Profile of HIDROX™

Executive Summary

- Multiple lines of converging evidence link polyphenols, particularly hydroxytyrosol and HIDROX™, to improved clinical outcomes.
- In human and animal studies, hydroxytyrosol and HIDROX™ have been shown to induce significant therapeutic effects on the cardiovascular system, improve the quality of life of patients with osteoporosis, and reduce markers of inflammation. These findings have all been reported in peer-reviewed, internationally-recognised academic journals.
- Hydroxytyrosol is well absorbed following oral administration, and rapidly reaches high levels in the blood.
- Hydroxytyrosol and HIDROX™ have strong safety profiles and are not associated with major adverse events. Patients receiving HIDROX™ are likely to have a strong level of adherence to the supplement.

HIDROX™

HIDROX™ is CreAgri’s trademarked name for hydroxytyrosol, which is produced using a unique, patent protected process. Hydroxytyrosol is a natural, potent, and protective polyphenol derived from olive juice and olive oil. However, olive juice has over 300 times the level of antioxidant polyphenols compared to olive oil.

Hydroxytyrosol is scientifically recognized for its powerful antibacterial, anti-inflammatory, antioxidant, and cardioprotective health benefits. Independent laboratory analyses have demonstrated that hydroxytyrosol is one of the most potent natural antioxidants yet discovered with the highest level of free radical protection activity ever reported for any natural antioxidant compound. Hydroxytyrosol’s therapeutics effects have been demonstrated in a large number of published scientific articles in internationally-recognized journals.

Dr Roberto Crea is the Chairman and Chief Executive Officer of CreAgri. Dr. Crea has over 30 years of experience in the biotechnology field as a scientist, investor, and entrepreneur. He is one of the scientific co-founders of Genentech, one of the leading global biotechnology companies. Dr Crea is also the founder of Creative Bio Molecules, Inc (1982), Creagen, Inc (1992), CreAgri (1998), Bioren (2002) and ProtElix (2005). Creagen, Inc was merged into Neurex in 1994, which was then sold to Elan Pharmaceuticals for over $700 million. Pfizer successfully acquired Bioren in 2005. Dr. Crea is the author of more than 25 international patents and has co-authored more than 50 scientific articles.

Effects of HIDROX™ and hydroxytyrosol on cholesterol

Numerous human and animal studies have shown that polyphenols, particularly hydroxytyrosol and HIDROX™, can safely and effectively improve blood cholesterol profiles and prevent the formation of plaques within the arteries (atherosclerosis). These effects could help reduce the risk of myocardial infarction (heart attack) and stroke.

In a 6-month clinical trial of HIDROX™ in patients with hyperlipidemia, twice daily administration of HIDROX™ was associated with a significant reduction in V-LDL cholesterol levels in trial participants (Bitler
et al., 20xx). V-LDL cholesterol contains the highest amount of triglyceride fat of all cholesterol subtypes; high levels of V-LDL cholesterol can significantly raise the risk of coronary artery disease and myocardial infarction. By reducing V-LDL levels in patients, HIDROX™ could significantly reduce the risk of cardiovascular mortality.

In the EUROLIVE clinical study (a major, multicentre trial conducted in five European countries), 200 healthy male volunteers received 25 ml of olive oil containing either low, medium, or high concentrations of polyphenols once a day for three weeks. High polyphenol olive oil resulted in significantly raised levels of HDL cholesterol (Covas et al., 2006). HDL levels in study participants increased by 0.045 mmol/L; previously in a separate study, a 0.026 mmol/L increase in HDL was associated with a 2-3 % decrease in heart disease risk (Gordon et al., 1989). Polyphenol-rich olive oil therefore has the potential to significantly reduce the incidence of cardiovascular mortality.

High polyphenol olive oil also invoked a significant reduction in the levels of oxidized LDL cholesterol; numerous studies have now linked oxidized LDL to the development of obstructive plaques within arteries. Oxidized LDL can enter arteries and form plaques which may constrict the flow of blood to the heart, invoke substantial tissue damage, and increase the risk of myocardial infarction and sudden cardiac death. Oxidation of LDL allows it to enter the wall of the artery where it can form plaques to obstruct blood flow. By significantly reducing levels of oxidized LDL in the body, high polyphenol content olive oil may be able to exert substantial cardioprotective effects.

