

# Analgesic and anti-inflammatory properties of a novel phycocyanin-rich microalgae extract, CyaninPlus™.

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## SUMMARY

CyaninPlus™ is an extract from microalgae. Its main component is Phycocyanin (PC), a known selective COX-2 inhibitor, with cardio- and neuro-protective effects. In addition to PC, CyaninPlus™ contains other anti-inflammatory compounds, different from PC, and with complementary anti-inflammatory properties.

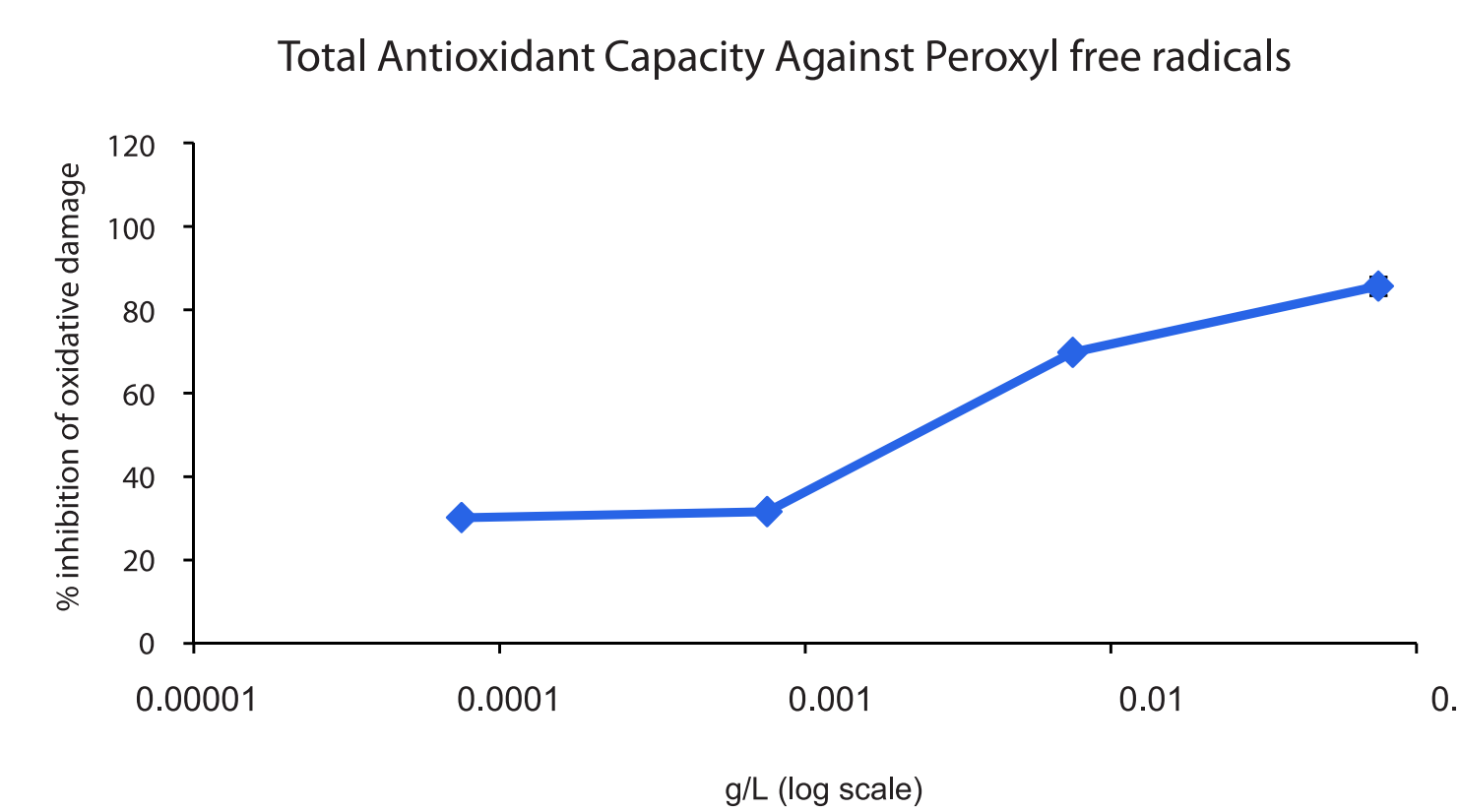
The objective of this pilot study was to evaluate the anti-inflammatory properties of CyaninPlus™ *in vitro*, and conduct a clinical pilot study on the pain reducing effects when human subjects consumed 1 gram daily.

We present data from a combined study of mechanisms of action, performed using *in vitro* bioassays, and a clinical pilot study.

