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# Nephroprotective activity of polysaccharide from red macroalga *Falkenbergia rufolanosa* against methyl thiophanate-induced oxidative stress and kidney inflammation

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**Abstract**
This study sought to investigate the protective potentials conferred by the polysaccharide extracted from red marine alga *Falkenbergia rufolanosa* (FRP) against methyl Thiophanate (MT)-induced oxidative injuries in kidney tissue. Therefore, adult wistar rats were divided in four groups: control; MT (700 mg/kg) injected intraperitoneally for 3 days; FRP (200 mg/kg) administrated via alimentation for 7 days followed by MT co-injection at the last 3 days and only FRP (200 mg/kg) administrated via alimentation for 7 days. Data revealed a significant perturbation in plasma biochemical and oxidative stress parameters associated with the Nephrology toxicity features. These results were confirmed by kidney his to-architecture consorted by detection of Bcl-2, IL-1beta, and P53 examined via immunohistochemistry revelation. Nevertheless, co-treatment with FRP regulated MT-induced Nephrology toxicity as shown by an improvement in kidney biochemical, histological and immunohistochemistry profiles. In conclusion, FRP could be considered as a good source of naturally occurring antioxidant and Nephrology protective agent.

**Keywords:** Polysaccharide, methyl Thiophanate, Nephrology toxicity, Nephrology protective

## Introduction
Nowadays, many polysaccharides obtained from various marine algae have attracted much of attention because of their different biological activities [1]. Most of these algal polysaccharides are generally associated with pharmacological properties such as anticoagulant, antitumor, Immunomodulation and antioxidant activities [2].
*Falkenbergia rufolanosa*, an edible red alga, contained various active substances such as protein, polysaccharides, amino acid, nucleic acid and vitamin [3]. Previous investigations of red algae have revealed the important of algal polysaccharides with multitudinous pharmaceutical properties. Currently, few studies have focused on the isolation of polysaccharide from the red marine alga *Falkenbergia rufolanosa.* Among their various potential application in biology and pharmacology, sulfated polysaccharides from red seaweeds are considered as the important and interesting natural sources occurring antioxidant effect [4].
Correspondingly, pesticides are one of the most known compounds that affect the disturbance of the balance oxidant/antioxidant inducing the installation of oxidative stress, which disrupt the mammalian health. Among pesticides, methyl Thiophanate (MT), is a systemic fungicide broadly used for the control of fungal diseases of crops. Many researches established that MT could affect the cell division by stopping some metabolizing enzymes and disrupting biological cell activities [5]. Interestingly, addition of natural antioxidants could be designed as an alternative method for stopping the installation of oxidative damages and limiting, as a consequence, several oxidative diseases.
Therefore, the present study aimed to investigate the protective effects of polysaccharide extracted from *Falkenbergia rufolanosa* against MT-induced kidney oxidative damage by evaluating the oxidative stress markers, plasma biochemical parameters, histopathological changes and the inflammatory microenvironment detected by kidney immunohistochemistry

Test and through Bcl-2, IL-1beta, and P53 genes expression.

## Material and Methods

### Plant material

The macroalga *Falkenbergia rufolanosa* was collected from S fax City (south of Tunisia), washed with distilled water to remove impurities. Then, the cleaned material was ground to powder and stored in plastic bags in a dry dark place before use.

The polysaccharide extraction protocol was realized according to Liu *et al* [6].

### Animals and experimental design

Adult Wistar rats, weighing 180±4 g were housed in plastic cages under standard conditions with a constant light/dark cycle at a temperature of 22±2 °C and 40% of humidity.

Rats were divided into four groups of 8 animals each:

- Group 1 (control group) rats received oil corn injection, used as vehicle;
- Group 2 received by intraperitoneally a single injection of 700 mg/kg of MT for 3 days;
- Group 3 received FRP at a dose of 200 mg/kg of body weight administrated via alimentation for 7 days followed by MT co-injection at the last 3 days;
- Group 4 received only FRP at a dose of 200 mg/kg of body weight administrated via alimentation for 7 days.

The treatment period and the dose of MT were determined according to previous studies to be toxic but not lethal [7]. The dose of algal polysaccharide was shown in previous studies to induce benefic effects without being toxic, referring to kammoun *et al* [8].

The experimental procedures were depicted according to the Natural Health Institute of Health Guidelines for Animal Care and approved by the Ethical Committee of S fax Sciences Faculty. All animal procedures were conducted in strict conformity with the "Institute ethical committee guidelines" for the Care and Use of laboratory animals [9].