Isolated hydroxytyrosol has been shown to have a direct, beneficial effect on the formation of atherosclerotic plaques in an animal model. Administration of hydroxytyrosol to rabbits for one month resulted in a prominent reduction of the size of plaques formed in the aorta; this effect of hydroxytyrosol is of significant clinical importance and could help reduce the risk of myocardial infarction and sudden death. Additionally, hydroxytyrosol resulted in a 50 % reduction in total cholesterol levels; these multiple beneficial effects of hydroxytyrosol were directly linked to the agent’s powerful antioxidant effects (Gonzalez-Santiago et al., 2006).

**Hydroxytyrosol prevents the formation of potentially lethal blood clots**

Blood clots (thromboses) are an important cause of myocardial infarction and sudden cardiac death. A clinical study conducted in a high risk patient population demonstrated that hydroxytyrosol could reduce an important biomarker of thrombosis, suggesting that it could exert important anticoagulant effects.

In a clinical study of hydroxytyrosol’s effects in type I diabetic patients, 12.5-25 mg of a hydroxytyrosol solution was administered once daily for 4 days. Hydroxytyrosol produced a significant decrease in plasma levels of thromboxane B2 (a metabolite of thromboxane A2, which is responsible for platelet aggregation and the formation of blood clots). This finding indicates that hydroxytyrosol may reduce the risk of thrombosis and myocardial infarction. Importantly, these beneficial effects occurred within a very short time frame (4 days) (Leger et al., 2005).

**Cardioprotective effects of hydroxytyrosol**

Chronic hypertension is associated with an increased risk of heart disease, stroke, and myocardial infarction. Dilation of the body’s major artery, the aorta, is an important safeguard against
hypertension, however, oxidative damage can prevent this from taking place. Hydroxytyrosol has been shown to protect the aorta from free radical damage and preserve its ability to dilate and therefore lower blood pressure.

A study has demonstrated that hydroxytyrosol’s potent antioxidant properties also exerted additional effects on the cardiovascular system. Nitric oxide is an important mediator of vascular relaxation and tone, and the authors of the study measured the ability of the aorta to dilate and lower blood pressure in the absence and presence of hydroxytyrosol. Oxidative damage to the aorta can prevent vascular dilation which can raise blood pressure and lead to cardiovascular morbidity and mortality. In this experimental study, cumene hydroperoxide (CHP) was used to mimic oxidative stress; CHP prevented aortic dilation, which was reversed with hydroxytyrosol. Hydroxytyrosol was also shown to be a potent antioxidant and free-radical scavenger. One single molecule of hydroxytyrosol was able protect against 30 molecules of CHP which ordinarily induced significant oxidative damage (Rietjens et al., 2006).

**Hydroxytyrosol’s effects on vitamin C levels**

In a clinical study of hydroxytyrosol’s effects in diabetic patients, daily administration of hydroxytyrosol raised the concentration of vitamin C; vitamin C is in itself an antioxidant and has been linked to immune function.

Hydroxytyrosol has been shown to increase levels of vitamin C in humans. Type I diabetic patients administered 12.5-25 mg hydroxytyrosol per day had raised plasma levels of vitamin C compared to baseline recordings. The authors of this clinical study hypothesized that hydroxytyrosol may exert antioxidant, protective effects on vitamin C (Leger et al., 2005). Vitamin C has been linked to multiple beneficial effects in the human body including immune health, however, its water soluble nature results in the body’s inability to store, and maintain consistent levels in the body. By apparently raising levels of vitamin C in the body, hydroxytyrosol could exert additional, supplementary therapeutic effects.

**Beneficial effects in patients with osteoarthritis**

In a clinical study, HIDROX™ decreased pain and inflammation associated with osteoarthritis.

An eight-week placebo-controlled clinical study of HIDROX in patients with osteoarthritis demonstrated that the supplement induced significant improvements in patient’s quality of life as measured by a health assessment questionnaire disability index (HAQ-DI). The HAQ-DI evaluated nine categories of functional activity; 69 % of osteoarthritis patients receiving HIDROX reported a greater than 20 % improvement in their HAQ-DI scores (Bitler et al., 2007). These results not only suggest that HIDROX is safe, but that the supplement helps to improve the daily living activities of patients with osteoarthritis.

**HIDROX™ reduces markers of inflammation**

A clinical study of HIDROX™ in patients with rheumatoid arthritis demonstrated that the supplement significantly reduced blood levels of C-reactive protein and homocysteine.
A placebo-controlled clinical study of HIDROX™ in patients suffering from rheumatoid arthritis revealed that the supplement significantly reduced blood levels of C-reactive protein after eight weeks (Bitler et al., 2007). C-reactive protein is an important biochemical marker of inflammation and it has been previously associated with rheumatoid arthritis and cardiovascular disease and mortality.