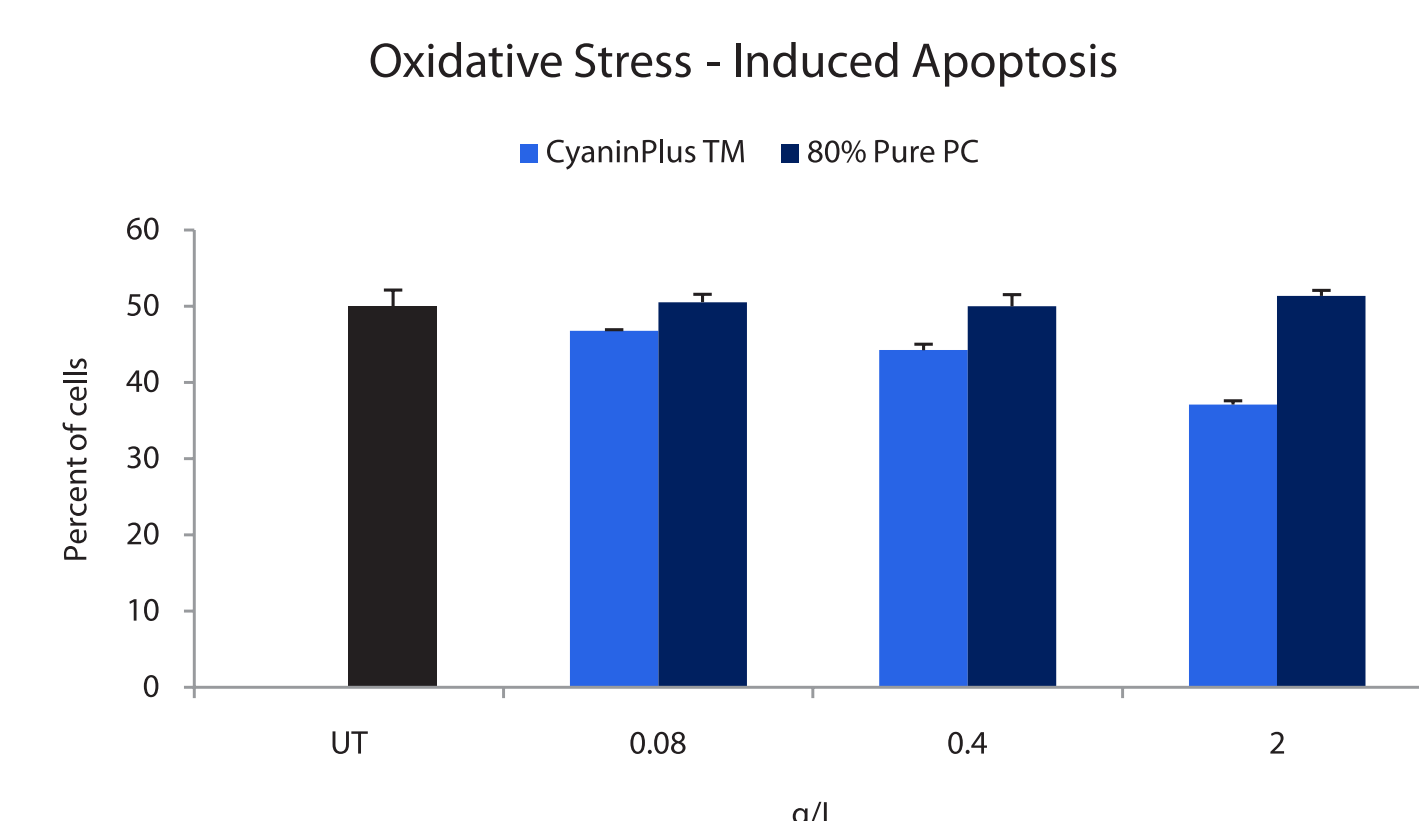
The data show that CyaninPlus™ inhibited both COX-2 and Lipoxygenase enzyme activities. CyaninPlus™ protected against reactive oxygen species (ROS) in several ways, both by a direct antioxidant effect, by reducing the formation of ROS by inflammatory cells, and by protecting cell viability under conditions of oxidative stress. The CyaninPlus™ mediated reduction of ROS formation was not seen when the same cell type was treated with pure phycocyanin, thus demonstrating non-PC compounds provided additional anti-inflammatory mechanisms of action. In addition, CyaninPlus™ treatment of inflammatory cells inhibited the migration of cells towards the inflammatory chemotactic compound Leukotriene B4 (LTB4). This property was seen also when PC was removed from the extract by ultrafiltration, indicating that other non-PC compounds added to the overall anti-inflammatory properties of the extract.

Based on this multi-faceted mechanism of action, involving both PC and non-PC compounds acting in synergy, a clinical pilot study was performed to examine effects on chronic pain. An open-label study design was used, involving 13 people consuming 1 gram CyaninPlus™ daily for 2 weeks. All subjects reported improvements from chronic pain shortly after study start. Weekly follow-ups showed rapid relief from both chronic pain and pain associated with exercise. In eight participants, marked improvements were noted as early as three days after consumption. After one week, a statistically significant reduction in perceived pain and use of medication for pain was observed. Other reported improvements included reduced swelling of hands and feet, better range of motion of affected areas, and improved ability to manage activities of daily living.

