livlonga.com

LivLonga®

A Potent Combination of Natural Actives for Liver Health

presented by

LIVLONGA®

SABINSA®

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INTRODUCTION

LivLonga® is the proprietary product containing a novel blend of Curcumin C3 Complex® (250 mg), Livinol® (Garcinol 20% - 50 mg) and the bioavailability enhancer BioPerine® (5 mg). Each ingredient in the blend has unique pharmacological properties and many preclinical and clinical studies have been carried out to know their impact on liver health.

Curcumin C3 Complex® is a proprietary commercial extract from the rhizomes of *Curcuma longa*, standardized for 95% curcuminoids. C3 Complex refers to the presence of three natural actives: Curcumin (75-81%), demethoxycurcumin (15-19%) and bisdemethoxycurcumin (2.2-6.5%), collectively known as curcuminoids. It is the most extensively studied and clinically documented product and is known to have a wide range of therapeutic actions, including antioxidant, anti-inflammatory, anticancer and lipid regulatory activities (Epstein *et al*. 2010). Curcuminoids were reported to have hepatoprotective activity mediated by the reduction of oxidative stress and attenuation of nuclear factor kappa B (NF-κB) mediated anti-inflammatory pathways (Wang 2015, Afrin *et al*. 2017).

Garcinol, a polyisoprenylated benzophenone isolated from the fruit rinds of *Garcinia indica*, is known to have antioxidant, antiglycation, anticancer, and protective action against drug-induced liver damage (Yamaguchi *et al*. 2000, Pan *et al*. 2001, Balasubramanyam *et al*. 2004, Hung *et al*. 2014). Livinol® is a standardized commercial extract of *Garcinia indica*, containing 20% Garcinol (w/w).

BioPerine® is obtained from the dried fruits of black pepper (*Piper nigrum*) and is standardized for 95% piperine. It is used as a nutrient bioavailability enhancer.

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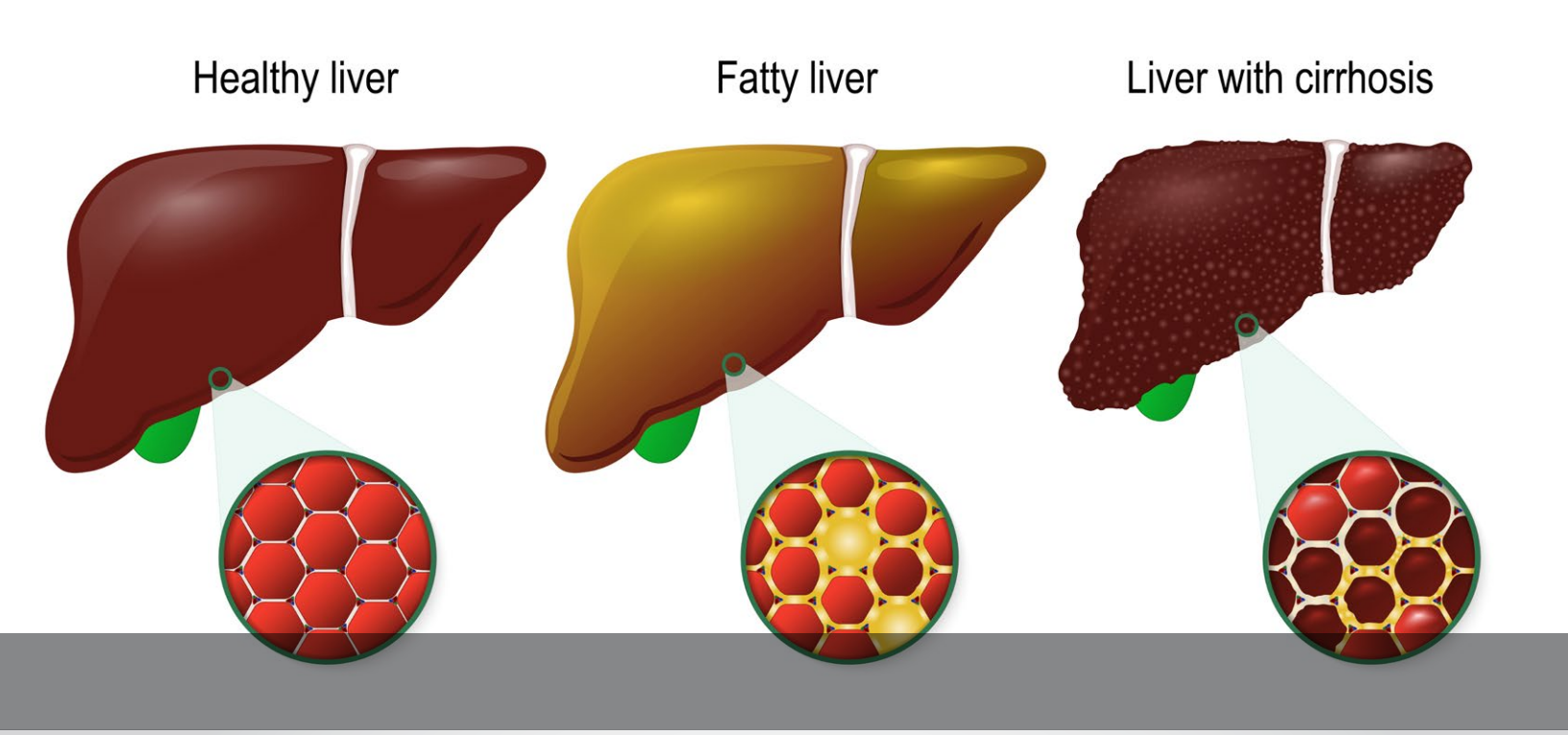
LIVER HEALTH

Liver is a vital organ that plays a key role in the homeostasis of the body by regulating digestion, protein synthesis, detoxification and metabolism. In recent decades, drastic lifestyle changes and health priorities have increased the prevalence of non-communicable chronic liver diseases all over the world. Liver normally contains some fat but when it exceeds 5-10% of the total weight of liver, it causes liver damage similar to that caused by alcohol abuse. The condition of excessive accumulation of fat in hepatocytes of individuals who do not drink heavily is called non-alcoholic fatty liver disease (NAFLD). It is the most common cause of chronic liver disorders (Lazo *et al*. 2013, Loomba and Sanyal 2013) and includes the spectrum of liver diseases, ranging from benign fatty liver to hepatocellular carcinoma. It is histologically further categorized into the nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH). NAFL, as the early-stage disease shows the presence



of excessive fat in the liver (hepatic steatosis) with no evidence of hepatocellular injury, while NASH is characterized by the accumulation of fat accompanied by infiltration of inflammatory cells and cellular damage, which can progress to cirrhosis, liver failure, and liver cancer (Chalasani *et al*. 2012). NASH is considered as the hepatic manifestation of metabolic disorder and is closely associated with type 2 diabetes, obesity, insulin resistance and systemic inflammatory state (Ratziu *et al*. 2010, Sanyal *et al*. 2010, Charlton *et al*. 2011, Williams *et al*. 2011, Yki-Jarvinen 2014). Consequently, NASH patients have a higher risk of cardiovascular events and neoplasia, resulting in a higher rate of mortality (Angulo *et al*. 2015, Ekstedt *et al*. 2015). It is usually a silent disease with minimum symptoms, while weight loss, fatigue, and weakness develop as the disease progresses.

NASH is a cryptogenic form of chronic liver disease commonly encountered worldwide which makes the liver more sensitive to subsequent insult, and promotes liver damage in conjunction with genetic and environmental factors. Macrovesicular accumulation of triglycerides in hepatocytes is generally caused by unidentifiable reasons but is usually seen associated with obesity, dyslipidemia, metabolic disorders, insulin resistance etc. but the etiology of the condition is poorly understood.

RISK FACTORS OF NASH

- ✓ **Central obesity**
- ✓ **Type 2 diabetes mellitus**
- ✓ **Dyslipidaemia**
- ✓ **Insulin resistance**
- ✓ **Metabolic diseases**
- ✓ **High body mass index (BMI)**
- ✓ **Age**

WHY DO WE NEED NATURAL MEDICINES FOR NASH TREATMENT?