A recent study has shown that despite having raised levels of HDL cholesterol, a subgroup of patients that also had raised levels of C-reactive protein had a high risk of suffering from recurrent cardiovascular events and sudden death. These findings suggest that reducing levels of C-reactive protein alongside raising levels of HDL is an optimal treatment strategy, and could help explain why a clinical trial of a drug that raised levels of HDL alone was prematurely terminated due to a high number of cardiovascular events and sudden death. By significantly reducing levels of C-reactive protein, HIDROX™ could be an important supplement to improve cardiovascular health.

The same clinical study of HIDROX™ in rheumatoid arthritis patients also demonstrated that the supplement significantly reduced levels of homocysteine after eight weeks. Homocysteine is also an important biochemical marker of inflammation and a number of large clinical studies have established homocysteine as an independent risk factor for venous thromboembolism, stroke, coronary heart disease, and death (Milani et al., 2008).

**Rapid absorption**

The pharmacokinetic profile of an isolated form of hydroxytyrosol has been previously investigated. Gonzalez-Santiago and colleagues (2010) demonstrated that an aqueous hydroxytyrosol solution was rapidly absorbed following oral administration; peak hydroxytyrosol plasma concentrations were detected within 13 minutes. The same study also reported that hydroxytyrosol transiently associated with LDL cholesterol; this effect is likely to play a key role in hydroxytyrosol’s effects to prevent LDL oxidation and the subsequent deposition of atherosclerotic plaques within blood vessels.

**Strong safety profile**

Data strongly suggest that daily consumption of polyphenol compounds, including hydroxytyrosol and HIDROX™, is free from major adverse events. Extensive toxicology studies of HIDROX™ have revealed an extremely strong safety profile; HIDROX™ administered in single or multiple dosages of up to 2000 mg/kg/day resulted in no adverse clinical, haematological, biochemical, or reproductive effects (Christian et al., 2004). It is important to note that the recommended daily dose of HIDROX™ is one or two 300 mg capsules twice daily, which would only equal a dose of up to 1200 mg per day; this dose equates to 17 mg/kg for a 70 kg individual and represents a HIDROX™ dose of under 1 % that was used in the above safety studies. Furthermore, HIDROX™ has been successfully used in a number of human studies, with no major adverse events reported. Clinical data also suggest that HIDROX supplementation has a very low risk for interactions with other medications; in a placebo-controlled clinical study of HIDROX, there were no major adverse events in patients receiving multiple concomitant medications (Bitler et al., 2007).

Previous studies of patients receiving polyphenol-rich olive oil, pure hydroxytyrosol, or HIDROX™ suggest that patients receiving daily HIDROX™ are likely to display strong levels of adherence to the supplement; this is a significant advantage which could optimize patient outcomes. It is noteworthy that adherence to pharmaceutical products is a major health care concern; patients assigned drugs that induce minor
adverse events, such as nausea or gastrointestinal disturbances, are more likely to discontinue their medications which can have a direct impact on patient outcomes.

**Scientific articles**


2. Bitler, CM et al. (20xx). Effects of hydrolyzed vegetation water on serum LDL levels and antioxidant capacity in male and female subjects.


5. González-Santiago M et al. (2006). One-month administration of hydroxytyrosol, a phenolic antioxidant present in olive oil, to hyperlipemic rabbits improves blood lipid profile, antioxidant status and reduces atherosclerosis development. *Atherosclerosis.* 188(1), 35-42.


**Biography**

**Dr. Matthew Killeen** - CreAgri’s Scientific Affairs Advisor. Dr Killeen received his undergraduate degree in pharmacology with highest honors and was awarded a Ph.D. in cardiovascular electrophysiology from the University of Cambridge. At Cambridge he studied the mechanisms underlying sudden cardiac death and identified a number of effective pharmacological treatment strategies. Following his Ph.D., Dr. Killeen was awarded research fellowships at Harvard Medical School and the Massachusetts General Hospital. Dr. Killeen has authored 19 peer-reviewed publications in leading international journals. He is a member of the Cardiac Safety Research Consortium, a collaborative initiative between the FDA and Duke University. Dr. Killeen developed the concept for and co-chaired an FDA Think Tank on pediatric drug safety. Dr. Killeen has also previously worked for Eli Lilly and he is the author of the forthcoming book, “Cardiac Drug Safety: A Bench to Bedside Approach,” which will be published in 2011.