After the sacrifice of rats, blood samples were collected in heparin tubes, which served to determine biochemical parameters. Kidneys were immediately dissected out and cleaned for oxidative stress markers. Other samples of the kidney were fixed in 10% formalin solution for histological studies.

### Protein quantification

Protein content in kidney homogenate was estimated according to Lowry *et al*. [10] using bovine serum albumin as a standard.

### Oxidative stress markers

### Determination of lipid peroxidation assay

The lipid peroxidation [11] and advanced oxidation protein product (AOPP) [12] levels were determined in kidney homogenate.

### Determination of enzymatic and non - enzymatic antioxidants

Superoxide dismutase (SOD) (13), glutathione peroxidase (GPx), reduced glutathione (GSH) (15-16) and catalase (CAT) (17) activities were depicted in kidney homogenate.

### Biochemical assays

The levels of urea, uric acid, and Creatinine in plasma and urine were determined spectrophotometric ally using commercial diagnostic kits (References: 20151, 20143 and 20091) purchased from Bio Maghreb (Ariana, Tunisia).

### Histological studies

Pieces of kidney from different groups of rats were fixed in a 10% formalin solution for 48 h. The fixed tissues were immersed in paraffin and catted in 5 µm thick sections. Different ssections were then colored with haematoxylin-eosin and visualized under a Motic AE2000 light microscope.

### Kidney immunohistochemistry

Tissue samples from kidney of each group were prepared as described by Cherif *et al* [18] the immunohistochemistry (IHC) samples were visualized using conventional image analysis software.

### Statistical analysis

The data were analyzed using the statistical package program Stat view 5 Software for Windows (SAS Institute, Berkley, CA). Statistical analysis was performed using one-way Analysis of Variance (ANOVA) followed by Fisher's Protected Least Significant Difference (PLSD) test as a post hoc test for comparison between groups. All values were expressed as means ± S. D. Differences were considered significant at $p< 0.05$.

## Results

### Lipid peroxidation and AOPP levels in Kidney

The present results revealed an enhancement in lipid and protein peroxidation in the kidney of MT-treated group as demonstrated by the elevation in the MDA and AOPP levels in kidney samples of adult rats (Fig 1). The co-administration of FRP to the MT group regulated these perturbations (Fig 1).

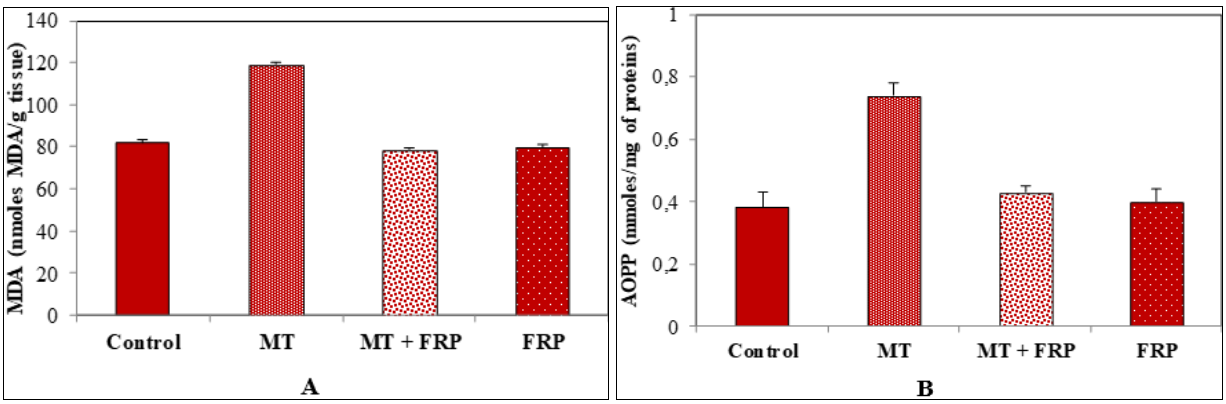


**Fig 1:** (A) Malondialdehyde content (MDA); (B) Advanced oxidation protein product (AOPP) levels in different treated groups

**Antioxidant enzymes**

Our results depicted the effect of FRP on enzymatic and non-enzymatic antioxidants of different experimental rats. A significant elevation in the activities of enzymatic antioxidants including SOD, CAT, and GPx in the kidney was noted (Table 1).