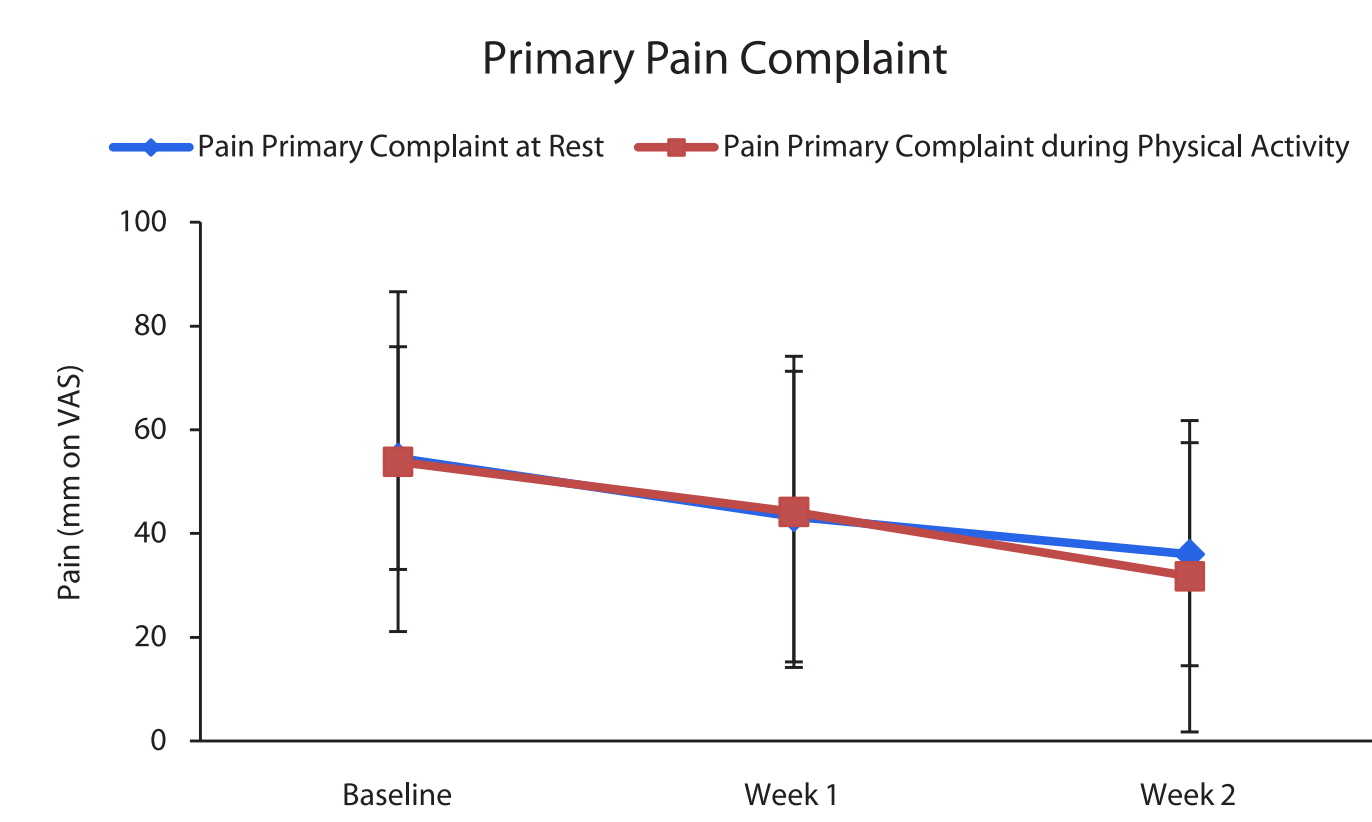
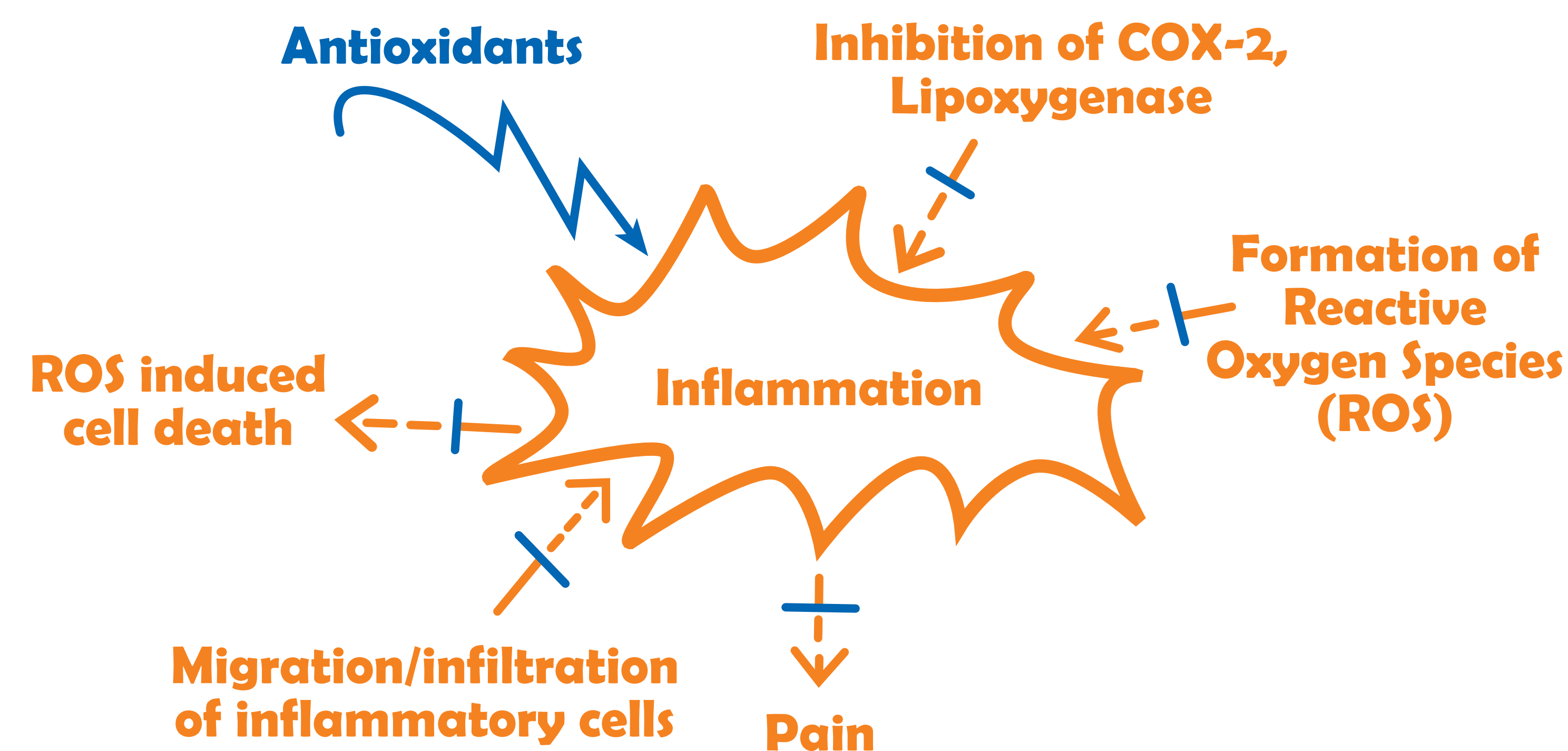
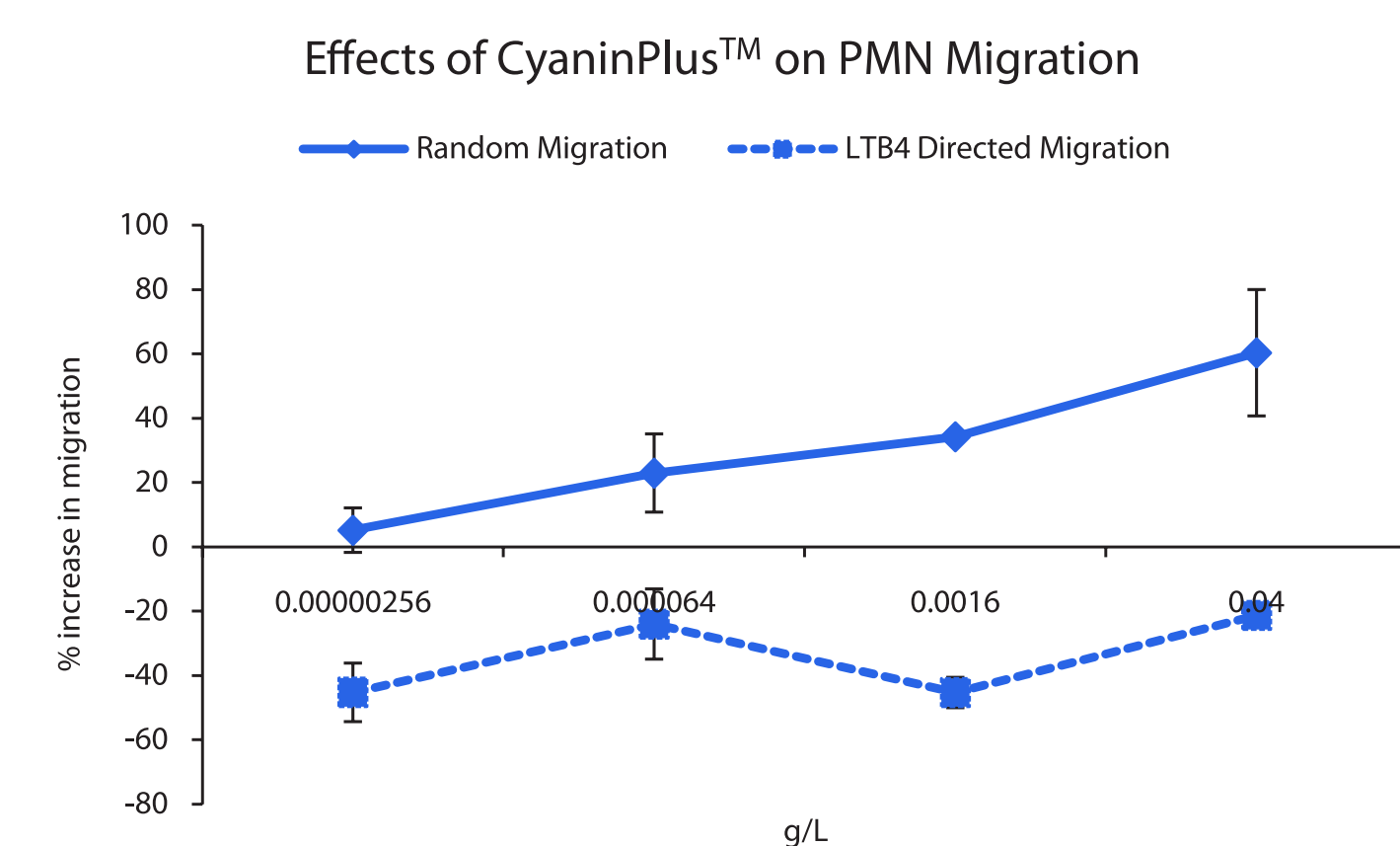
**Figure 1.** The antioxidant protection of CyaninPlus™ was tested in the Total Antioxidant Capacity Against Peroxyl free radicals (TACAP) assay, which unlike other antioxidant assays does not use organic solvents. This allows testing of compounds such as PC. The IC50 value of CyaninPlus™ was 0.004g/L.



