



N E W E A R T H <sup>TM</sup>

# Wild-Harvested AFA Microalgae from Klamath Lake

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## Cellular and Immune Protection

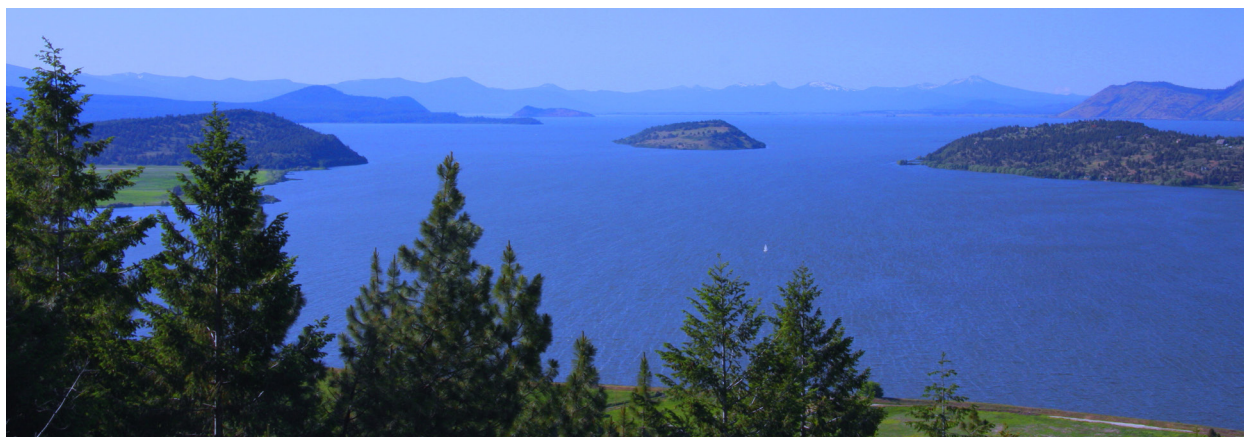


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# 1. The Klamath Lake Ecosystem

Upper Klamath Lake, in southern Oregon, presents a unique ecosystem, due to the combination of the volcanic geology, high altitude, and the properties of the water in the lake. The lake is located in the High Cascades, a volcanic part of the Cascade Range in western North America, stretching through Washington, Oregon, and Northern California. From Klamath Lake, there are 5 major visible volcanoes: Mount Shasta, Mount McLoughlin, Medicine Lake Volcano, and most notably, the former Mount Mazama, a volcano that erupted and collapsed approximately 7,700 years ago. The collapse of Mt Mazama led to the formation of Crater Lake, the deepest freshwater lake in the United States.



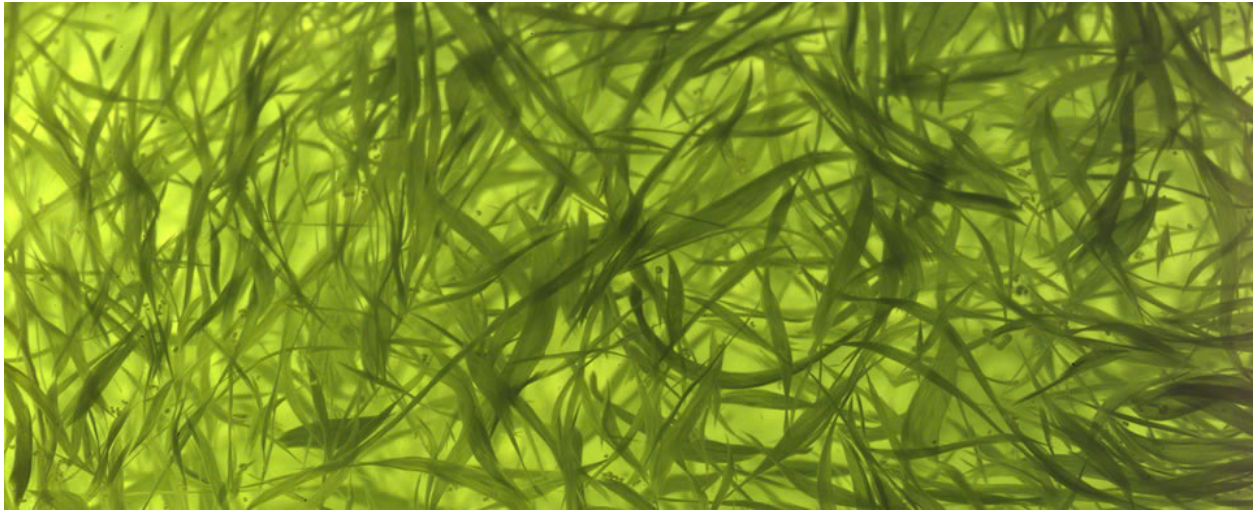
Upper Klamath Lake gets its water from Crater Lake in a unique manner. The volcanic shores of Crater Lake rise sharply up to form the former outline of Mount Mazama reaching over 8,000 feet above sea level, and 4,000 feet above Upper Klamath Lake. There are no rivers flowing out of Crater Lake. However, the lake water seeps through loose volcanic rock, and creeks and rivers on the outside of the caldera. Some of these rivers flow through grassland and forests before reaching Upper Klamath Lake. It is from such rivers that Upper Klamath Lake gets its water.