Supplementation of FRP ameliorated the enzymatic antioxidant activities, which reached normal values. The FRP group indicated no noticeable variation in the activities.

**Table 1:** Effect of the treatment on enzymatic and non-enzymatic antioxidant markers in the different groups

| Parameters | Treatment groups | | | |
|---|---|---|---|---|
| | Control | MT | MT + FRP | FRP |
| GSH (mg/g tissue) | 543.15±2.27$^{d}$ | 356.25±2.77$^{a}$ | 525.21±2.22$^{b}$ | 504.32±2.08$^{c}$ |
| GPx (n moles of GSH/min/mg protein) | 1.024±0.12$^{a}$ | 1.89±0.056$^{d}$ | 1.21±0.052$^{b}$ | 1.13±0.071$^{c}$ |
| Catalase (m moles $H_2O_2$ degraded/min/mg protein) | 5.21±0.73$^{b}$ | 5.51±0.49$^{a}$ | 5.35±0.83$^{b}$ | 5.16±0.16$^{b}$ |
| SOD (U/mg of protein) | 96.091±1.15$^{b}$ | 122.51±1.75$^{c}$ | 95.73±1.32$^{b}$ | 92.36±1.13$^{a}$ |

Results are expressed as mean of three experiments ± SD. The number of determinations was n = 3. $^{a.\ b.\ c.\ d}$ In the same column indicate significant differences ($p< 0.05$)

**Plasma and kidney biochemical markers**

Creatinine and Urea levels in the MT-treated rats were higher in plasma and lower in urine when compared to normal rats (Table 2). Contrarily to uric acid which noted a significant reduction in plasma and urine (Table 2). However, a significant regulation in these parameters was noted due to FRP addition.

**Table 2:** MT, MT + FRP and FRP on plasma and urine levels of Creatinine. Uric acid and urea

| Paramètres | Treatment groups | | | |
|---|---|---|---|---|
| | Control | MT | MT + FRP | FRP |
| Creatinine *(mmol/L)* | | | | |
| Plasma | 142.46±1.028$^{a}$ | 251±1.58$^{c}$ | 145.16±1.47$^{b}$ | 143.08±1.14$^{a}$ |
| Urine | 3241.56±1.77$^{d}$ | 2251±1.58$^{a}$ | 3145.16±1.47$^{b}$ | 3193.08±1.14$^{c}$ |
| Uric acid *(mmol/L)* | | | | |
| Plasma | 335.04±1.12$^{d}$ | 213.6±1.14$^{a}$ | 319.38±1.15$^{b}$ | 326.77±1.32$^{c}$ |
| Urine | 1576.25±1.17$^{d}$ | 920.82±1.63$^{a}$ | 1488.35±1.96$^{b}$ | 1513.27±1.59$^{c}$ |
| Urea *(mmol/L)* | | | | |
| Plasma | 4.35±0.31$^{a}$ | 8.37±0.34$^{c}$ | 4.96±0.23$^{b}$ | 4.65±0.24$^{b}$ |
| Urine | 4.41±0.12$^{c}$ | 2.37±0.34$^{a}$ | 4.038±0.084$^{b}$ | 4.25±0.12$^{c}$ |

Results are expressed as mean of three experiments ± SD. The number of determinations was n =3.$^{a.\ b.\ c.\ d}$ In the same column indicate significant differences ($p < 0.05$)

**Histological studies**

Histological changes in the kidney sections are shown in Figure 2. In the MT-treated rats, kidney histological pictures showed numerous abnormalities (Figs. 2B) observed in glomeruli and in tubules, when compared to controls (Figs. 2A). In addition, this fungicide provoked multiple foci of hemorrhage, inflammatory leukocyte infiltration, and vacuolization of tubules in the kidney his to-architecture. According to microscopic examinations, co-administration of FRP attenuated kidney histopathological perturbation seen in the MT-treated group (Figs. 2C). The histological observation was normal in rats treated only with FRP (Figs. 2D).

