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Evaluation Of MT-Liver Protective And Antioxidant Effects Of Red Algae

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Background: Antioxidants include minerals, anthocyanins, flavonoids, and phenolics. They can help prevent and treat diseases associated with oxidative stress and reactive oxygen species.
Objective: This study aims to investigate the potential of the red sea macroalga Falkenbergia rufolanosa to shield animal models against MT-induced liver injury.
Methods: Studies done in the past have shown that the dose and length of MT treatment were bad, but they did not cause death. Studies in the past have shown that giving a certain amount of methanolic fluid from algae can have positive effects with no negative effects.
Results: This research showed that the animal model with liver damage had stronger antioxidant defences against free radicals. This work reports the first results about how the algae can protect mammalian models from the harmful effects of MT on their blood, DNA, and cells. Oxidative stress markers were found in the supernatants after the samples were mixed and washed. Extra liver tissues were sealed in paraffin so that they could be studied histologically.
Conclusion: The study mentioned above shows that Falkenbergia rufolanosa protects against MT-induced hepatotoxicity in rats. Our work shows this for the first time. This effect is because of the algae's antioxidant properties and the variety of antioxidant parts it has, like polyphenols.

Keywords: Hepatoprotective, anti-oxidative, preclinical research, liver disease.

INTRODUCTION:

Scientists think that high amounts of reactive oxygen species (ROS) in cells are linked to a number of diseases in humans, including diabetes, heart disease, cancer, and getting older. High levels of oxidative stress eventually cause apoptosis, which damages DNA, oxidizes proteins, and kills parts inside cells [1-3]. So, using possible antioxidants is good for people's health. Antioxidants can stop and treat oxidative stress and lower the damage that oxidation does to cells. Trichloromethyl free radicals are made when a chemical that can cause cancer, like CCl4, breaks down. Reactive oxygen species (ROS) levels rise because of these radicals, which hurt hepatocytes. An increase in reactive oxygen species (ROS) causes hepatocyte necrosis and apoptosis after an acute liver injury. This causes a lot of cell damage to the liver [2-4].

CCl4 can cause immediate liver damage that needs to be stopped or treated by lowering the levels of reactive oxygen species (ROS) and stopping the oxidative chain reaction that CCl4 starts [4-6]. Compounds that work as antioxidants and lower the amount of ROS inside cells could be used as medicines to stop and treat oxidative damage. Methylthiophanate (MT) is a benzimidazole fungicide that is often used to protect crops from a number of serious fungal diseases. Reactive oxygen species (ROS) are molecules that contain oxygen. They play a part in many biological processes, like how the immune system works and how cells talk to each other normally [5-7].

High amounts of reactive oxygen species (ROS) may be a major factor in the development of solid tumors, hematological diseases, and cancer. Because of this, reactive oxygen species (ROS) are created when there is a mismatch between oxidants and cell antioxidants because of oxidative stress [6-8]. This causes biomolecular oxidation, which includes DNA damage, lipid peroxidation, and protein oxidation. The ongoing study showed that bone and red blood cells exposed to MT had higher amounts of MDA, AOPP, and LDH activities. Over time, free radicals have been shown to damage proteins and lipids through oxidation damage [7-9]. A lot of the time, high amounts of MDA, increased osmotic fragility, and higher LDH activity were signs of intravascular hemolysis. Erythrocytes are especially susceptible to oxidative damage because they have a lot of oxidative metabolic activity. This can make them better at dealing with oxidative stress or put their lives at risk [8-10]. The study also looked at whether the level of antioxidants in both organs made the ways that MT is harmful easier. Falkenbergia rufolanosa, a type of red algae, has been shown to protect against liver damage caused by methyl thiophanate in rat models by reducing free radicals and protecting the liver.

MATERIAL AND METHODS:

Materials:

For the study, the air-dried powdered leaf had to be collected and extracted. At first, 300 ml of methanol solvent was added to 20 g of dried algae powder crude extracts. Following that, the mixture was stirred and left to sit at room temperature for 18 hours. The solution was evaporated, and then a Whatman filter paper was used to separate it. As the last step, the waste that had been dried out was kept at a temperature of 4 °C. This was used in the studies that

followed.

Experimental Design:
Previous research has shown that the amount of MT used and the length of time it was used were harmful but not deadly. Studies in the past have shown that giving a certain amount of algae methanolic extract can have positive benefits with no negative effects. The wistar rats were put to sleep after the dose was given, and blood samples were taken using heparin tubes to look at molecular markers. The livers were quickly taken out, cleaned, and weighed. This was found in the supernatants after they had been homogenized and rinsed: signs of oxidative stress. To do histology studies, extra liver samples were put in a 10% formalin solution and then covered in paraffin to keep them safe [10-12].