NASH is estimated to be present in 2 – 5% of the general population and it is estimated that more than 20% of patients with NASH will develop cirrhosis in their lifetime. NASH has been recently identified as the most leading indication of liver transplantation in the U.S. in patients with or without liver cancer and is expected to overtake alcoholic liver disease in next few years (Younossi *et al*. 2021). Currently, the prevalence of NAFLD and NASH is not only seen in geriatric population but in young adults and children too. About 25% to 30% of the adult population is thought have NAFLD and NASH while more than 50% of individuals with type 2 diabetes and 90% of the morbidly obese have NAFLD (Younossi *et al*. 2016, 2019). NASH patients have higher risk of cardiovascular events leading to increased mortality.

NASH is a significant burden to the public health system, with no approved drugs for treatment. The primary mode of treatment is lifestyle management, while pioglitazone and Vitamin E have been used to reduce cellular injury, fibrosis and improve steatohepatitis (Dyson *et al.* 2014). Several novel medications, including agonists of peroxisome proliferator-activated receptor (PPAR)-alpha and PPAR-gamma and semisynthetic bile acid analogs, targeting different stages of the disease are in the pipeline (Ratziu *et al.* 2016). Moreover, low disease awareness and asymptomatic existence of NASH delay the diagnosis and contribute to hepatocellular carcinoma with high mortality. Herbal medicines to treat various types of liver diseases are in existence from ancient times and in recent years, they are gaining importance in the management of NAFLD and NASH. Even though several polyherbal formulations are available in the market meant for curing NASH, only a few are systematically evaluated in human clinical studies.

IN VITRO STUDIES

Livinol® and Curcumin C3 Complex® alone and in combination (LivLonga®) were found effective in the management of inflammation and steatosis *in vitro* studies. Human hepatocyte cell line, HepG2, was treated with free fatty acids (FFA) and respective samples to analyse the intracellular lipids via Oil Red O staining which showed significant reduction in lipid accumulation (Figure 1). Further, the levels of antioxidant markers such as superoxide dismutase (SOD) and nuclear factor-erythroid factor 2-related factor 2 (NRF2) were significantly elevated in cells treated with LivLonga® compared to control indicating the potency of LivLonga® in the management of NASH progression (Majeed *et al*. 2020).

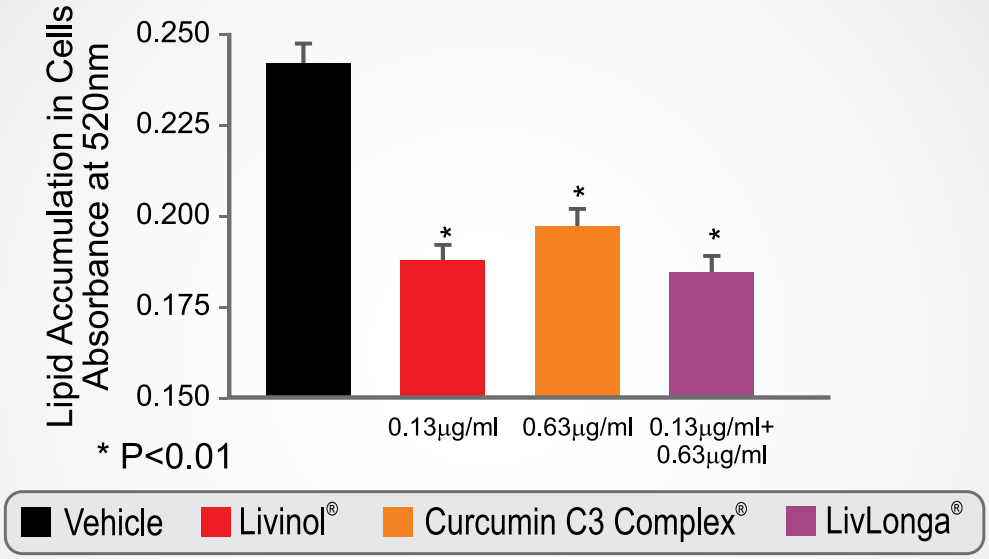


FIGURE 1. *IN VITRO* ANALYSIS OF LIPID ACCUMULATION. THE INTRACELLULAR LIPIDS WERE STAINED BY OIL RED O (ORO) AND QUANTIFIED BY ABSORBANCE AT 520 nm.

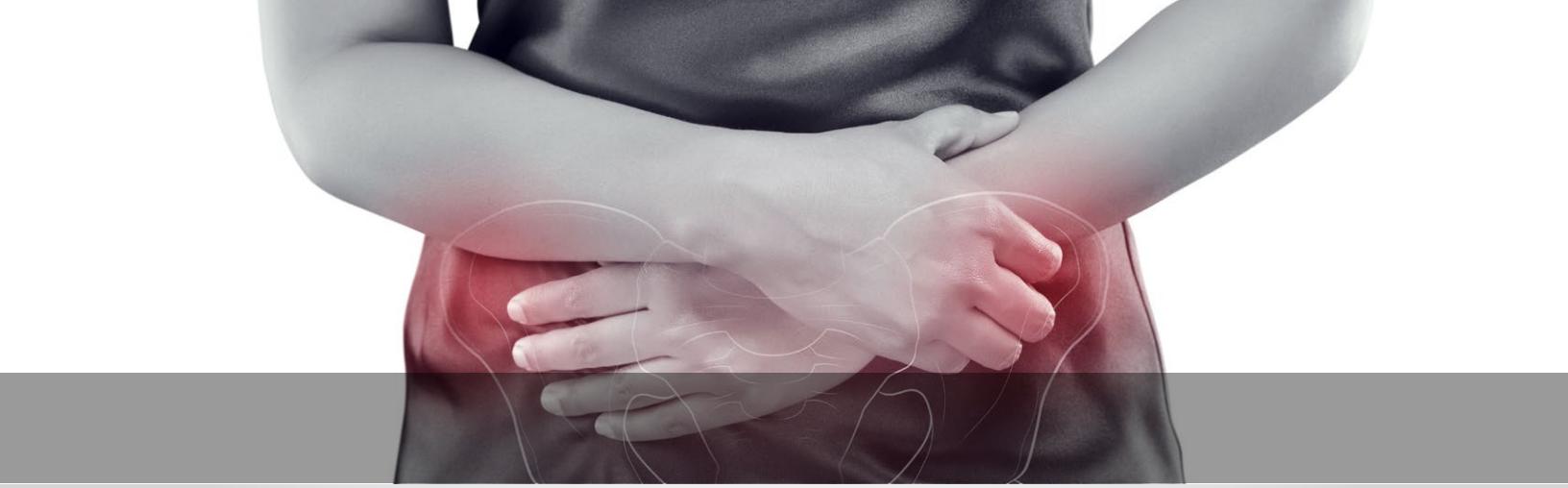
Anti-inflammatory activity was evaluated in THP1 human monocytes stimulated with lipopolysaccharide (LPS) in the presence of Livinol®, Curcumin C3 Complex® or LivLonga®. It was observed that phosphorylated NFκB level significantly reduced by the treatment of LivLonga®.

PRECLINICAL STUDIES

Stelic animal model (STAM™) is well established and recommended mouse model to test pharmacological drugs for anti NASH activity. The STAM™ mouse model developed by Fuji *et al*. shows pathological progression of chronic liver illness from steatosis to NASH, then to fibrosis and finally to carcinoma and is quite similar to that seen in humans (Fujii *et al*. 2013). The efficacy of the novel combination LivLonga® was analysed in STAM™ mice (Majeed *et al*. 2020).

LIVLONGA® AND NAFLD ACTIVITY

The lipotoxicity induced by the accumulated triglycerides in hepatocytes, activates the inflammatory pathways that results in the hepatocellular ballooning, infiltration of inflammatory cells and expression of pro-inflammatory cytokines by both hepatocytes and Kupffer cells. Hepatocyte ballooning was not observed in 50% of STAM™ animals treated with Livinol® and Curcumin C3 Complex® individually while LivLonga® showed 75% inhibition. Moreover, the NAFLD activity score reduced from 4.8 ± 0.7 to 3.0 ± 1.3 in the LivLonga® group showing a promising reduction of 37.5% (Figure 2).