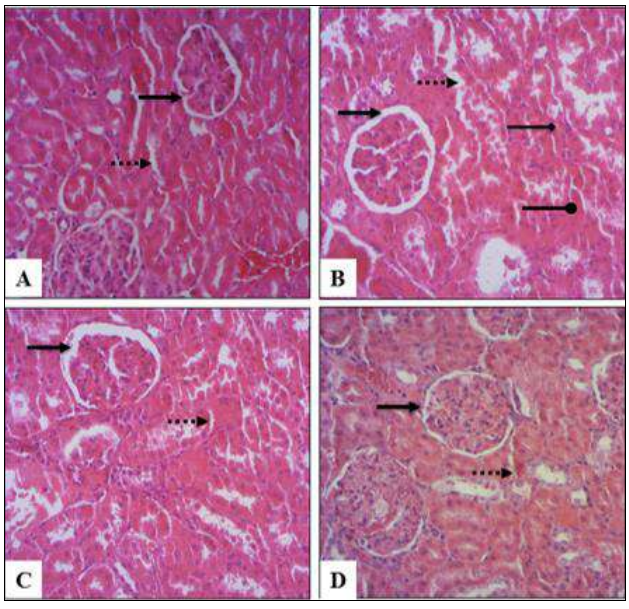


**Fig 2:** (A-D) Histological changes of the kidney tissues stained with H&E at magnification (x200).

**Arrows indicate**

→ Tubular lumen;
- - > glomeruli;
—● Hemorrhage;
—◆ Leucocyte

**Kidney immunohistochemistry**

Figure 3 illustrated the results of immune his to chemical evaluation of IL1-béta, Bcl2 and P53 expression in the different experimental groups. In fact, the immunostaining positive cells of these proteins were considered a positive, when the cells' nuclei became stained a dark brown color (Fig. 3). Data showed that the rats treated with MT (Fig 3 A2) have the most intense IL1-beta expression, when compared with the control group (Fig 3 A1).

In addition, Fig. 3B and Fig. 3C summarized the results of immune his to chemically evaluation of Bcl-2 and P53 expression in the different experimental groups. Data revealed that these anti-apoptotic factors were not expressed in normal renal tissue (Fig. 3 B1 and Fig. 3C1), while there were highly presented in MT treated rats (Fig (Fig. 3 B2 and Fig. 3 C2). However, the co-treatment with FRP (Fig. 3 A3-B3 and C3) attenuated IL1-béta, Bcl2 and P53 expression in the renal tissue of rats exposed to MT, when compared with the control group.

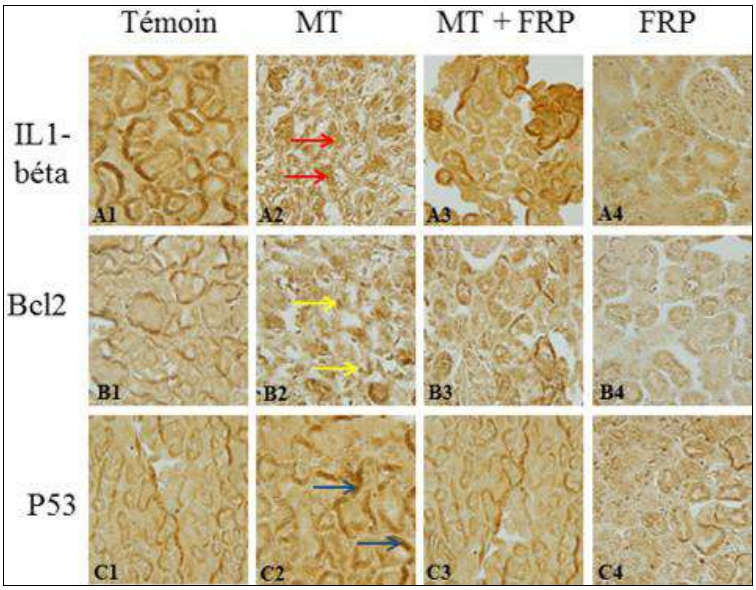


**Fig 3**: (A1-A4) IL-1 beta expression analysis by immune his to chemical staining in kidney of rat at magnification (x200); (B1-B4) Bcl-2 expression analysis by immune his to chemical staining in kidney of rat at magnification (x200); (C1-C4) P53 expression analysis by immune his to chemical staining in kidney of rat at magnification (x200).

**Arrows indicate**

→ IL-1 beta positive Cell;
→ Bcl-2 positive Cell;
→ P53 positive cell.

**Discussion**

The kidney is a dynamic organ controlling the body homeostasis. Renal tissue could be altered by many chemical compounds and drugs, including xenobiotic [19]. The present investigation studied the protective effect of FRP against MT-induced Nephrology toxicity.