The nutrient level in Klamath Lake is very high. A large amount of volcanic ash from Mt Mazama's eruption is settled on the lake bottom, where the layer of ashes in some places is over 60 feet (20 meters) deep. The volcanic ashes, made up of small particles of volcanic rock combined with other components including potassium, is ejected during a volcanic eruption. The volcanic ash releases nutrients into the lake water. This supports the massive algal blooms.

Upper Klamath Lake is located in the High Cascades at a moderately high altitude at 4,200 feet, meaning that there is a higher exposure to UV radiation than at sea level. The climate in the high desert is arid, and there is an average of 300 days of sunshine per year, further increasing the UV exposure compared to more rainy Oregon coastal climates.

## 2. The Algae: *Aphanizomenon flos aquae*

The unique ecosystem in the waters of Klamath Lake includes the microalgae *Aphanizomenon flos aquae* (AFA). This alga represents the most ancient and primordial living beings on our planet, stretching back 3 billion years. Each living cell is microscopic, but they live in colonies that are organized in long strands. The long strands stick together, and when we look at the lake water with a bloom of AFA, it looks like floating fresh-cut grass.



*Photograph of large colonies of AFA floating in the lake waters.*

The AFA alga is edible and has been harvested and dried by native Americans for thousands of years. There have been numerous applications of algae as food and medicine for centuries, and they contribute greatly to human health. Currently, large-scale commercial harvesting of this wild-grown algae takes place on Klamath Lake, and even with the intensive harvesting, it does not disturb the ecosystem, because of the abundance of the AFA algae. It literally produces tons of biomass every 24 hours.

In the late fall and early winter, AFA prepares for hibernation, producing special Akinete cells that sink to the lake bottom, protected from frost. In the spring, the storage cells become active again, and AFA blooms typically start in June.

### 3. Immune Health

Our immune system provides protection from pathogens, including viruses and bacteria. Immune cells must travel throughout the whole body to look for threats to our health. Consumption of nutraceutical products can support the natural processes of immune surveillance and activation status within hours. Consuming AFA has such effects.<sup>1</sup>

Polysaccharides from AFA have immune-activating properties,<sup>2</sup> and increases the communication within the immune system via cytokines.<sup>3</sup> Natural killer cells treated with a polysaccharide-rich extract from AFA show a higher state of alert.<sup>4</sup>