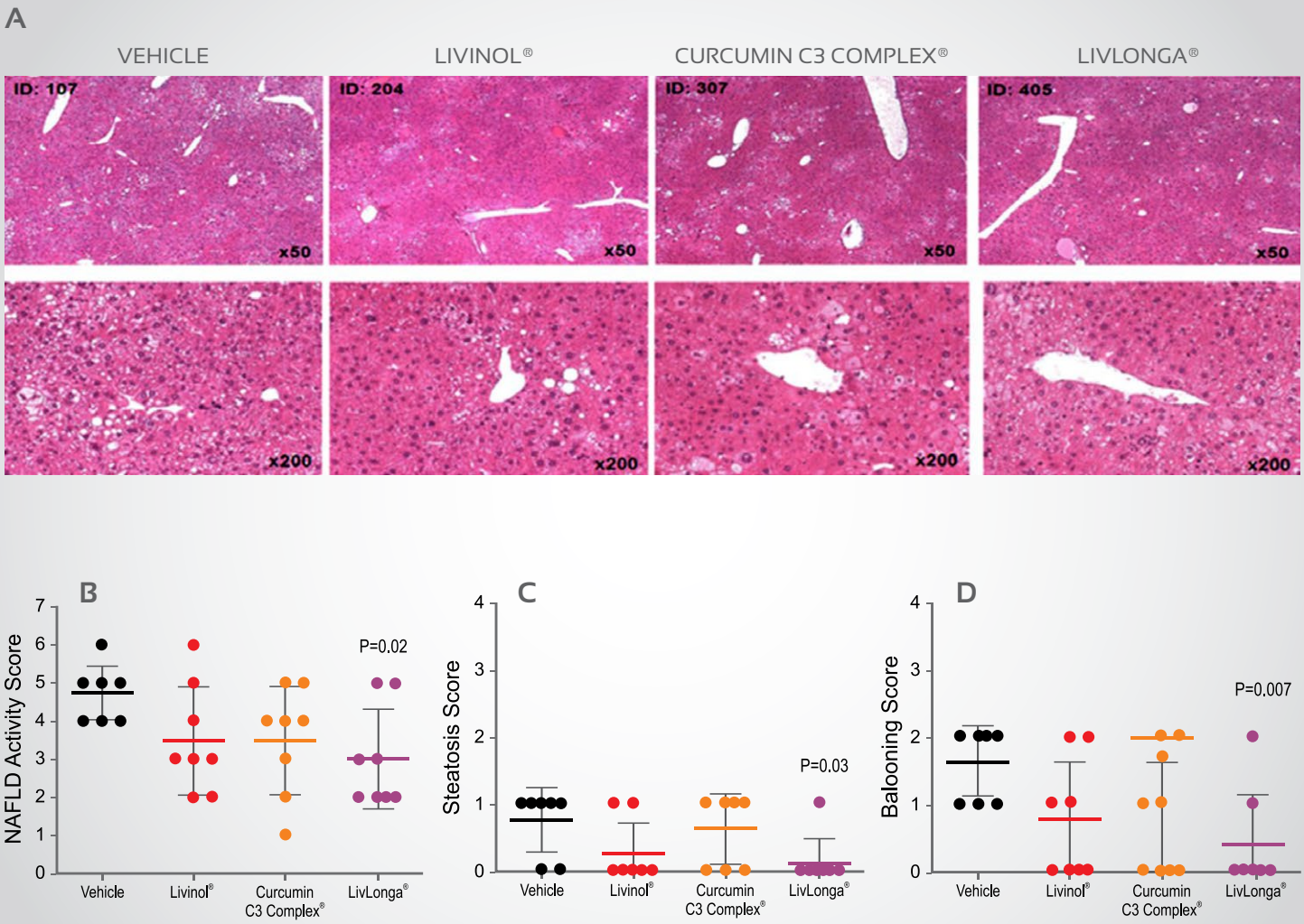


FIGURE 2. REDUCTION IN NAS IN STAM™ MICE FOLLOWING THE TREATMENT: (A) REPRESENTATIVE HEMATOXYLIN AND EOSIN STAINED (50X AND 200×) LIVER SECTIONS OF TREATED NASH STAM™ MICE COLLECTED AT WEEK 9; (B) NAFLD ACTIVITY SCORE (NAS); (C) STEATOSIS SCORE; (D) HEPATOCELLULAR BALLOONING SCORE; N = 8 IN EACH GROUP.

LIVLONGA® AND LIVER FIBROSIS

The extracellular matrix (ECM) of liver is the array of macromolecules like collagen, elastase, structural glycoproteins, hyaluronic acid, proteoglycans etc. that constitutes about 0.5% of total weight of the organ (Bedossa and Paradis 2003). Under normal physiological conditions, the synthesis and degradation of ECM is well balanced so that a constant level is maintained. Chronic inflammations of liver such as NASH change the composition of ECM rending it more resistant to degradation resulting in the replacement of hepatic parenchyma by scar tissue and by vascular architectural distortion (Tanwar *et al.* 2020). Fibrosis may progress to irreversible damage called cirrhosis which adversely affects the liver functions.

Transforming growth factor (TGF)-β is considered as one of the major biomarkers of hepatic fibrogenesis which drives hepatic stellate cells (HSC) activation and induces ECM production (Dewidar *et al.* 2015). It also increases the trans-differentiation of fibroblasts to myofibroblasts by increasing the expression of α-smooth muscle actin (α-SMA) and collagen-1 (Zhang *et al.* 1994). The fibrosis area (Sirius red-positive area)

was reduced by 22.47% with Livinol®, 34.83% with Curcumin C3 Complex® and 30.33% with LivLonga™ compared with the untreated vehicle group. Liver hydroxyproline concentrations and the mRNA transcripts of collagen 1 and TGF-β were lower in the treated groups compared with the vehicle. LivLonga® downregulated the expression of TGF-β and collagen 1 more effectively compared to Livinol®, Curcumin C3 Complex® (Figure 3).

