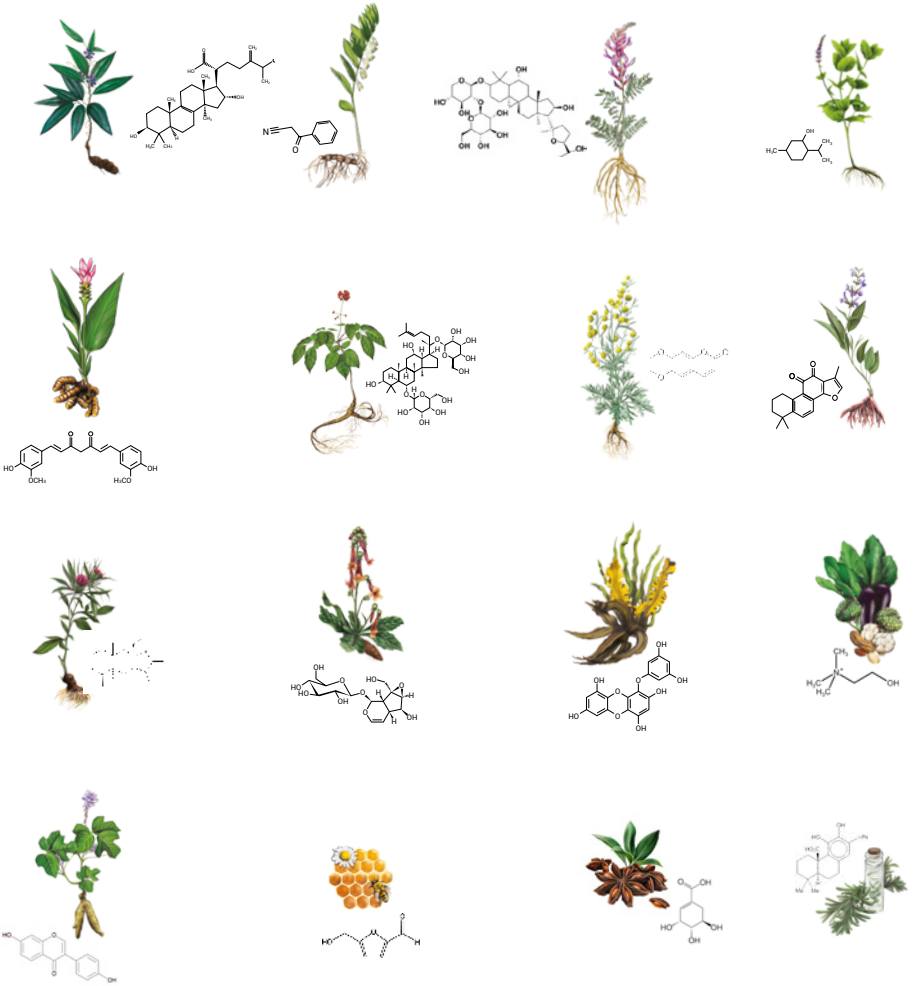
The Preclinical and Clinical evidence for de-liver-ance®



de-liver-ance®

Let's improve the 500+ functions of your liver.

"The most mighty hath created medicines out of the earth, and a wise man will not abhor them"

ECCLESIASTES

Introduction

In evaluating any new therapeutic intervention it is important to assess the safety as well as the efficacy of the product. This statement applies equally to both allopathic and naturopathic approaches.

Metabolism dysfunction-Associated Fatty Liver Disease (MAFLD) currently effects 40% of the adult population and is a global problem. There is therefore an urgent need for a “medicine” that leads to the resolution of MAFLD and does so reliably, and without inducing side-effects that may limit the application of said therapy.

The aim of this document is to summarize existing data on file that supports the use of de-liver-ance® in MAFLD.

Furthermore it will outline the future research plan for de-liver-ance® including both preclinical & clinical elements.

Preclinical Studies

Preclinical studies refer to those that occur before a human is exposed to a new agent. These are typically performed on cells and in rodents to demonstrate that the new product is effective in its stated goal and achieves this goal without prohibitive toxicity.