### 4. Oxidative Stress

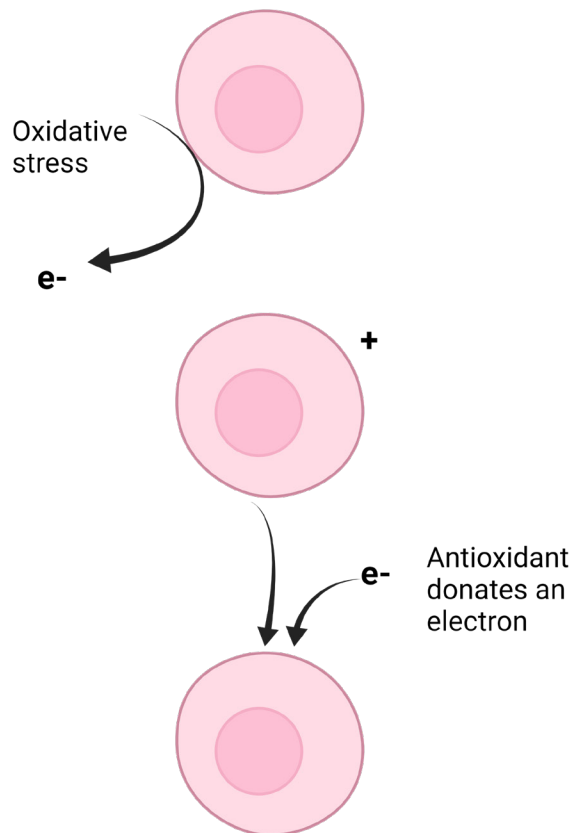
Oxidative stress is a condition that occurs when there are too many unstable molecules called free radicals in the body which are produced during normal metabolic processes as well as other factors such as exposure to ultraviolet (UV) radiation, pollution, illnesses, and inflammation. The free radicals are oxygen-containing molecules typically known as reactive oxygen species (ROS) containing unpaired electrons. They play an essential role in fighting off parasites and other pathogens in the body. Despite this, when there is an imbalance between the body's antioxidant defenses and its ROS production, there can be potential consequences for cells, tissues, and organs, as well as DNA. The consequences can include aging, cancer, cardiovascular and inflammatory conditions, and other ailments in the body. Essentially, oxidative stress occurs when the body does not have enough antioxidants.

AFA contains multiple compounds with antioxidant properties. The blue light-harvesting pigment, phycocyanin, contributes to the antioxidant capacity of AFA.<sup>5 6</sup> In addition, it has been demonstrated that other yet unknown compounds in this class of microalgae have complementary antioxidant and anti-inflammatory properties, acting in synergy with phycocyanin.<sup>7</sup>