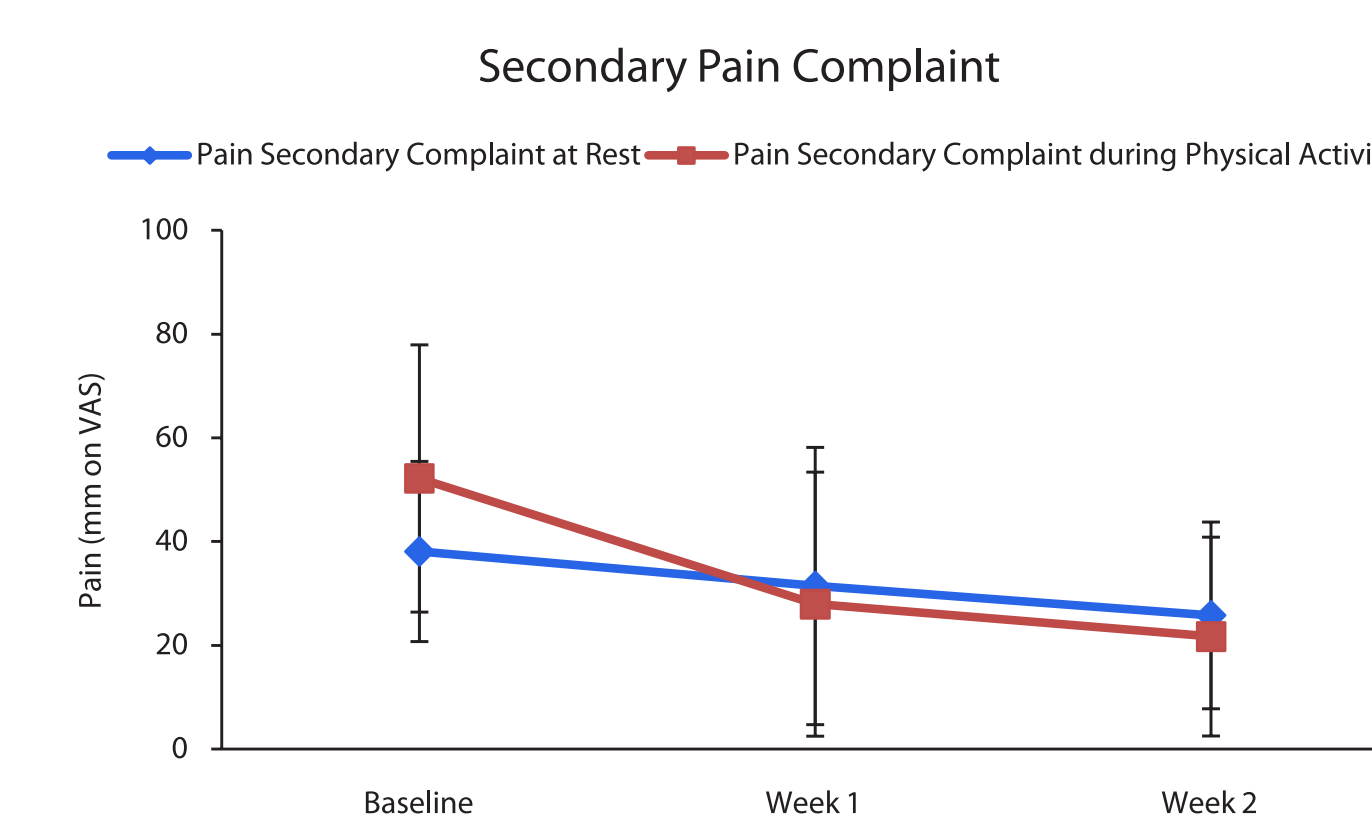
**Figure 2.** CyaninPlus™ protected human polymorphonuclear (PMN) cells from oxidative stress-induced programmed cell death (apoptosis). PMN cells were cultured overnight with or without CyaninPlus™. The cells were stained with Annexin V FITC, which binds to phosphatidyl choline externalized on the membranes of apoptotic cells. Determination of apoptosis was performed by flow cytometry. A dose comparison was performed to an 80% pure PC extract. This showed that CyaninPlus™ was better at protecting PMN cells from apoptosis than the more concentrated PC extract, indicating that additional, non-PC compounds were responsible for some of the protective effect.