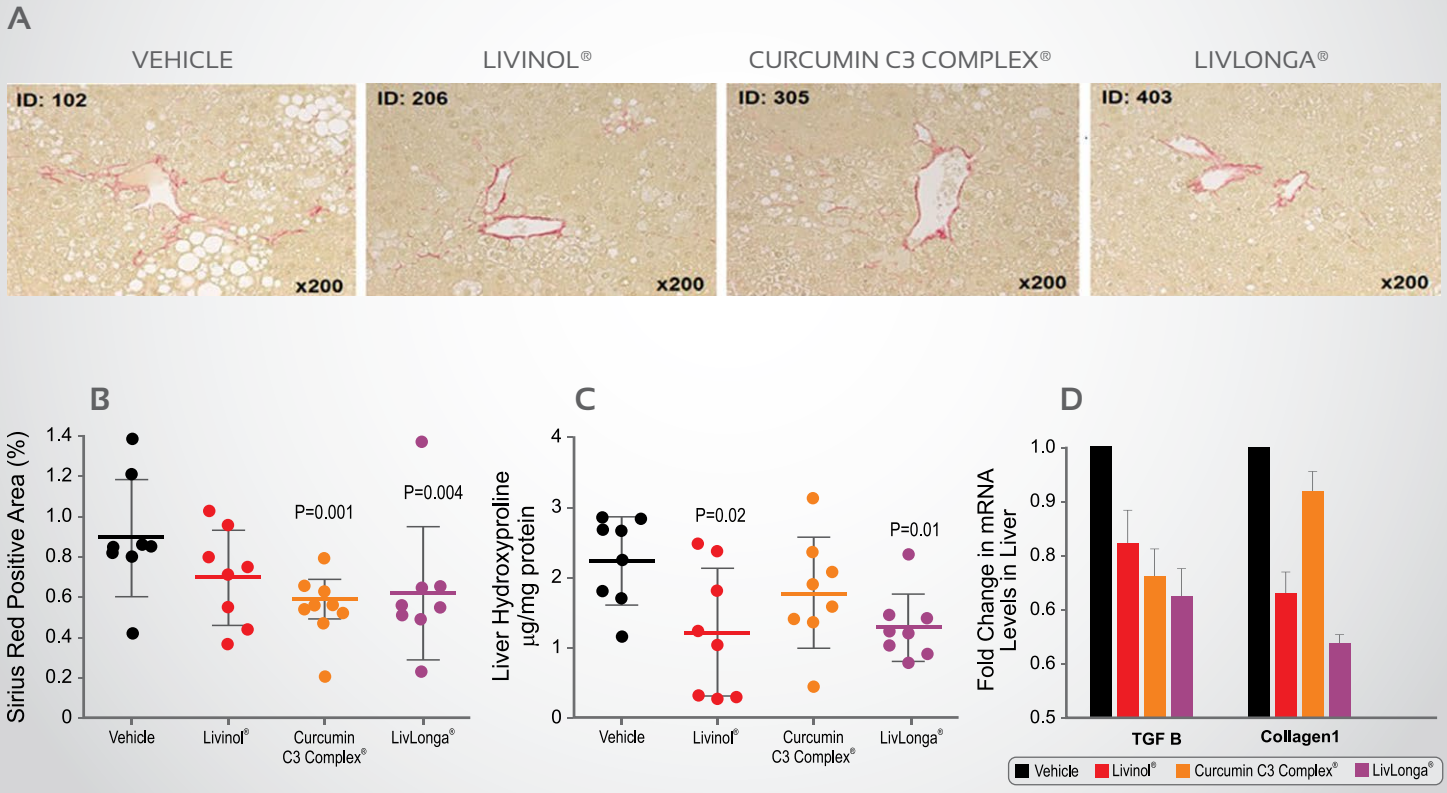
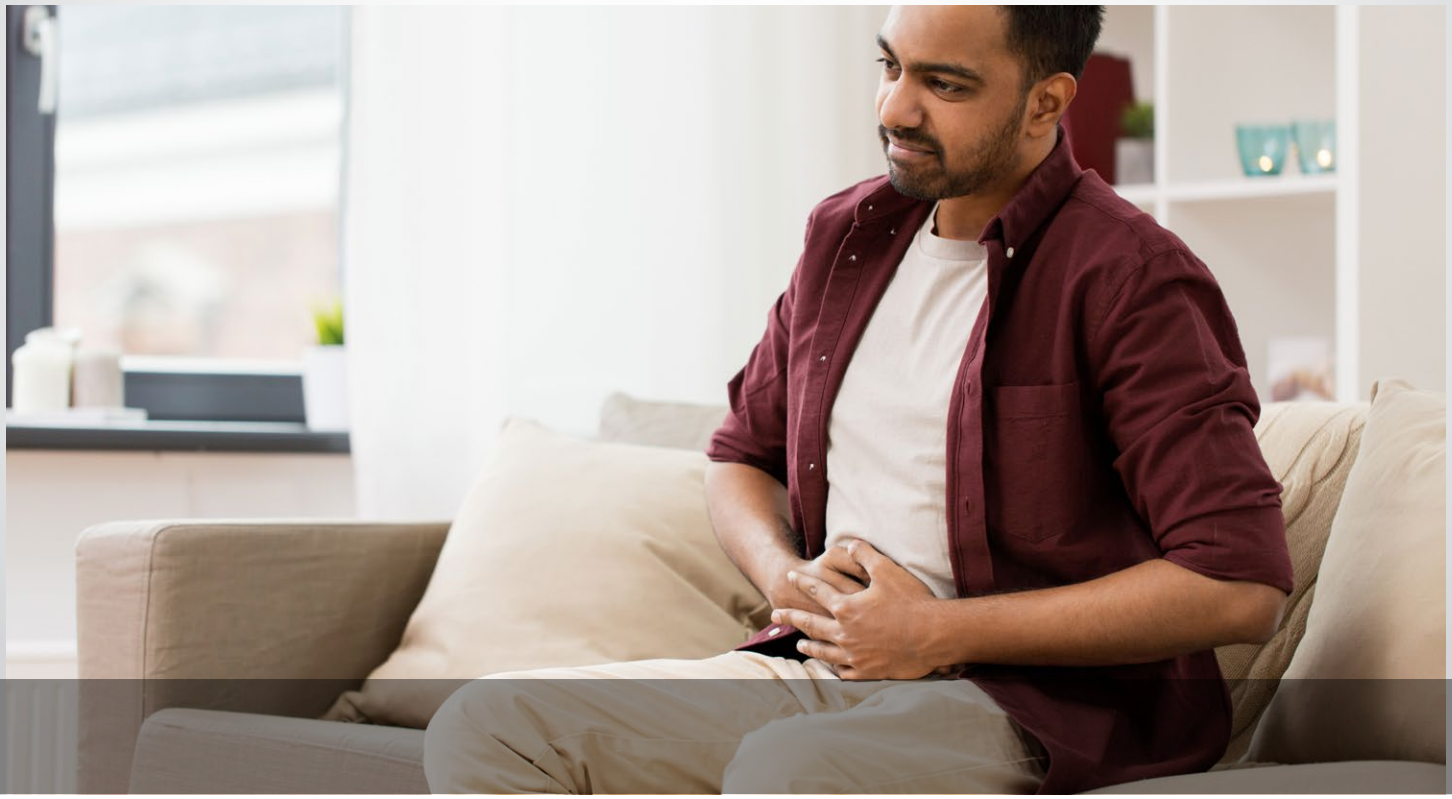


FIGURE 3. REDUCTION IN MARKERS RELATED TO FIBROSIS FOLLOWING LIVINOL®, CURCUMIN C3 COMPLEX® OR LIVLONGA® TREATMENT. (A) REPRESENTATIVE PICROSIRIUS STAINED (200×), LIVER SECTIONS FROM TREATED NASH STAM™ MICE COLLECTED AT WEEK 9; (B) FIBROSIS SCORE; (C) LIVER HYDROXYPROLINE CONCENTRATION; (D) RELATIVE mRNA LEVELS OF TGF B AND COLLAGEN 1 IN THE TREATED GROUPS IN COMPARISON TO VEHICLE. N = 8 IN EACH GROUP. * P < 0.05.

LIVLONGA® AND LIVER INFLAMMATION



Among the various cytokines, the pro-inflammatory interleukin (IL)-1, monocyte chemoattractant protein 1 (MCP-1) and C-reactive protein (CRP) have emerged as the key players of hepatosteatosis as they regulate various inflammatory events including the activation of monocyte and macrophages (Tilg 2001). The relative mRNA expression of tumor necrosis factor α (TNF-α) and CRP in liver were down regulated significantly by 1.41 and 1.73 folds when the animals were treated with LivLonga®. Nuclear factor kappa B (NF-kB) has central role in inflammation by regulating the expression of proinflammatory cytokines. Hence, targeting NF-kB mediated pathway is proposed to have potential benefit in NASH (Tilg *et al*. 2016). Treatment with LivLonga® down regulated the expression of NFkB mRNAs by 1.62 folds. Further LivLonga® significantly reduced the TNF-α protein concentration in the liver as compared to the vehicle group (Figure 4).