This fungicide could over generate reactive oxygen species (ROS), reacting directly with proteins, lipids, cell biomolecules and DNA (20-21). In fact, MDA is considered as one of the most specific oxidation product of lipid peroxides mostly generated by lipid oxidation. Our results depicted an elevation in MDA and in AOPP contents in the kidney homogenate of the MT-treated group. In addition, it may be assigned that the increase in lipid peroxidation level induced by this fungicide treatment may result from a perturbation in antioxidant enzyme activities. Among the enzymatic antioxidants, SOD, CAT, and GPx are considered as the first line of defense enzyme. In fact, the enhancement in the activity of these enzymes after MT exposition could be related to the overproduction of ROS, which surpassed the capability of detoxification mechanisms. In the other hand, a decrease in the non-enzymatic antioxidant, including GSH levels, was noted in MT treated groups, improving the installation of oxidative stress. Interestingly, the co-administration of FRP avoided the oxidative perturbations induced by MT intoxication in Wistar rats. The present data confirmed the beneficial effect of algal polymer due to the presence of bioactive compounds and to its capability to scavenge ROS.

In a second set of analyses, biochemical parameters in kidney such as urea, uric acid, and Creatinine were evaluated in urine and plasma. Generally, Creatinine and urea are considered as the most common parameters in the evaluation of renal dysfunction [22]. In the present study, plasma urea and Creatinine levels augmented, while that of uric acid diminished in rats exposed to MT. These modifications in renal biochemical parameters are generally related to urinary tract obstruction, nephritis, and certain extra-renal diseases [23-24]. In the other hand, the decrease in plasma uric levels are generally related to a decrease in antioxidant defense systems [25]. Interestingly, the perturbation in plasma and urine biochemical parameters in the MT-treated group was restored by parallel

administration of FRP, which neutralize the formation of ROS and induce a reduction in the oxidative stress injuries. This data confirmed the previous researches revealed by a various *in vitro* experiments as well as *in vivo* animal, confirming the potential effect of polysaccharides in human and animal health

These biochemical modifications were confirmed by renal histological observation. Data indicated an elevation in the glomerular area in the rats treated with MT. Histopathological lesions were characterized by the presence of leucocytes infiltration and a depletion in glomerular filtration rate in the kidney tissue. Histopathological changes could be related to the overproduction of ROS and the elevation in lipid peroxidation levels. The supplementation of FRP treated rats attenuated the above mentioned data. The present study is supported by previous research of Jaballi *et al* [26], who declared that algal polysaccharide had efficient Nephrology-protective effects on rat models.

In addition, the investigation of inflammatory microenvironment in kidney, related to IL1-beta expression was determined by immunohistochemistry test on kidney tissue sections. These results depicted that the most intense positivity of IL1-beta expression was observed in exposed rats. As a response to the inflammation reaction, the proinflammatory cytokines, such as IL1-beta stimulated the inflammatory processes triggering, as a consequence apoptosis. Once apoptotic pathways have been activated, cytoplasmic P53 binds and the anti-apoptotic Bcl-2 proteins DE clenched the apoptotic process [27]. As known, Bcl-2 and P53 pertains to apoptosis-related gene products, crucial for the regulation and the control of cell cycle arrest and apoptosis, which correlate with the generation of ROS [18]. Data revealed that these anti-apoptotic factors were not expressed in normal kidney tissue, indicating that normal functioning of these cells is not dependent on these proteins. However, Bcl-2 and P53 were observed in the MT treated groups. The significant elevation in these genes expression due to MT exposition is an indication of the damage of kidney cells explained by the failed attempt of the anti-apoptotic proteins to repair the damaged cells. Interestingly, the co-treatment with FRP attenuated the proinflammatory cytokines and the anti-apoptotic proteins expression in the rat kidney tissue, when compared with the control. The supplementation of this polymer appeared to be an efficient method for counteracting the apoptotic toxicity induced by MT through the antioxidant influence of the polysaccharide by inhibiting the phosphorylation of P53 and thereby suppressing NF-κB activity [28].

## Conclusion

To conclude, our study demonstrates, for the first time, that polysaccharide extracted from *Falkenbergia rufolanosa* controlled and regulated the MT-induced Nephrology toxicity in rats as demonstrated by the balance of oxidant/antioxidant markers, plasma biochemical parameters and kidney histological and immunohistochemistry observations. In fact, the algal polymer can be a useful and effective agent against Nephrology toxicity and renal failure.

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