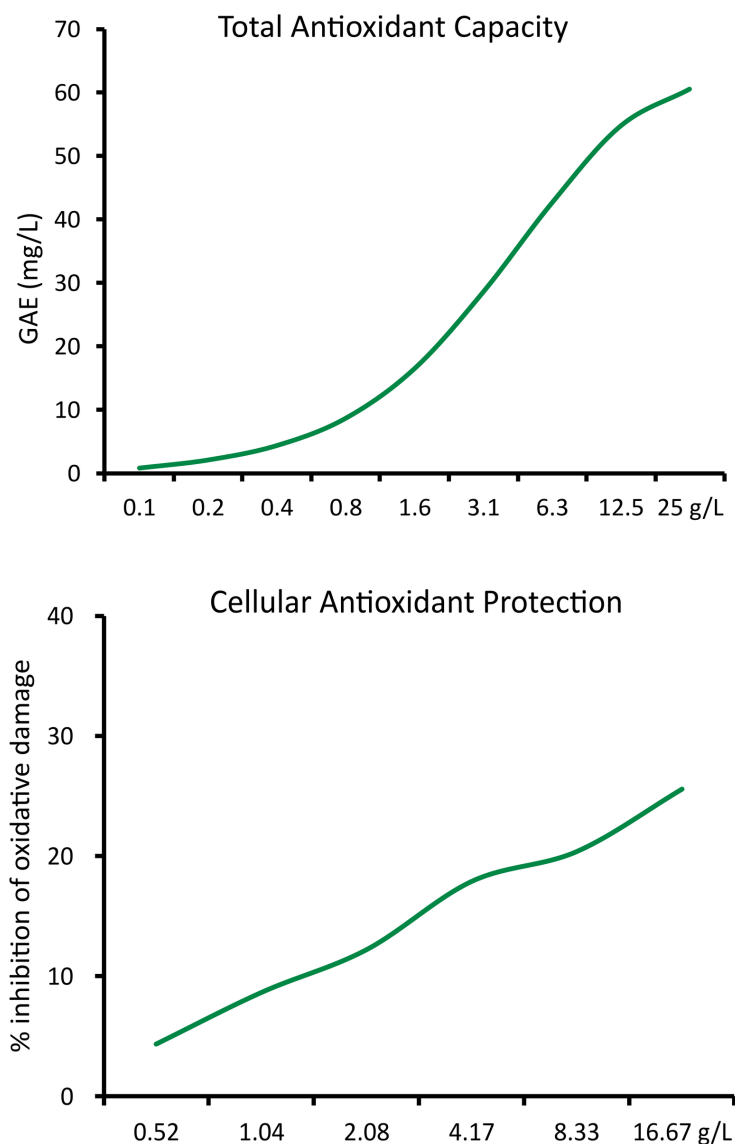
## 5. Antioxidants

Antioxidants are substances that help to protect our cells, tissues and organs, as well as DNA from damage caused by harmful free radicals (ROS) that play a role in oxidative stress which is associated with a variety of health problems. Antioxidants work by neutralizing free radicals, preventing them from causing damage to our DNA and other important cellular components.



*Figure 1. Diagram showing the loss of electrons under conditions of oxidative stress, creating an unbalanced electrical charge on cells and molecules. When an antioxidant donates an electron the system gains stability again.*

The data generated from our studies showed that a proprietary Wild AFA® Extract contains antioxidants. The total antioxidant capacity of AFA increased with an increase in dosage up to 25 g/L AFA in a chemical reaction. Furthermore, we know that some of the antioxidants in AFA can enter into living cells and protect the cells from the inside-out. This is seen from the cellular antioxidant protection of living cells by AFA, when cells were exposed to oxidative stress.



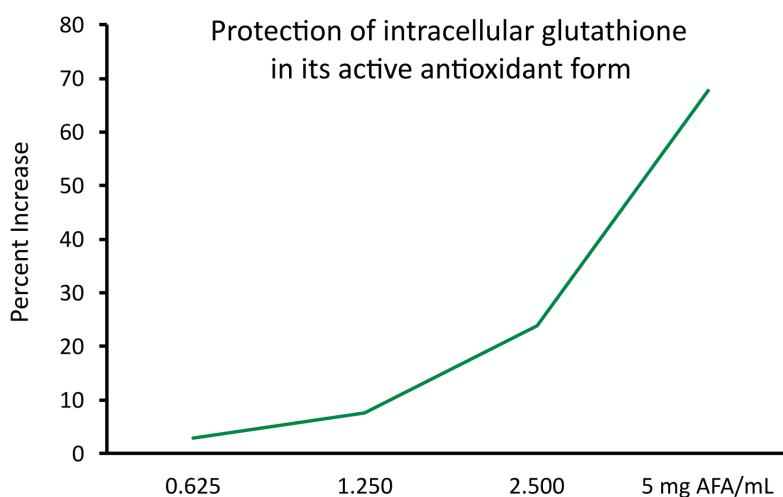
*Figure 2. Antioxidant properties of AFA. TOP: The total antioxidant capacity of AFA is shown. BOTTOM: The cellular antioxidant protection when living cells were exposed to severe oxidative stress in the laboratory.*

## 6. Protection of Active Glutathione

An important protection in our body comes from the antioxidant glutathione. This unique molecule is abundant inside all the cells in our body. It neutralizes free radical stress by donating an electron after which it needs to be restored to its antioxidant form. It is rapidly converted back to its active antioxidant form and continues to provide cellular protection.

Glutathione is also involved in detoxification of toxic metabolites produced under oxidative stress in the body when inflammation is present. It supports other antioxidants in the body to remain in their active antioxidant forms, including Vitamin C and E.

We tested the ability of Wild AFA Extract to support the active antioxidant form of Glutathione. We found that Wild AFA Extract provided robust protection, with over 70% increase in the relative abundance of active antioxidant Glutathione at the highest dose tested in human cell cultures.