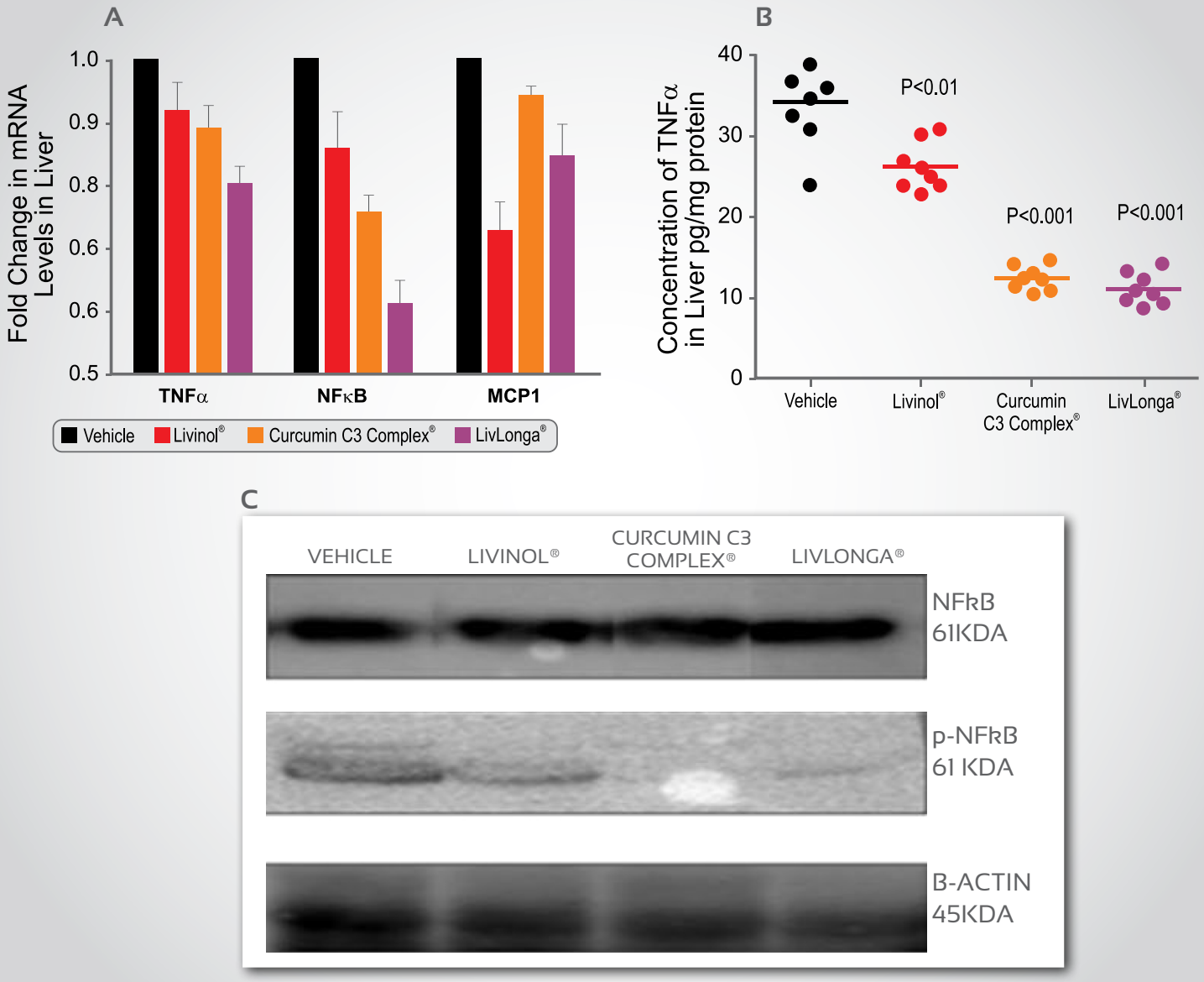


FIGURE 4. REDUCTION OF INFLAMMATORY MARKERS IN STAM™ MICE. RELATIVE mRNA LEVELS OF (A) TNF-A, NFKB, AND MCP1; (B) CRP; (C) IMMUNOBLOT ANALYSIS OF NFKB TOTAL AND PHOSPHORYLATED PROTEIN LEVELS IN THE LIVER HOMOGENATE IN THE TREATED GROUPS IN COMPARISON TO VEHICLE.

LIVLONGA® AND ANTIOXIDANTS

Free radicals are formed in the liver as a result of hepatic metabolism. Overproduction of free radicals causes harmful effects in the biological system incrementing oxidative stress. It causes cellular damage and plays an important role in the development of several chronic liver diseases like NASH. The endogenous antioxidants like superoxide dismutase (SOD), glutathione (GSH), catalase and glutathione peroxidase (GPx) play an important role to scavenge the free radicals maintaining the redox homeostasis. Livinol® and LivLonga® showed an increase in the levels of nonenzymatic antioxidants such as GSH and antioxidant enzyme, GPx. The SOD activity was not influenced by Livinol® and Curcumin C3 Complex® individually but was significantly increased by LivLonga®. Malondialdehyde (MDA) is an indicator of lipid peroxidation, activates the inflammatory response and, consequently, causes cellular damage. LivLonga® treatment significantly reduced the MDA activity as compared to the vehicle group (Figure 5).

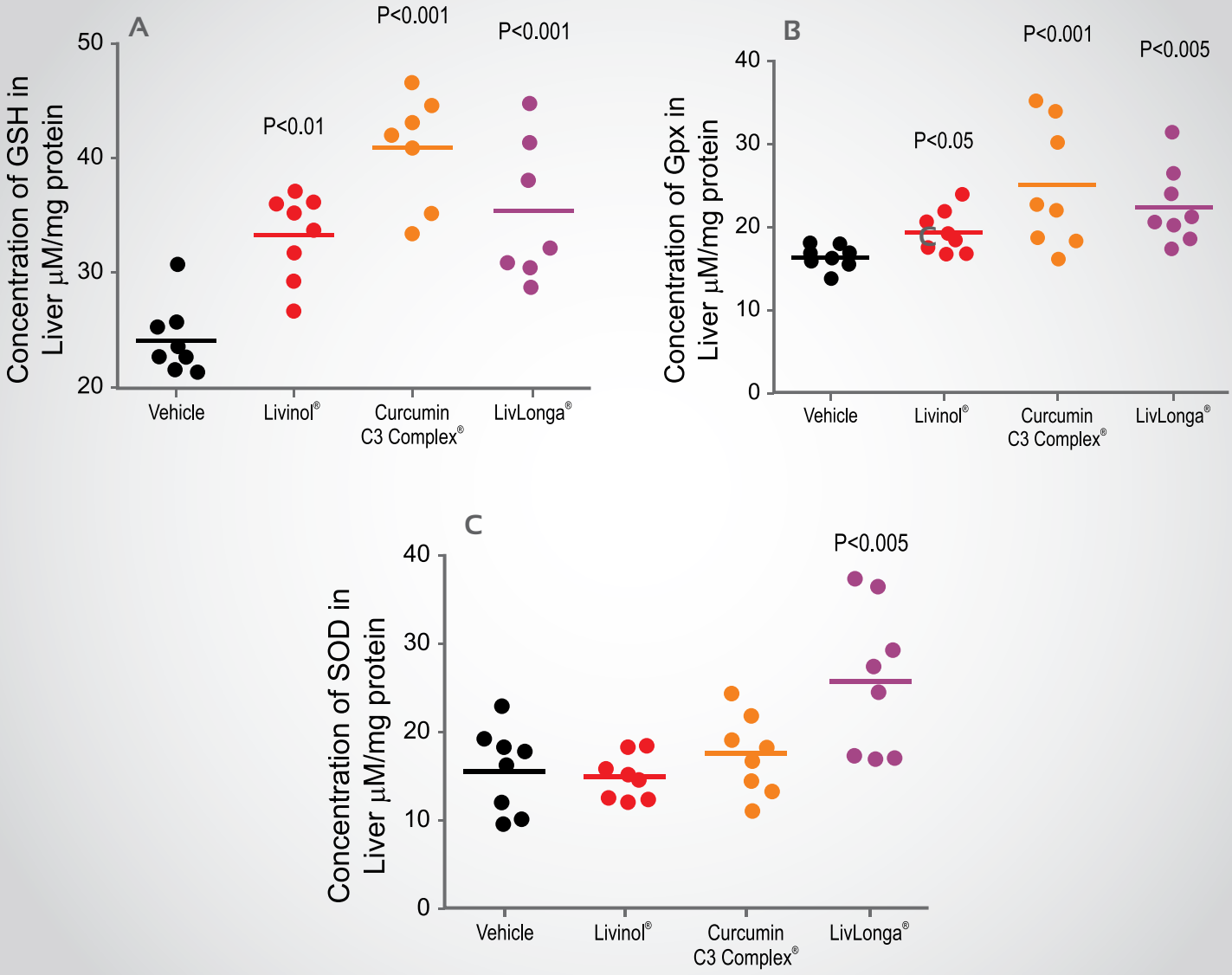
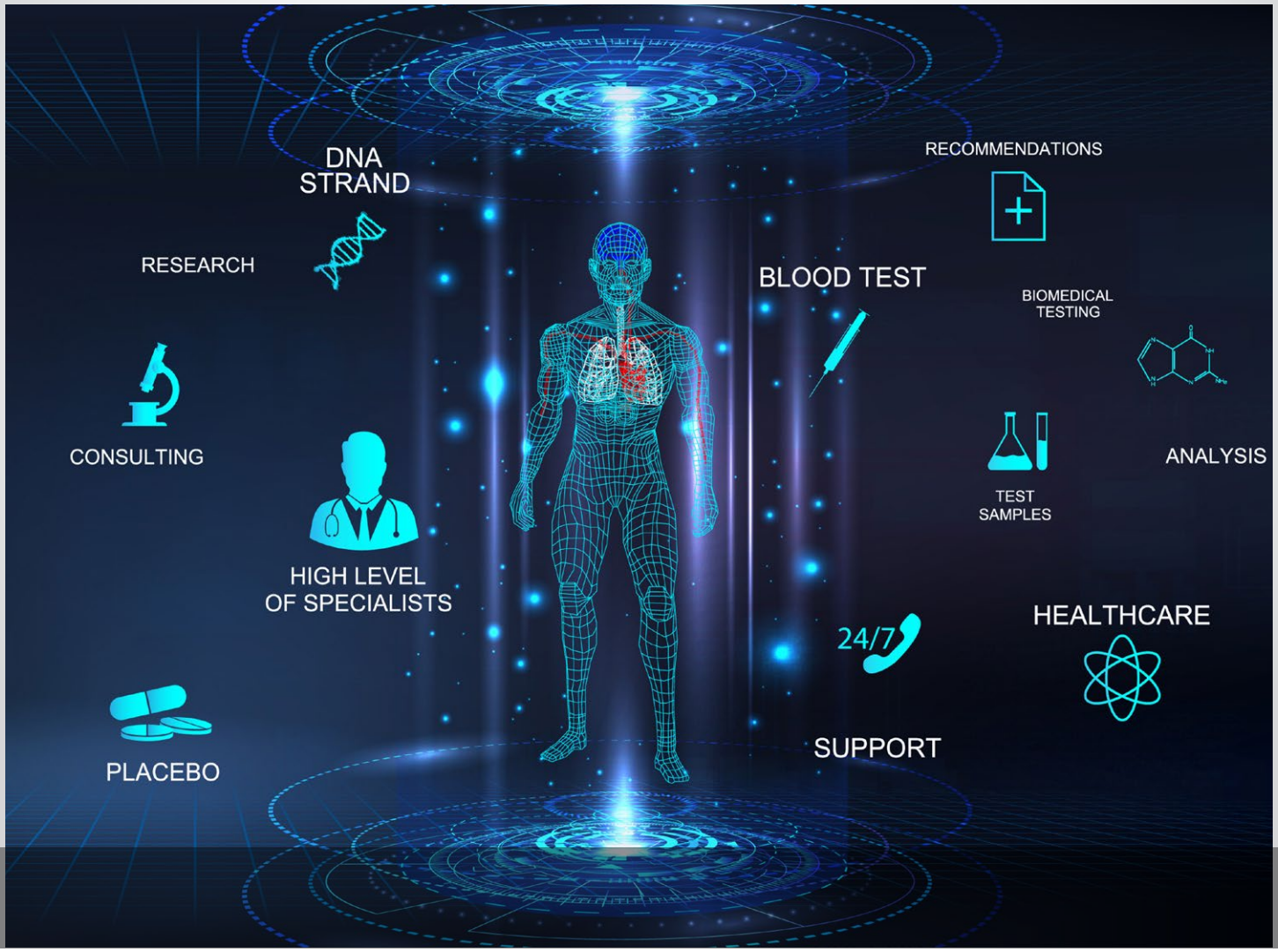