As a phytomedicine, and being classed as a food supplement, the Food & Drug Administration does not require formal testing of de-liver-ance®. However, the performance of select and targeted experiments provides additional evidence as to the potential benefits of de-liver-ance® when subsequently provided to individuals with MAFLD.

Herein are described experiments which show that when administered to mice predisposed to the development of fatty liver disease that de-liver-ance® can both reverse fatty changes, and when administered prophylactically prevent the development of MAFLD.

In addition to standard parameters such as liver fat content, and blood lipid profiles, the effects of de-liver-ance® on transcriptomic profiles was also assessed. Transcriptomics is the study of all RNA in the cell and reflects how genes are expressed as proteins at a specific time under specific conditions such as in a disease state such as MAFLD or following implementation of therapy as in de-liver-ance®.

N.B. Thes studies were conducted prior to the change in nomenclature form NAFLD to MAFLD.

Prevention & Treatment

Aims: The aim of this study was to determine the effects of de-liver-ance® in a mouse model of NAFLD.

Study Design: The fat-, fructose- and cholesterol-rich liquid diet (FFC) in which energy is supplied as carbohydrates (60%) fat (25%), and protein (15%) was used to induce NAFLD in C57BL/6 mice. Male mice were randomly allocated to FFC or control groups (6-9 weeks duration) as displayed below (Figure 1).

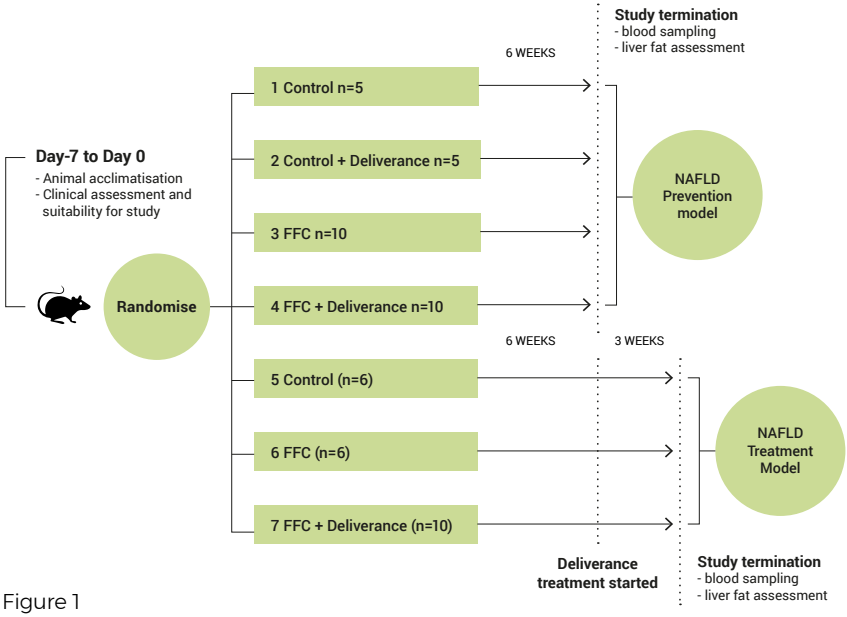


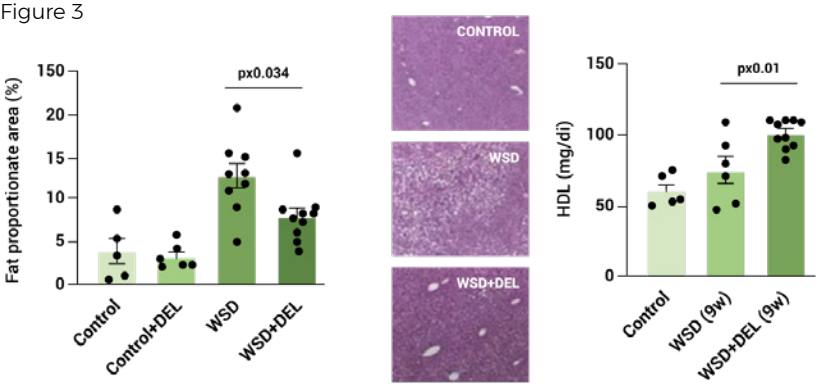
Figure 1

Animals were fed control diet or FFC for 6 weeks and then randomized to de-liver-ance® treatment or no additional treatment from baseline (NAFLD prevention model, groups 1-4). Additionally, three further groups were extended for a total of 9 weeks (control or FFC) with de-liver-ance® added at