*Figure 3. Percent increase in the abundance of the active antioxidant form of Glutathione (i.e., reduced Glutathione) inside human red blood cells in cell cultures.*



## 7. Immune Defense

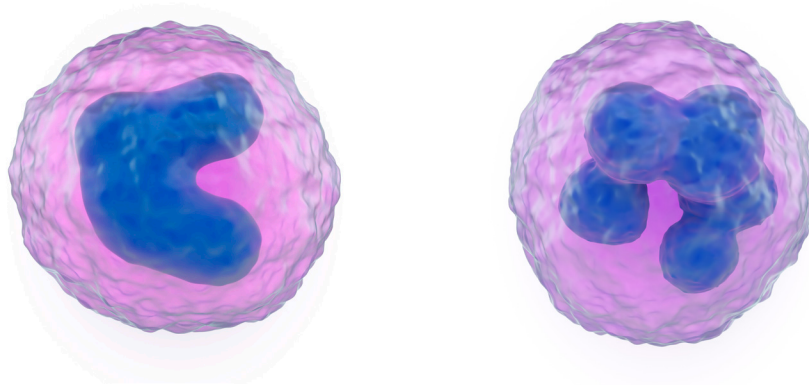
Immune protection is necessary for our health and survival. Much of this protection happens every minute throughout our body. Immune cells patrol our body to search for bacteria, virus-infected cells, and cancer cells.

An important part of this protection involves elimination of bacteria that can cause infection and illness. Specialized immune cells called phagocytes (*phago*: eat, *cyto*: cells), where these immune cells engulf bacteria, kill, and digest them.

We tested the ability of the Wild AFA Extract to enhance this phagocytic activity. We studied two different types of human phagocytes:

- Mononuclear phagocytes;
- Polymorphonuclear phagocytes.

The Wild AFA Extract caused a high degree of immune activation of phagocytic cells, which are part of our innate (immediate) immune defense mechanisms against invading pathogens.

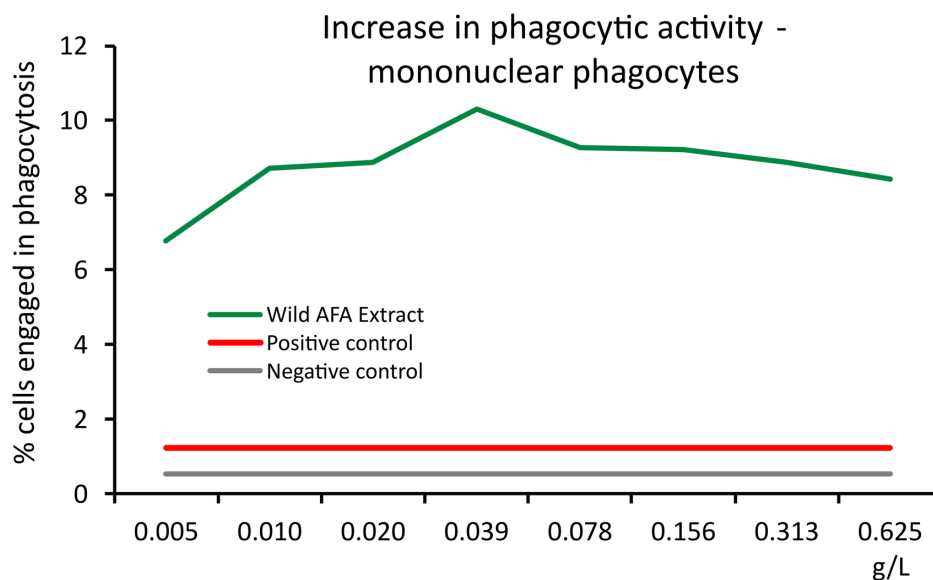


*Figure 4. Artistic rendering of the two cell types:  
LEFT: Mononuclear phagocyte. RIGHT: Polymorphonuclear phagocyte.*

Mononuclear phagocytes are also known as monocytes and are found in tissues all over the body where they play a crucial role in initiating and coordinating immune defense activity. They circulate the bloodstream and migrate to tissues where they differentiate into macrophages, which are highly efficient at engulfing and digesting foreign particles.

When the mononuclear phagocytes have digested foreign particles, they are able to present small, digested bits to other immune cells, in a concerted effort that leads to production of immunoglobulins and generates immunological memory. The memory ensures that if the same foreign particle invades the body again, the immune system will be at a higher alert and will respond quicker than the first time.