FIGURE 5. INCREASE IN ANTIOXIDANT ENZYMES AND REDUCTION IN OXIDATIVE STRESS IN STAM™ MICE FOLLOWING LIVINOL®, CURCUMIN C3 COMPLEX® AND LIVLONGA® TREATMENT. (A) CONCENTRATION OF GSH (GLUTATHIONE); (B) CONCENTRATION OF GPX (GLUTATHIONE PEROXIDISE) IN LIVER HOMOGENATE; (C) CONCENTRATION OF SOD (SUPEROXIDE DISMUTASE).

CLINICAL STUDY



The safety and efficacy of LivLonga® was evaluated in patients with NASH but without liver cirrhosis.

A randomized, double blind placebo study was conducted in 72 patients aged 30-65 with NASH in which the participants administered two doses of LivLonga® daily for 90 days.

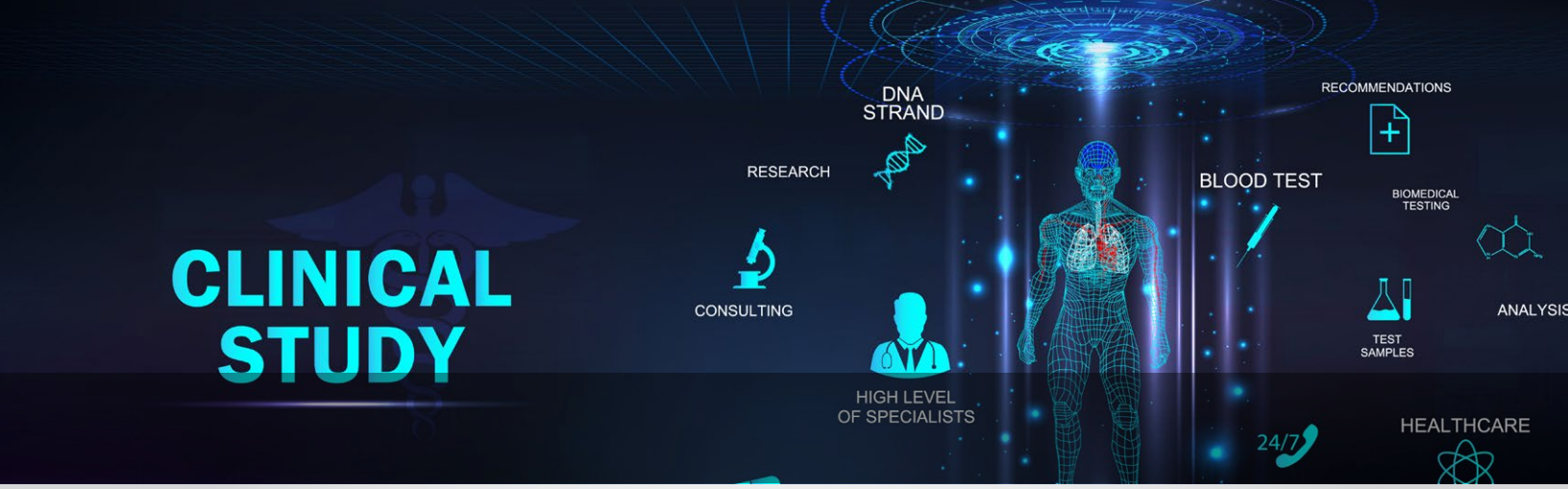


FIGURE 6. HEALTH BENEFITS OF LINLONGA® AS OBSERVED IN THE CLINICAL STUDY.

Improvement of fatty acid metabolism is an effective strategy to treat NASH. In the clinical study, the treatment group showed better lipid metabolic parameters like improved blood lipid profile including decrease in the levels of triglycerides (TG), total cholesterol (TC) and low-density lipoprotein (LDL-C), V-LDL. LivLonga® induced significant reduction in liver enzymes like alanine aminotransferase (ALT) and aspartate aminotransferase (AST), compared to the placebo group which were assessed as the markers of reduced liver injury (Figure 7).

Thus, the study proved the efficiency of LivLonga® in improving dyslipidemia and fatty acid metabolism which are considered as the milestones in NASH management.