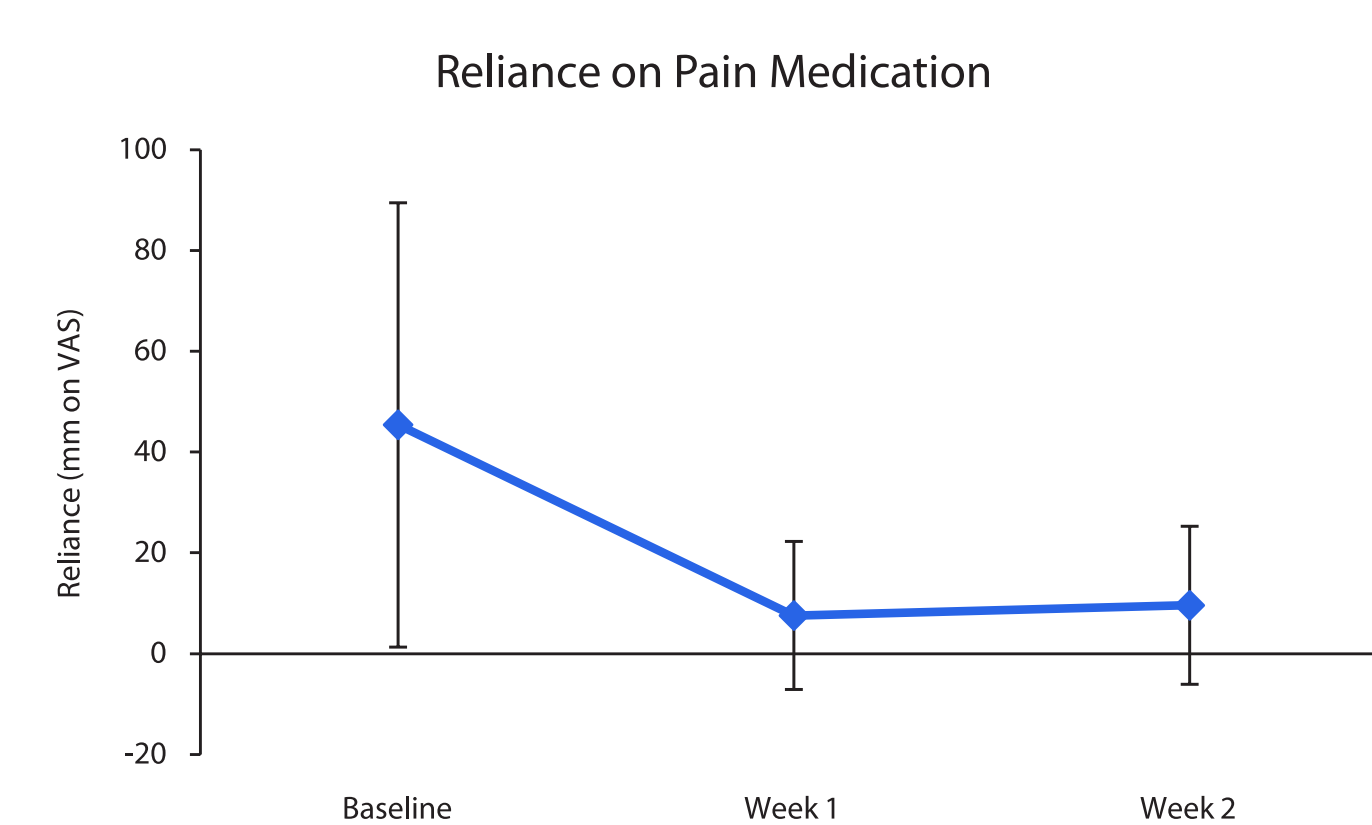
**Figure 3.** CyaninPlus™-treated PMN cells were cultured in a transwell migration plate and random migration (solid line - reflecting immune surveillance activity) was allowed for 18 hours. In parallel, CyaninPlus™-treated PMN cells were cultured in a transwell migration plate and allowed to migrate for 18 hours towards a culture medium with the inflammatory mediator Leukotriene B4 as a chemoattractant (dashed line), thus mimicking the recruitment of inflammatory cells. Migratory cells were quantified by staining with CyQuant. CyaninPlus™ increased the immune surveillance behavior of PMN's when compared to untreated cells. In contrast, CyaninPlus™ reduced the migration of PMN cells towards this inflammatory cytokine when tested in parallel to untreated cells.



