.57 µg GSH/mg of protein with *Murraya koenigii* treatment, and showed non-significant levels in Group III (ascorbic acid). SOD levels in control and toxic groups were 11.63±1.1 and 5.5±0.9 SOD units/mg protein, respectively. *Murraya koenigii* significantly raised SOD levels to 12.78±1.6 SOD units/mg protein, providing significant protection. However, no notable difference was observed between Groups IV and III. TBARS activity in Group 2 (NaF-treated) significantly increased ($p<0.001$) to 646.3±7.41 compared to the control (382.2±9.9). Conversely, Group IV (*Murraya koenigii* treatment) and Group III (ascorbic acid treatment) showed substantial TBARS reductions ($p<0.001$ and $p<0.01$, respectively), with values of 496.4±7.51 and 494.1±5.96.

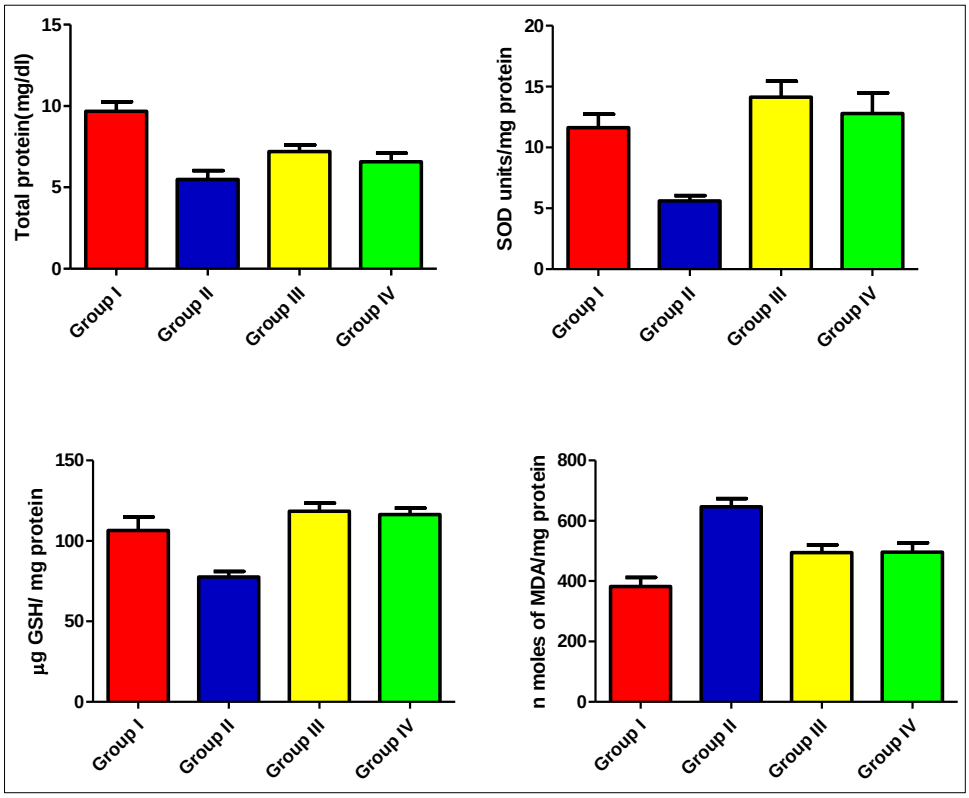

Fig 2: Graphical representation of Total protein, SOD, GSH and MDA.

Discussion

Oxidative stress results from an imbalance between ROS production and their defense mechanisms, potentially causing harm. Increased MDA signals lipid peroxidation, reduced glutathione and decreased SOD activity - clear indicators of oxidative stress [11]. The NaF group exhibited elevated MDA levels, but these were significantly reduced by both ascorbic acid and *Murraya koenigii* treatment. *Murraya koenigii*, rich in Phyto constituents, can generate ROS but also contains antioxidants like GSH, crucial for maintaining cellular integrity. GSH effectively eliminates free radicals by donating hydrogen atoms [12]. SOD plays a protective role by converting superoxide anions into harmless oxygen and hydrogen peroxide. In the NaF group, GSH levels dropped, but *Murraya koenigii* treatment effectively restored them [13]. This study's findings align with research, demonstrating a decrease in MDA levels, an increase in GSH, and the restoration of SOD activity following *Murraya koenigii* treatment [11].

Conclusion

Murraya koenigii, at a concentration of 100 µg, has exhibited remarkable antioxidant capabilities, effectively countering oxidative damage induced by sodium fluoride.

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