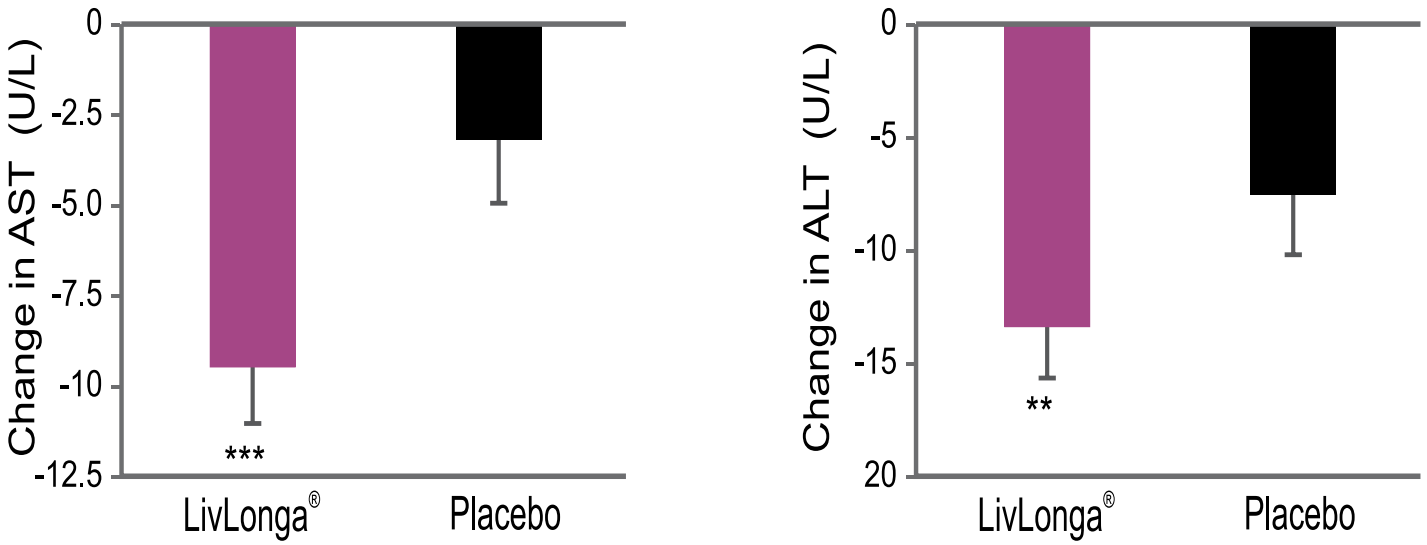


FIGURE 7. THE MEAN LEVELS OF (A) AST; (B) ALT AFTER 90 DAYS OF TREATMENT.

Fibroscan with controlled attenuated parameter (CAP) was used measure the stiffness of the liver as well as fat deposition. High attenuation rates of signal waves indicates advanced grade steatosis. Supplementation of LivLonga® resulted in promising improvement in liver fibrosis and also showed significant reduction in liver fat accumulation in the test subjects (Figure 8). Reduced liver stiffness and fat attenuation indicate significant improvements in liver fibrosis (NF-kB).

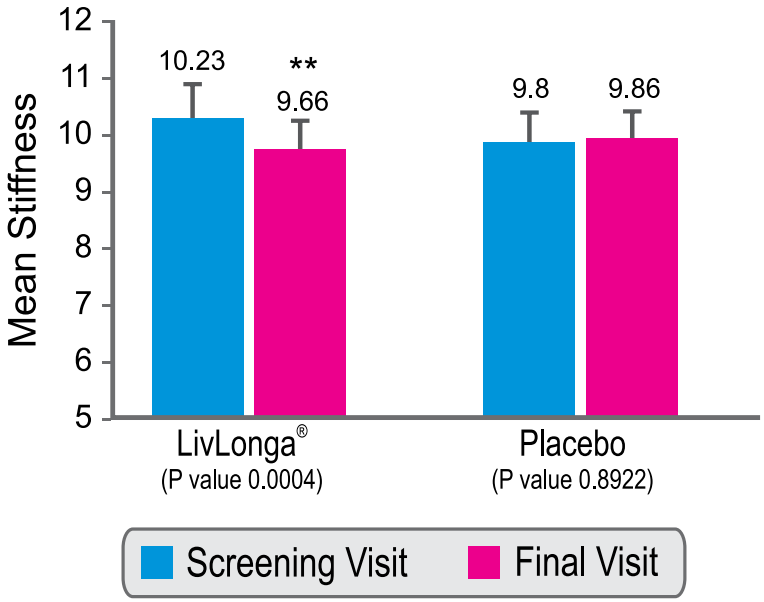
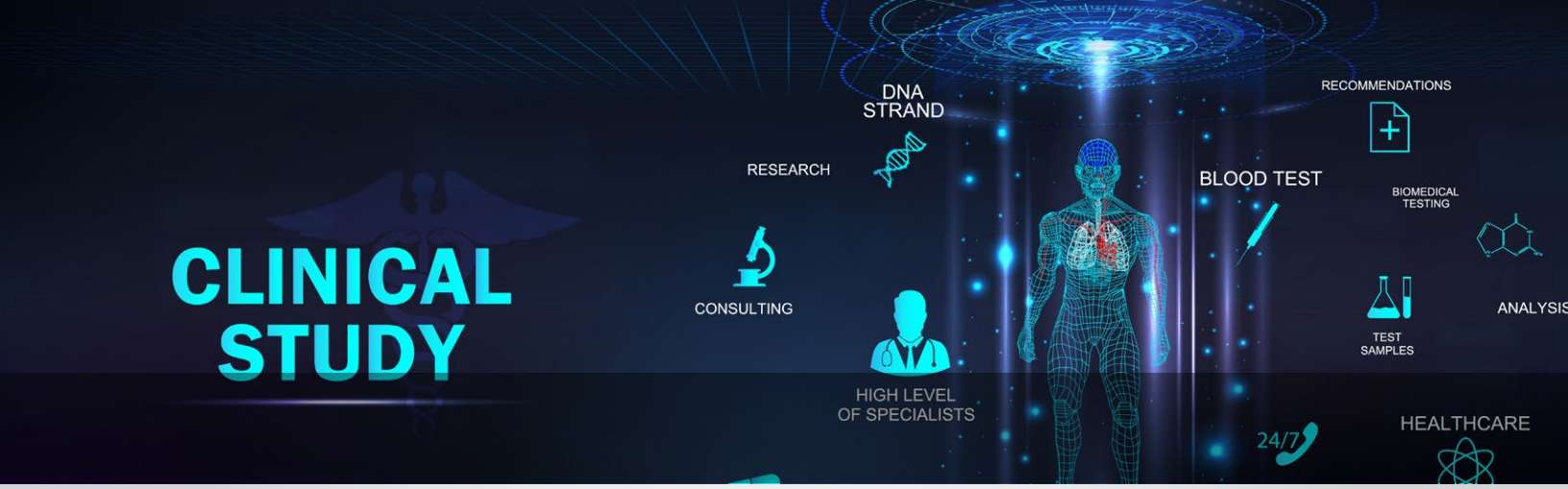


FIGURE 8. THE MEAN CHANGE IN LIVER STIFFNESS MEASURED BY FIBROSCAN FROM BASELINE TO END OF STUDY WAS SIGNIFICANT (P-VALUE<0.0001) IN LIVLONGA® COMPARED TO PLACEBO.



Interestingly after 3 months of supplementation, the participants supplemented with LivLonga® showed improvements in body weight, body mass index (BMI) and waist circumference. Adiponectin is an important hormone that plays a critical role in the regulation of fat and glucose metabolism. Down regulation of adiponectin is associated with obesity and insulin resistance (Kadowaki *et al.* 2006). The subjects who administered LivLonga® for 90 days showed increased level of adiponectin at the end of the study period which directly sensitizes the body to insulin. Further, LivLonga® supplemented group showed reduced levels of inflammatory markers such as hsCRP and IL-6 in the serum of NASH patients indicating effective management of liver inflammation (Figure 9).