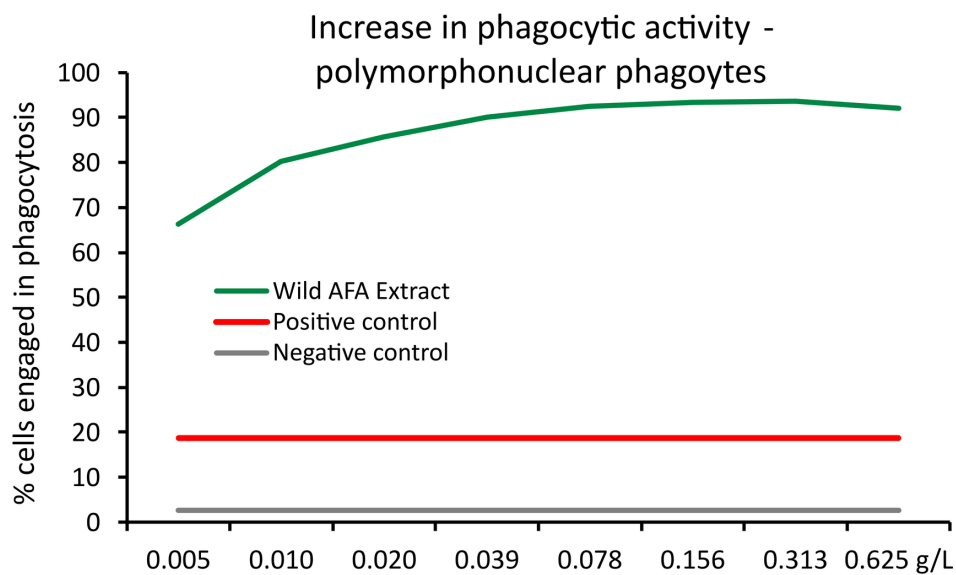
Mononuclear phagocytes treated with Wild AFA Extract showed a 10% increase in phagocytic activity. This is important, since the mononuclear phagocytes play a role in communication and coordination with the rest of the immune defense.



*Figure 5. Phagocytic activity of mononuclear phagocytes. The number of phagocytic cells treated with the wild AFA extract is shown with a green line, untreated cells with a grey line, and cells treated with an inducer of phagocytic activity with a red line. Cells treated with the wild AFA extract showed a 10% increase in this immune defense activity (green line). This was many times higher than the positive control (red line).*

Polymorphonuclear phagocytes, also known as neutrophils are smaller in size than the mononuclear phagocytes. They are the most abundant type of white blood cells and are the first in the line of defense against invading pathogens. They are able to quickly migrate to sites of infection or injury and engulf and digest invading pathogens.

Polymorphonuclear phagocytes treated with Wild AFA Extract showed over 90% increase in phagocytic activity.



*Figure 6. Phagocytic activity of polymorphonuclear phagocytes. The number of phagocytic cells treated with the wild AFA extract is shown with a green line, untreated cells with a grey line, and cells treated with an inducer of phagocytic activity with a red line.*