week 6 in one FFC group (NAFLD treatment model, groups 5-7). De-liver-ance® was dosed (added to liquid diet) according to the allometric approach which considers the differences in body surface area and animal weight, at 1.7ml/mouse daily (allometric equivalent to the 22ml human dose). Endpoints included histological liver fat quantification (fat proportionate area, FPA), and serum high density lipoprotein (HDL).

Results: In the prevention model, the FPA was significantly lower in the de-liver-ance® treated arm (Figure 2) and the HDL levels we significantly higher compared to control animals. In the treatment model the HDL levels we significantly higher in the de-liver-ance® arm compared to control mice (Figure 3) and there was a trend towards a reduced FPA in the treated group.

Figure 3



Conclusions: The studies demonstrated the value of de-liver-ance® both in the prevention of the development of NAFLD in a mouse model predisposed to the disease, and in the resolution of NAFLD following its manifestation. In both groups, de-liver-ance® also improved the cholesterol profile increasing HDL levels in each of the treated cohorts.

Transcriptomics

Aims: The aim of this study was to determine the gene expression patterns associated with de-liver-ance® to better understand its impact on metabolic homeostasis and its potential benefits in NAFLD in terms of both treatment and prophylaxis.

Study Design: The livers assessed had been preserved following the Prevention & Treatment study. RNA was isolated from 36 liver samples using a column-based extraction method using QIAGEN RNeasy®. RNA was quantified using a NanoDrop UV spectrophotometry and its integrity determined through gel electrophoresis. Gene expression was assessed using NanoString technology and evaluated 893 mouse genes within a metabolic pathway panel. 100ng of each RNA sample was hybridized to probes tagged with fluorescent barcodes and an nCounter digital analyzer was used to count the probe bound RNA molecules by detecting individual barcodes. Data was analyzed with nSolver advanced analysis software.

Results: The gene expression data obtained captured the effects of de-liver-ance® on the liver. The FFC diet resulted in overexpression of transcripts associated with inflammation, fat accumulation and oxidative stress. These included genes associated with lysosomal degradation, Nuclear factor – kappa B (NF-kB), antigen presentation, and fatty acid synthesis all of which correlating with the main features of NAFLD. de-liver-ance® elixir did not affect gene counts beyond threshold levels indicating that oxidative stress, inflammation, and fat accumulation were

not evident. Furthermore, the administration of de-liver-ance® did not perturb normal metabolic pathways and maintained homeostasis. Below are depicted Directed Global significance heat maps for the prophylactic arm (Figure 4) and treatment arm (Figure 5). Covariates assessed were de-liver-ance® - treated versus untreated (left panel) and diet - FFC versus control (right panel).

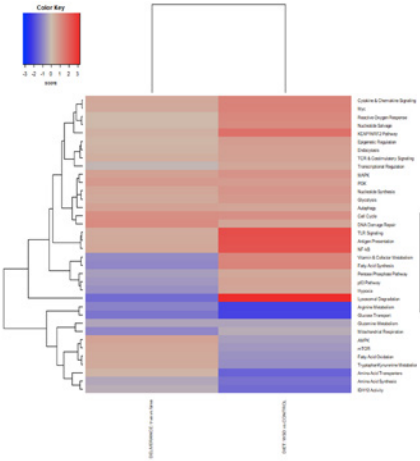


Figure 4

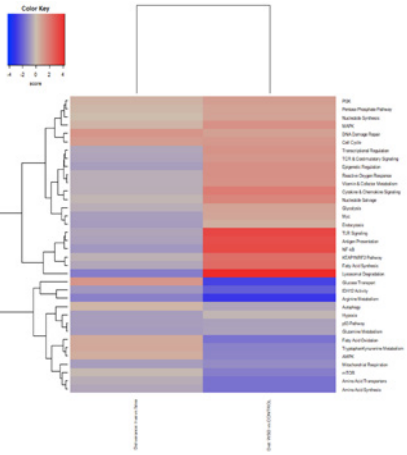


Figure 5

Conclusions: The administration of de-liver-ance® had the opposite effects as the administration of a FFC diet corroborating data that it may have an important role in the prevention and treatment of NAFLD.