Plasma biomarkers levels:
Spectrophotometry was used to measure the amount of thiobarbituric acid reactive chemicals in the liver homogenate. The results were then shown as the amount of malondialdehyde present, which shows how much lipid peroxidation there was. A wavelength of 532 nanometers was used to measure the absorption and nanomoles of malondialdehyde (MDA) were used to measure the amounts of MDA per milligram of protein. The method involved using spectrophotometry at a wavelength of 340 nm to find out the amounts of advanced oxidation protein product. The amount of AOPP was found and given as μmoles/mg protein using the extinction coefficient of 261 cm-1 mM1 [11-13].

The Antioxidant Activity:
The deep violet DPPH radical is a stable free radical. An antioxidant lowers the radical's strength by giving DPPH an electron, which changes its color from violet to yellow. The amount of staining shows how well the antioxidant works. It is mixed with a DPPH solution that is either ethanol or methanol-based. A spectrophotometer is used to measure the absorbance at a certain frequency of 517 nm after a set amount of time has passed. The drop in absorption shows that the antioxidants are working [12-14].

Histopathological and hematological Analysis:
Histopathological analysis is a key technique used in pathology to examine tissues and cells under a microscope to identify abnormalities and diagnose diseases. Here's a detailed overview of the methods involved in histopathological analysis. When rats were given MT, their white blood cell counts were higher than those in the control group, but their red blood cell, hemoglobin, and platelet amounts were lower. In rats that were given MT, the mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin content did not change. When MT was given along with algae extract, it significantly fixed the changes that happened compared to the control group [13-15].

Results:

Morphological Study:
In this study, the rats in any of the treated groups did not change their behavior in a noticeable way, either right after treatment or later on. This includes not being in pain, having trouble

breathing, moving or shaking, or having catalepsy. Additionally, no deaths were reported until the experiment was over. Also, both the absolute and relative liver weights dropped a lot when MT was added to the group that was not treated. According to these results, the rats that were given MT drank and ate a lot less. The groups of rats that were given alga extract along with MT had much better body weight gains than the groups that were only given MT (Table 1). [14-16]

Oxidative stress marker:

The study we did showed that MT treatment made lipid peroxidation levels rise significantly in the livers of rats. In the experimental rats that were given MT, the liver tissue homogenate had a lot more MDA levels than the control group. This is because of lipid peroxidation. Also, the amounts of AOPP and PCO in the liver homogenates of rats that were given MT were slightly higher than those in the control group (5% and 10%, respectively). Adding algae extract to the diet of animals lowered the amounts of PCO and AOPP, which in turn lowered the damage to liver tissue proteins. Following the administration of algal treatment, there was no discernible change in the levels of these two liver markers, as shown in Table 1 [15-17].

Table 1: Treatment effects on plasma biochemical markers in treated groups

Parameters	Alga	MT	MT+ alga	Control
HDL	0.25±0.08	0.42±0.14	0.39±0.50	0.12±0.65
LDL	1.30±0.02	1.49±0.13	1.52±0.40	1.13±0.14
CT	2.22±0.41	2.60±0.54	2.40±0.74	2.47±0.65
AST/ALT	2.90±2.13	2.45±1.36	5.10±0.50	4.20±0.70
Bilirubin	1.17±1.33	2.13±0.48	2.20±0.78	1.45±0.38
AST	120.34±24.89	140.78±15.29	122.44±15.12	118.78±12.59
ALT	42.4±12.65	54.15±2.44	41.47±17,25	28.35±2.60
GGT	2.8±0.50	3.58±1.41	3,40±2,53	2.70±1.57
TG	1.51±0.12	1.70±0.34	1,61±0,05	1.50±0.10

Plasma biomarkers levels:

The amounts of AST, ALT, GGT, and bilirubin in the plasma of people who had been exposed to MT were significantly higher than those in the control group (Table 2). The levels of AST were 50% higher, ALT levels were 20% higher, GGT levels were 45% higher, and bilirubin levels were 28% higher. Still, giving algae methanolic extract at the same time changed the amounts of plasma enzymes in a big way. The problems were much better in the group that was supplemented with the alga methanolic extract than in the group that was treated with MT. The rates of improvement reached the same level as in the control group [16-18].

Table 2: Treatment effects on enzymatic antioxidant activity in several treated

Parameters	Alga	MT	MT + alga	Control
Catalase	9.14±0.70	8.71±0.55	8.13±0.69	9.40±0.49
GSH	51.24±0.81	49.45±0.81	52.79±0.81	60.69±0.89
SOD	15.82±1.34	24.22±1.40	19.14±1.16	16.51±1.59
GPx	8.16±0.31	12.14±0.51	10.55±0.26	9.40±0.21

Histopathological and hematological Analysis:
When the slides from the group that was given MT were looked at under a light microscope, they showed a lot of very bad problems. The liver control pictures showed a central vein, sinusoidal gaps, and clear hepatic cells, all of which pointed to a normal cell structure. The livers of rats that were given MT, on the other hand, showed a lot of degenerative changes, including apoptosis, hepatic steatosis, hepatocyte vacuolization with increased sinusoidal gaps, and an infiltration of inflammatory leucocytes around the central vein. But giving the algae methanolic extract at the same time made the histology much better, as seen by the smaller amount of dead areas and the lack of vacuolization and steatosis. Figure 1 shows that the structure of the liver was very similar to the normal structure of the liver [17-19].