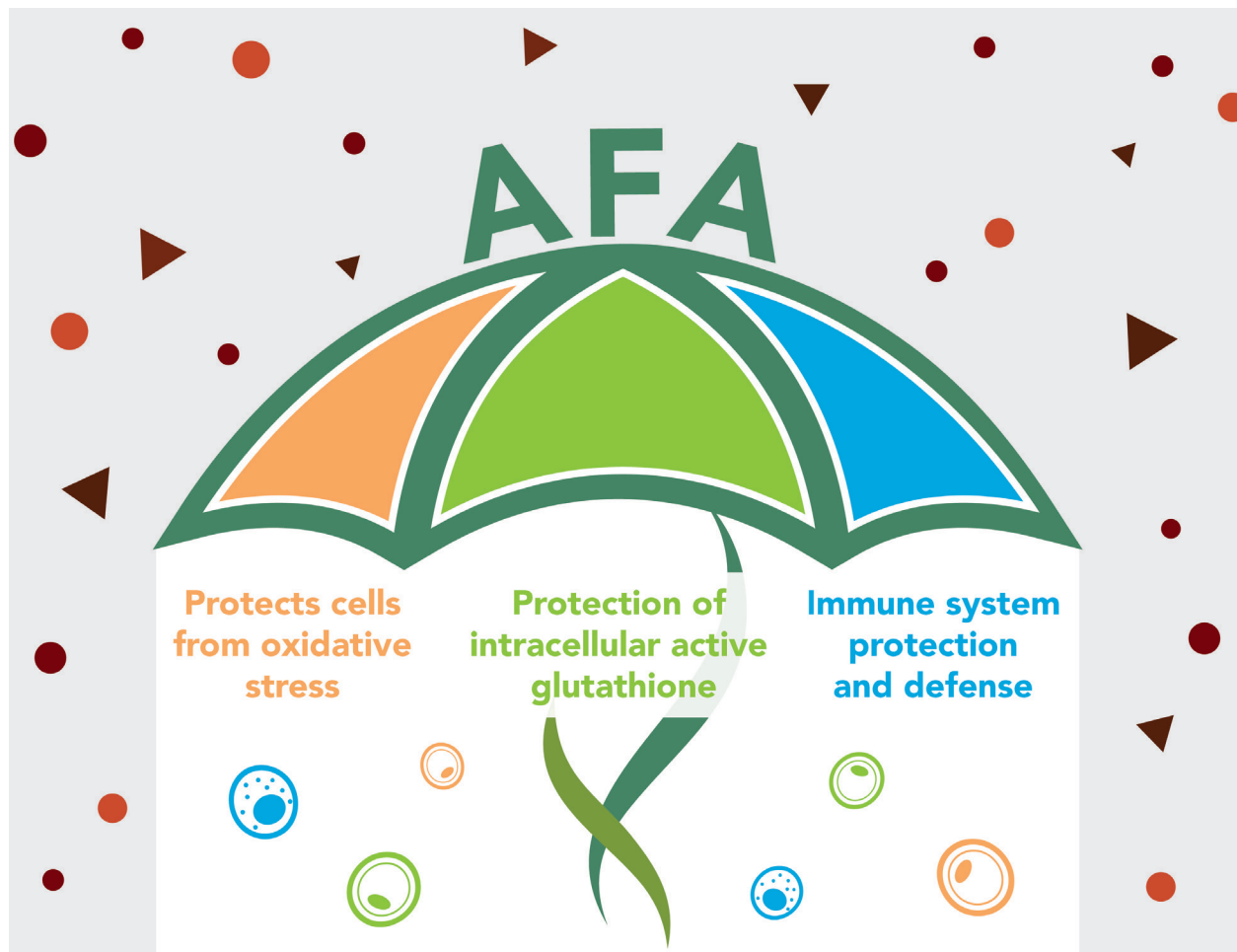
*Cells treated with the wild AFA extract showed over 90% increase in this immune defense activity (green line). This was many times higher than the positive control (red line).*

## 8. Tying It Together

The effects of AFA at the cellular level show an interesting triage, related to cellular and immune protection.

- Cellular protection from oxidative stress;
- Protection of intracellular active glutathione;
- Cellular protection from challenges by pathogens, through increased immune cell activity.

This is important, since oxidative stress is an underlying factor for accelerated aging, cognitive decline, and many inflammatory problems pertaining to cardiovascular health. Furthermore, the increased immune activity suggests that consuming Wild AFA Extract provides support of the immune system.



## 9. References

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- <sup>2</sup> Pugh N, Ross SA, ElSohly HN, ElSohly MA, Pasco DS. Isolation of three high molecular weight polysaccharide preparations with potent immunostimulatory activity from *Spirulina platensis*, *aphanizomenon flos-aquae* and *Chlorella pyrenoidosa*. *Planta Med.* 2001 Nov;67(8):737-42. doi: 10.1055/s-2001-18358. PMID: 11731916.
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- <sup>5</sup> Benedetti S, Benvenuti F, Pagliarini S, Francogli S, Scoglio S, Canestrari F. Antioxidant properties of a novel phycocyanin extract from the blue-green alga *Aphanizomenon flos-aquae*. *Life Sci.* 2004 Sep 24;75(19):2353-62. doi: 10.1016/j.lfs.2004.06.004. PMID: 15350832.
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