Clinical Studies

Clinical studies are investigations carried out in human volunteers or patients, in this case, patients with MAFLD. Such studies aim to confirm that the that the agent under evaluation, having been shown to be effective in preclinical models remains as safe and effective in humans.

A study performed to document the clinical efficacy of de-liver-ance® in MAFLD is reported on the forthcoming pages. In addition to the initial clinical study, de-liver-ance® has now been provided as a therapy to reverse MAFLD in more than 1,000 clients over the past 7 years.

To-date, there have been no serious adverse reactions documented. There have the occasional cases of sensitivity to curcumin, the principle curcuminoid of turmeric, and one of the components of de-liver-ance®. All have occurred in individuals with known sensitivity to this polyphenol.

An extensive review of the 17 components of de-liver-ance® in the naturopathic literature, revealed no components with known risk of anaphylaxis. 4 of the elements are recommended not for use in the first trimester of pregnancy and as such de-liver-ance® is not advocated in pregnancy.

Furthermore, de-liver-ance® has also been evaluated in the clinical setting as a means of reducing the toxicity of alcohol. These data are also reported.

Clinical Results in MAFLD

Aims: The aim of this study was to evaluate the safety and efficacy of de-liver-ance® in patients with NAFLD.

Study Design: 44 males and 27 females (mean age of 49 years) and NAFLD confirmed on FibroScan® were treated over a 6-month period with de-liver-ance®. Data assessed at baseline and 6-months follow-up included symptoms indicative of MAFLD (sleep, energy, and cognition) and lipid profiles (Total cholesterol, HDL, low density lipoprotein [LDL], and triglycerides [TG]). FibroScan® results including controlled attenuation parameter (CAP) score indicative of fatty change and stiffness score were recorded at baseline and follow-up. For a subgroup (n-20) with deranged liver function, the concentrations of the enzymes: alanine aminotransferase (ALT); aspartate aminotransferase (AST); and gamma glutaryl transferase (□GT) were documented.

Results: Clinical symptoms improved significantly in the vast majority of study patients (Figure 6).

Parameter	Improvement
Sleep	**100%▲**
Energy	**90%▲**
Cognition	**80%▲**

Figure 6

There were significant reductions in plasma cholesterol, LDL, and TG levels, and an increase in HDL (Figure 7).

Parameter	Improvement
Total Cholesterol	65%▼
HDL	28%▲
LDL	47%▼
TG	59%▼

Figure 7

73% of individuals showed reduced fat levels on follow-up FibroScan®. An example of transformation from stage 3 steatosis to normal within 6-months is illustrated in Figure 8.

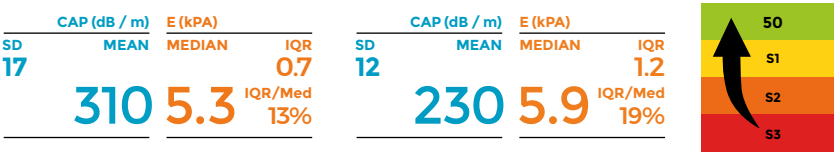


Figure 8

Median levels of all enzymes improved over a 40-day assessment period (Figure 9).

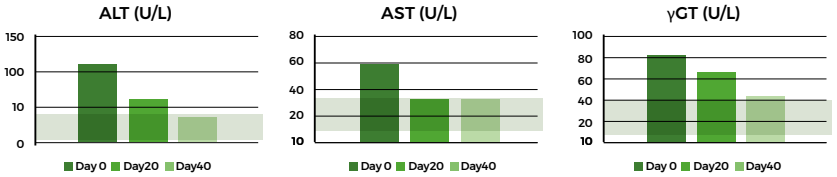


Figure 9

Conclusions: The consumption of de-liver-ance® lead to significant improvements in symptoms as well as in lipid and liver profiles and in CAP scores as assessed by FibroScan®.