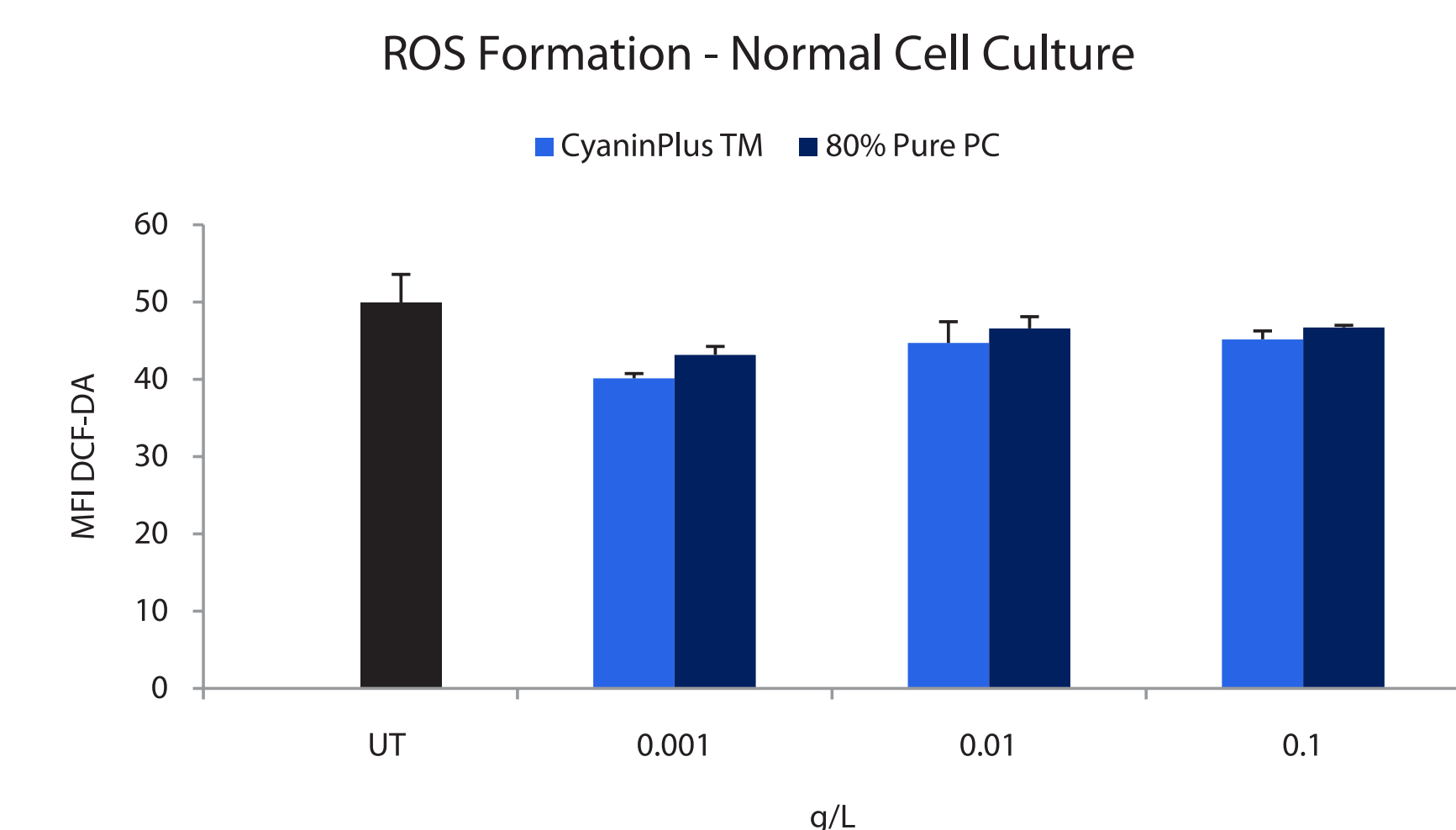
**Figure 5.** The pain-related problems for the study population are listed in Table 1. At the beginning of the study, participants were asked to describe their worst chronic pain area, and this area was described as primary pain for that person. Over the course of the study, all participants reported a reduction in their primary pain during resting (blue line) and physical activities (red line) as measured by Visual Analogue Scales.