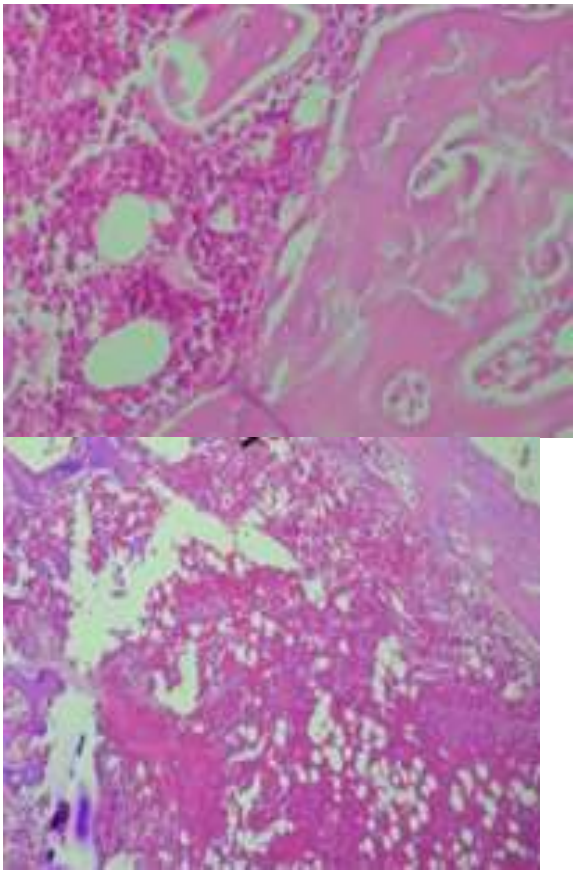

Figure 1: Alterations in the histology of the liver

Discussion:
Natural antioxidants are becoming more and more popular as a way to treat liver failure caused by high levels of reactive stress. When there is an imbalance between antioxidants, reactive oxygen species are made. These can cause a number of illnesses. The production of more free radicals can also cause protein structures to oxidize and crosslinks to form between proteins, which could break proteins into smaller pieces and lower the number of sulphhydryl groups

in amino acid chains [18-20]. In our work, we found that MT therapy caused reactive oxygen species to be released in the liver tissue, which damaged proteins through oxidative damage. In turn, this caused the amounts of protein carbonyl groups and advanced oxidation protein products to rise. These are well-known signs of protein oxidative damage. On the other hand, adding algal methanolic extract stopped liver damage caused by MT and stopped lipid peroxidation and protein oxidation in rats. This finding proved that adding algae did stop the oxidative chain reaction or get rid of the free radicals that were made in the liver [19-21].
The GSH antioxidant enzymes were much less active in the group that was exposed to MT than in the control group. There may be a link between the drop in GSH activity and more lipid peroxidation and less substrate available. The results show that the studies' conclusions are correct. But the gains seen were much bigger in the rats that were treated with MT along with the control group [20-22]. A drop in lipid peroxidation and a rise in antioxidant state showed this to be true. Studies have shown that the natural antioxidants in red algae can successfully reduce and repair liver damage. These new results back up those earlier studies. Based on our most recent research, we saw that after four weeks of MT treatment, the amounts of CT, LDL, and TG went up while HDL went down significantly [23-25].
Heart disease is greatly sped up by having high cholesterol. This is because it hurts the cells that line blood vessels (endothelial cells) and makes more harmful reactive oxygen species (ROS). This makes the development of atherosclerosis worse and may also hurt the liver (hepatotoxicity) [24-26]. It was shown that the algae extract was helpful because it lowered CT, TG, and LDL cholesterol levels and raised HDL cholesterol levels. MT can lead to a number of pathological problems in the liver, including bleeding, changes that happen over time, the widening of the sinusoids, swollen central veins, and changes in the structure of the tissue. The liver slices from the control animals showed a normal lobular pattern, a central vein that could be seen clearly, and a normal arrangement of hepatocytes with no signs of necrosis. Animals may be less likely to get tissue damage from MT if they are also given algae extract, which is a strong antioxidant. Polyphenols, flavonoids, and anthocyanins were found in large amounts in the algae fluid, according to earlier research [27-34].

CONCLUSION:

Our study showed that bone structure got better, protection against harmful free radicals got better, and bone and blood mineral metabolism got better controlled. This is the first study to look into how the algae can protect bone and blood from the damaging effects of MT, such as hematotoxicity, genotoxicity, and oxidative damage. Finally, because red marine algae has a lot of health benefits and is high in nutrients, there is proof to support using it wisely to treat conditions linked to oxidative stress.

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Conflict of Interest:
None

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