Blood Alcohol Metabolism

Aims: The aim of this study was to determine the effects of de-liver-ance® on blood alcohol metabolism (BAM) in healthy volunteers. through determination of blood alcohol concentrations (BAC)

Study Design: 10 healthy adult volunteers aged 20-60 years underwent 3 assessments at intervals of 3 days thus acting as their own controls. None had consumed alcohol within 72 hours of Test 1. No food was to be consumed within 2 hours of commencing or completing the test.

Test 1 - Participants consumed 80-100mL of 47% alcohol over a period of 60 minutes.

Test 2 - Participants consumed de-liver-ance® 30 minutes before consumption of alcohol (same dose of alcohol and duration as in Test 1).

Test 2 - Participants consumed de-liver-ance® during last 15 minutes of their alcohol consumption (same dose of alcohol and duration as in Test 1).

BACs were determined at baseline and for the next 5 consecutive hours for each volunteer during each test. All measurements were conducted by an independent observer. The baseline measurements were 0 mcg/100mL confirming adherence to the study protocol in each case. The second measurement at 1 hour was immediately after completion of alcohol intake.

Results: The results demonstrated that de-liver-ance® significantly reduced BAC and that the change was most pronounced when de-liver-ance® was consumed prior to alcohol (Figure 10). The peak alcohol concentration was 3 to 4 times greater when no de-liver-ance® was provided after or before alcohol respectively. The percentage reduction was most noticeable in females and in participants with mild-moderate intake. No participant reported a hangover after taking de-liver-ance®.

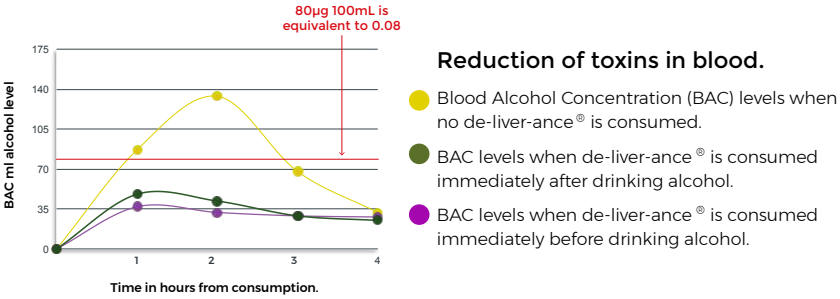


Figure 10

Conclusions: The administration of de-liver-ance® significantly BAC with the best results obtained when taken prior to consumption of alcohol. It is presumed that the effect is related to induction of alcohol dehydrogenase and further studies are planned to confirm this mechanism.

Antioxidant Properties

A key property of de-liver-ance® is its ability to combat oxidative stress, a critical feature in the pathogenesis of MAFLD. As a result of the combinatorial effect of the 17 natural ingredients in de-liver-ance®, its antioxidant score as assessed by the ABEL-RAC™ peroxynitrite assay is considerably greater than other well-known antioxidants as per Figure 11.

item	Antioxidant score*
de-liver-ance®	94.5 million
Rosemary	819,00
Grapeseed extract	390,000
Ginko extract	100,632
Curcumin	43,000
Bee pollen	9,520
Broccolli extract	6,600

Figure 11

*ABEL-RAC Units of Peroxynitrite

Another means of assessing the effects of de-liver-ance® in MAFLD is to assess the activity of neutrophils, the cell type driving inflammation. This test measures the production of reactive oxygen species (ROS) by neutrophils in a sample of whole blood diluted 1:1000. Release of the superoxide free radical, occurs only after the enzyme NADPH oxidase is activated. fMLP (formyl-methionyl-leucyl-phenylalanine) a receptor activator of the NADPH oxidase is used. When the fMLP is injected into the diluted blood it binds to receptors on the neutrophils, activates the NADPH oxidase resulting in the extracellular release of superoxide. The superoxide reacts

with a light emitting molecule (Pholasin®). The amount of ROS generated, and the rate of production is visualized in a light response curve. The shape of the curve and peak light value are used to analyze the result. The normal reference curve and peak light range are derived from the average light response from 385 samples. The results are given as a light response curve, designated a shape based on the best fit from the library of curves and are also given a peak light response value. Both of these results are interpreted to indicate the effects of de-liver-ance on neutrophil function.