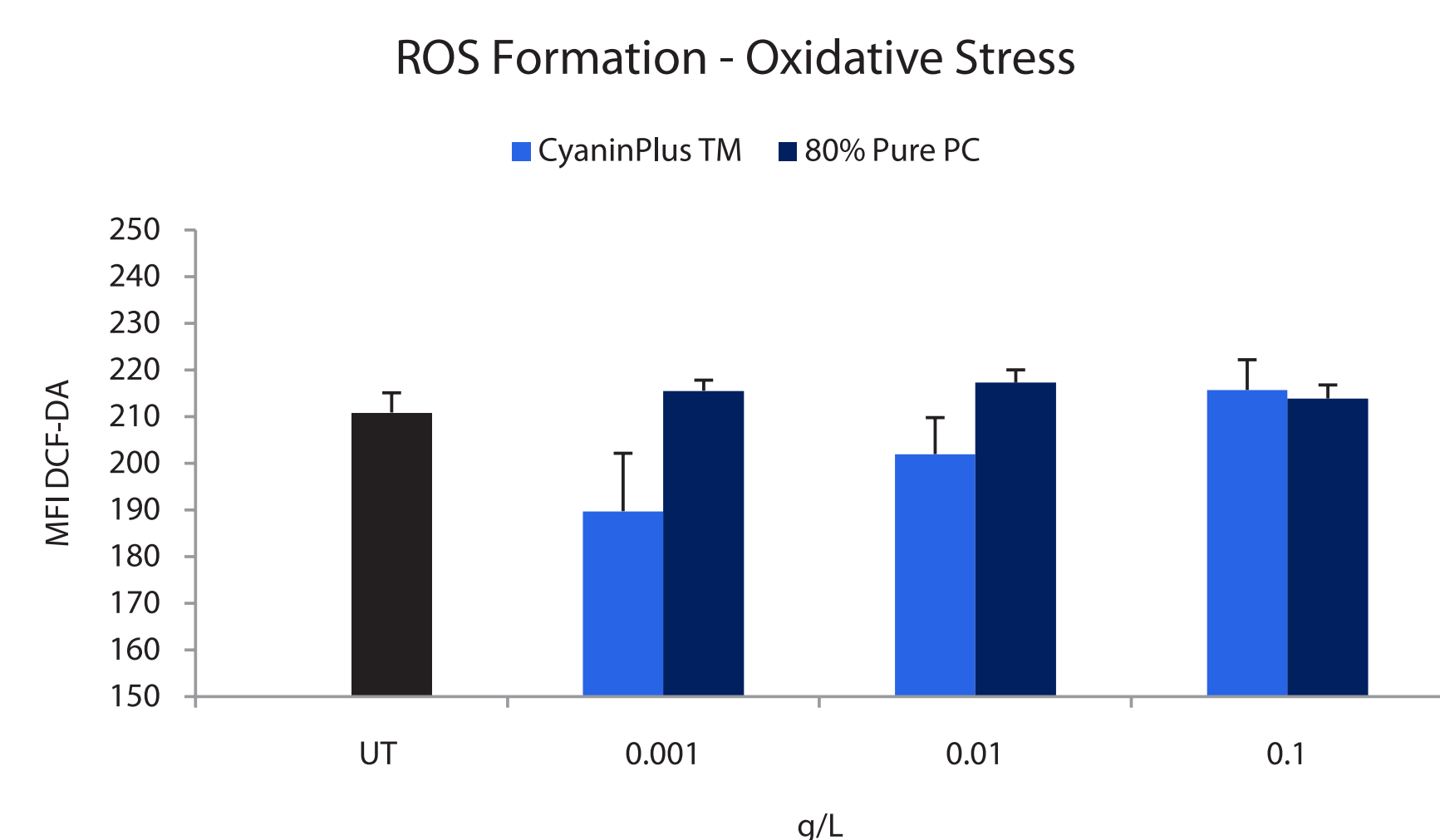
**Figure 6.** Upon entry into the study, participants were asked to identify their secondary pain complaint. Within 1 and 2 weeks, 13 patients determined that their secondary pain locations were reduced below baseline. It was also observed that the secondary pain locations were less painful for these individuals during stressful activities. At the beginning of the study, the secondary pain complaint was aggravated by physical exercise in most study participants. It was observed that by the end of week 1, the secondary pain was no longer aggravated by physical exercise above the discomfort experienced at rest.