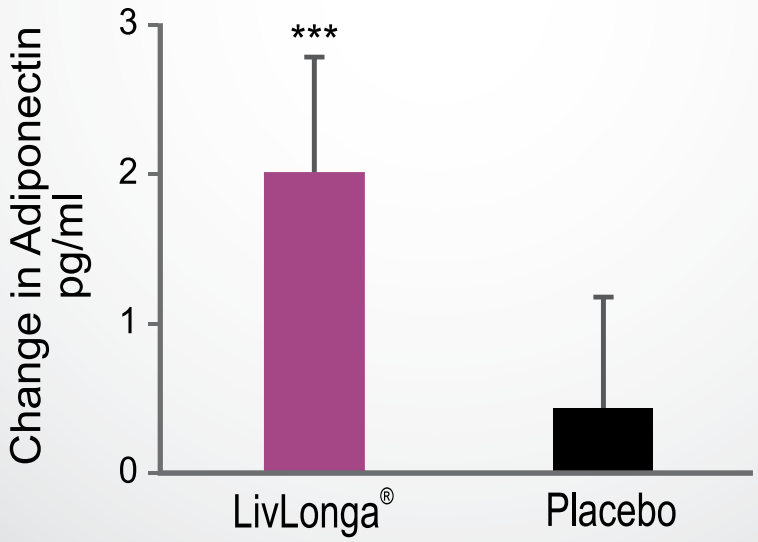


FIGURE 9. THE MEAN ADIPONECTIN LEVEL AT THE END OF STUDY WAS SIGNIFICANTLY HIGHER IN LIVLONGA® COMPARED TO PLACEBO.

HOW SAFE IS LIVLONGA® FOR HUMAN CONSUMPTION?

Curcumin is approved as a food additive with "Generally Recognized As Safe" (GRAS) status by the United States Food and Drug Administration (FDA). It is widely in use not just as a medical treatment in the form of capsules and tablets, but as a supplement in ointments, energy drinks, soaps, and cosmetics worldwide. Further, Livinol® was found as safe with no reported chronic, sub-chronic and reproductive toxicity in rats when administered at a concentration of 100 mg/kg/day (Majeed *et al.* 2018). LivLonga®, was found to be safe in the clinical study with no changes in the vital parameters, hematological and biochemical parameters like

- ✓ Hematology
- ✓ Serum electrolytes
- ✓ Renal function test (serum creatinine, uric acid)
- ✓ Urine analysis
- ✓ Thyroid function test

THE OVERALL SAFETY PROFILE WAS SIMILAR TO THAT OF PLACEBO WITHOUT ANY ADVERSE EVENTS INDICATING THAT LIVLONGA® IS SAFE FOR HUMAN CONSUMPTION.

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CONCLUSION

Supplementation with LivLonga® showed significant improvement in NASH and liver damage in preclinical and clinical studies unmistakably pointing out to its crucial benefits in liver health. The novel formulation of LivLonga® is found effective in improving key pathological events associated with lipid/glucose metabolism, NASH, liver function, hepatosteatosis and fibrosis. Further, it is found as safe for human consumption without any adverse events. Hence, LivLonga® supplemented either alone or in combination with other therapeutic approaches might provide feasible clinical benefits to patients with chronic liver diseases like NASH.

TREATMENT WITH LIVLONGA® REDUCED NASH BY

- ☑ Reducing hapatocyte ballooning
- ☑ Reducing pathological deposition of collagen in liver
- ☑ Reducing inflammation, TGF-β and collagen 1 expression
- ☑ Reduction of oxidative stress
- ☑ Reducing metabolic syndrome in liver

SABINSA®

LivLonga® is a combination of *Garcinia indica* extract containing 20% Garcinol (Livinol®) and Curcuminoids (Curcumin C3 Complex®) with bioavailability enhancer BioPerine®. LivLonga® acts on different pathways of NASH pathogenesis and has synergistic hepatoprotective activity.

PATENTS

Liver Protectant Composition and Therapeutic Applications

- ✓ US 10,653,643
- ✓ SA2020/06540

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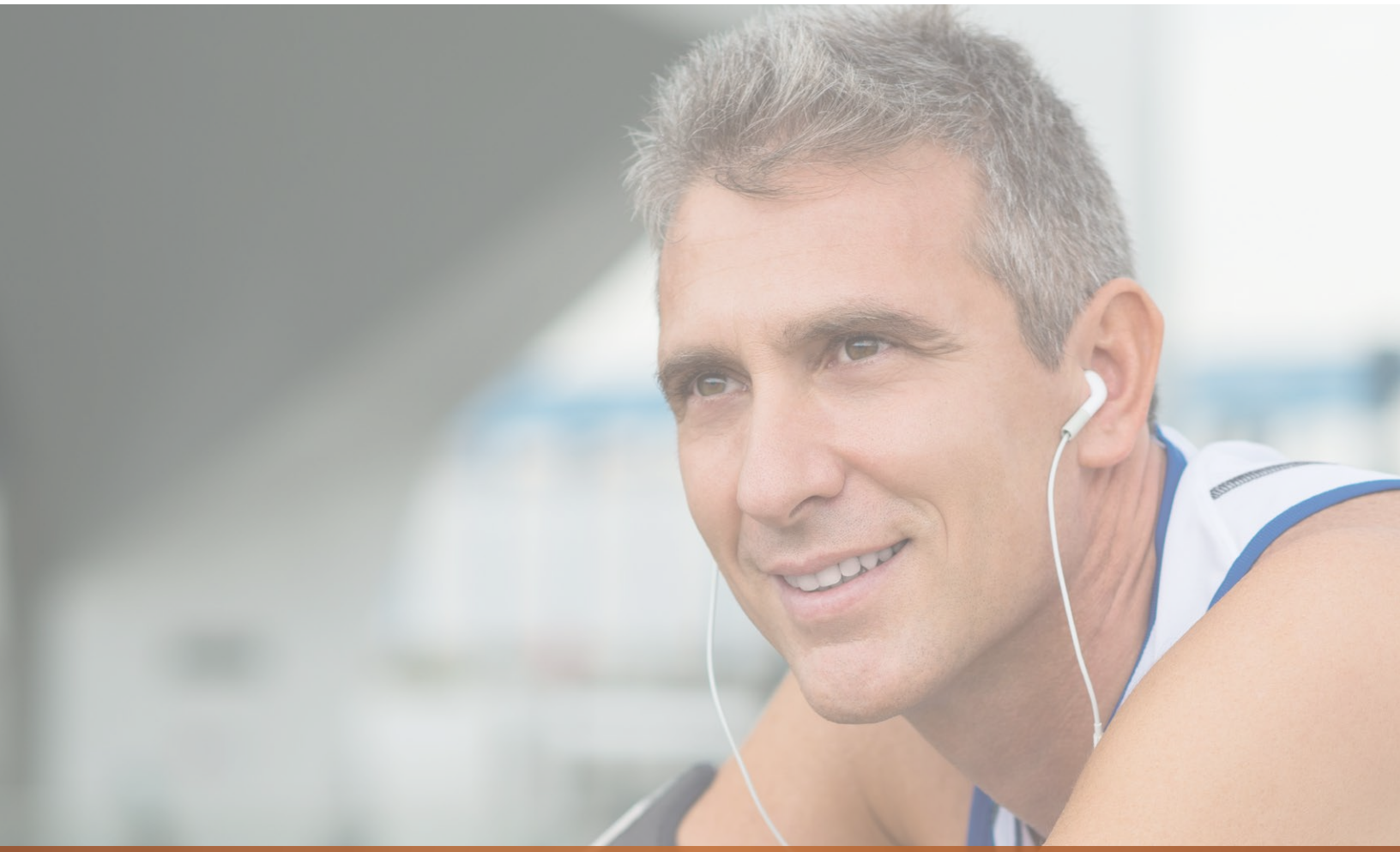

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Sabinsa, founded in 1988, provides alternative and complementary natural products for human nutrition and well-being. Sabinsa has pioneered the introduction of more than 120 ingredients, ranging from standardized botanicals, natural cosmeceuticals, to multi-enzyme blends and production of a shelf-stable probiotic. To support these products, there are numerous privately funded clinical studies in conjunction with prestigious institutions studying these products in a very consistent manner. Sabinsa is globally positioned with 1,000 people working in manufacturing and distribution facilities, and 120 full-time scientists conducting on-going research in India and the United States. Ingredients by Sabinsa are both Kosher and Halal certified.

For more information, contact info@sabinsa.com.

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