The appearance of an ABEL cell activation assay is shown in Figure 12.

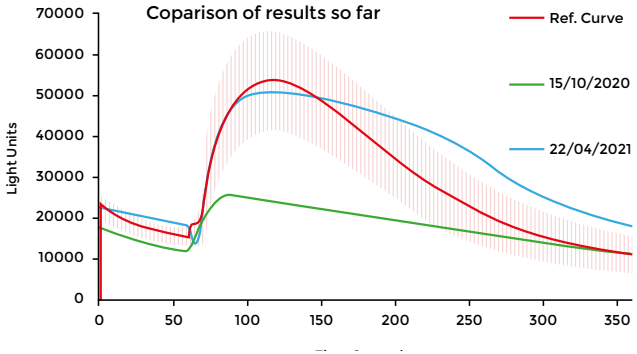


Figure 12

The baseline assessment (green) demonstrates an exhausted neutrophil response indicating a chronic inflammatory state. Following 6-months of de-liver-ance® as indicated in the blue trace, the peak response has normalized indicating improved neutrophil function. This correlated with a resolution of steatosis within the liver.

Future Studies

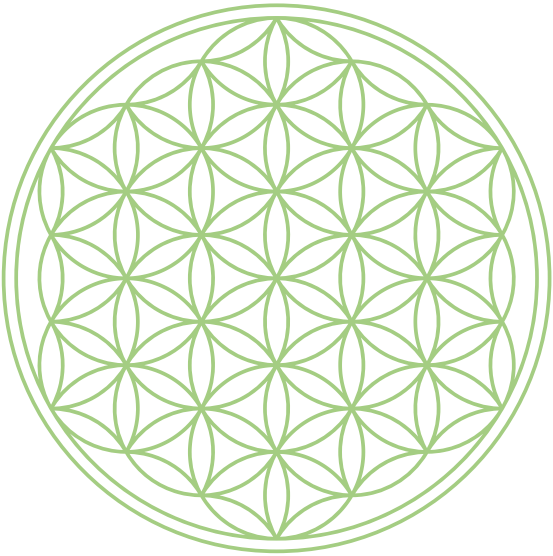
Preclinical

1. Repeat prevention and treatment studies with larger numbers and increased duration of therapy
2. Metabolomic analysis of those gens that were up or down regulated in the transcriptomic study.
3. Assess effects of de-liver-ance® on hepatic Phase I/II detoxification systems and on on mitochondrial function.
4. Assessment of the effects of de-liver-ance® in inflammation-driven cancers in cell and animal models.

Clinical

1. Randomized trial of standard of care (SOC) namely diet and exercise versus diet and exercise supplemented with de-liver-ance® in individuals with stages 2 & 3 steatosis.
2. Observational study of de-liver-ance® combined with StemRegen® in patients with stage 3 & 4 fibrosis.
3. Observational study of de-liver-ance® in symptomatic perimenopausal women
4. Observational study of de-liver-ance® in men with low testosterone in conjunction with metabolic syndrome and steatosis confirmed on FibroScan ®.
5. Observational study of de-liver-ance® in women with polycystic ovarian syndrome

6. Observational study of de-liver-ance® in patients on statins to document incidence of fibrosis and steatosis and to observe effects of therapy
7. Observational study of de-liver-ance® combined with Glp-1 inhibitors in patients with obesity with the aim of reducing inflammation-driven complications
8. Assess the gut-liver axis in MAFLD by examining the effects of de-liver-ance® on the microbiome.



“The greatest medicine
of all is teaching people
how not to need it.”

HIPPOCRATES

To learn more about
your own liver
scan the QR code.

loveyourliver.com

ROOTS TO HEALING
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