**Figure 7.** At study start participants were asked to list the frequency of consuming pain relieving medication. When most waking hours were supported by pain medication, a score of "100" was given. The consumption of pain relieving medication was significantly reduced at 1 week when compared to baseline.



**Figure 4.** Production of Reactive Oxygen Species (ROS) by PMN cells was evaluated under A) normal culture conditions, and B) conditions of oxidative stress. ROS formation was analyzed by loading the cells with DCF-DA (a non-fluorescent precursor that becomes fluorescent when exposed to ROS). The mean fluorescence intensity (MFI) of untreated PMN cells versus cells treated with serial dilutions of CyaninPlus™ was measured. CyaninPlus™ reduced the amount of ROS formation when compared to untreated cells. It was also found that CyaninPlus™ reduced ROS levels below that of an 80% pure Phycocyanin extract, indicating the anti-inflammatory role of non-PC compounds in CyaninPlus™.



Volunteer	Age	Primary Complaint	Secondary Complaint
V01	56	Hands/Wrist	Neck
V02	53	Lower Back	Upper Back
V03	40	Hips	Knees
V04	55	Feet	Knees
V05	60	Knees	Right Ankle
V06	62	Right Hip	Both Thumbs
V07	46	Left Thumb	Lower Back
V08	69	Lower Back	Left Foot
V09	57	Hands	Ankles
V10	51	Left Shoulder	Right Wrist
V11	53	Back	Right Ankle
V12	44	Left Knee	Right Leg
V13	60	Back	Muscle Stiffness

## Conclusion

CyaninPlus™ has many parallel anti-inflammatory mechanisms of action *in vitro*. Furthermore, CyaninPlus™ demonstrated rapid and efficient pain relieving and anti-inflammatory effects *in vivo*.

## Acknowledgements

This study was conducted at NIS Labs, an independent contract research laboratory specializing in natural products testing. The study was sponsored by Desert Lake Technologies LLC.

