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## Research Article

# Efficacy of a multicomponent nutraceutical on the normalization of liver functional parameters in patients with NAFLD: a double blind, randomized, clinical trial

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## ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in Western countries. The main preventive/therapeutic tool of NAFLD is currently the improvement of lifestyle. The purpose of this study is to evaluate the impact of a combined nutraceutical approach on the clinical and laboratory features of NAFLD patients. We consecutively enrolled 80 NAFLD patients in a double-blind, placebo-controlled, randomized clinical trial. After a 4 weeks stabilization diet period, we randomized them to assume a combined multivitamin/multimineral/botanical nutraceutical (Metaclear™) 2 tablets per day or an identical placebo for 3 months. Patients were rechecked after further 30 days. Liver parameters significantly improved in the combined nutraceutical treated subjects. In particular, in a generalized linear mixed model with Fatty Liver Index (FLI) as dependent variable a significant period by treatment effect was observed (F-value = 22.5,  $p < 0.001$ ). For the patients treated with the combined nutraceutical, FLI decreased with on average 11.9 units and 13.6 units at the end of treatment and the follow-up visit compared to baseline, respectively. The decrease in FLI from baseline to the end of treatment and to follow-up was on average 5.9 units higher (95% CI 3.3-8.4,  $p < 0.001$ ) and 8.6 units higher (95% CI 6.0-11.1,  $p < 0.001$ ) for the combined nutraceutical treated group in comparison to the placebo group, respectively. The short-term treatment with the combined nutraceutical has been associated with a significant improvement of NAFLD biomarkers.

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## Introduction

Non-alcoholic fatty liver disease (NAFLD) is a potentially evolutive condition characterized by a progressive infiltration of fat in the hepatocytes and comprises a wide spectrum of hepatic disorders that are not linked to other pathological conditions, such as viral hepatitis, alcohol consumption (>20 gr/day) and chronic drugs use [1]. In the last years, the incidence of NAFLD has shown an exponential increase in western countries. The prevalence of NAFLD in developed countries is

estimated to be about 30% of adult population, 15% of children, and more than 50% of overweight, obese, and type 2 diabetics [2]. However, also in Asia the disease is becoming increasingly common, with a prevalence up to 15% reported in China [3].

The clinically aggressive and irreversible variant of NAFLD, non-alcoholic steatohepatitis (NASH), affects about 5% of the general adult population, and 20% of obese people [4]. It's characterized by inflammation and progressive tissue degeneration of the liver

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parenchyma: in general, about 1/3 of the cases of NAFLD tend to become NASH, and 15% of these can degenerate into cirrhosis. In addition, NAFLD is itself a risk factor for the development of cardiovascular disease and type 2 diabetes, and preliminary data suggest that it may also be associated with a greater incidence of hepatic and extra-hepatic cancers [5-7]. A meta-analysis of six studies (25,837 people, of whom 5953 were affected by NAFLD) showed that patients with NAFLD had a relative risk of total CV events of 1.77 (95% CI 1.26–2.48,  $p < 0.001$ ); the relative risk increased to 2.26 (95% CI: 1.04–4.92,  $p < 0.001$ ) with regard to coronary artery disease and to 2.09 (95% CI: 1.46–2.98,  $p < 0.001$ ) relative to ischemic stroke. Furthermore, the presence of NAFLD seems to significantly increase the relative risk of mortality due to cardiovascular causes (RR 1.46, 95% CI 1.31–1.64,  $p < 0.001$ ) [8].

On the other hand, overweight/obesity, insulin resistance/type 2 diabetes, hypertriglyceridaemia and the related dietary-behavioural triggers the high intake of beverages as well sweetened with fructose are the main risk factors for NAFLD [9]. As reported by observational studies, consumption of sugared soft drinks per se increases the risk of developing NAFLD of around 55% [10]. Since NAFLD pathogenesis involves both genetic, epigenetic and environmental factors, it is widely accepted that the disease can be considered a multifactorial pathology [11]. Even if NAFLD is often related to poor lifestyle habits, among the emerging risk factors there are the smoking habit and the Obstructive Sleep Apnoea Syndrome (OSAS), but also insomnia and excessive daytime sleepiness unrelated to nocturnal sleep apnoea [12, 13]. Finally, also the strong association between hypothyroidism and NAFLD has recently been confirmed by a meta-analysis of 13 prospective studies [14].

Treatment of NAFLD remains difficult pending specific drug therapies. The cornerstone of treatment is achieving weight control and reduction in cardiovascular risk factors. Currently the most effective treatment is weight loss by a combination of dietary modifications and exercise in order to reduce insulin resistance [15, 16]. Since NAFLD has many risk factors similar to the ones for CVD, the general indications are the same, including a relatively low-calorie diet (caloric quantity proportional to energy consumption), with carbohydrates predominantly with a low glycemic index and minimizing the consumption of fructose, alcohol, saturated and trans-unsaturated fats [17, 18]. The adherence to Mediterranean Diet seems to be an important predictor of liver fat content in people with NAFLD and also the coffee consumption is related to a lower risk of fibrosis as summarised in a recent meta-analysis regarding coffee drinkers patients with NAFLD [19, 20]. At the same time, physical activity is fundamental: a recent meta-analysis shows that the difference in risk of developing NAFLD among sedentary and physically is 21% in observational studies and 57% in case-control studies [21]. On the other hand, regardless of diet, the greater the frequency and intensity of physical activity, the greater the reduction of transaminase levels and the degree of hepatosteatosis, especially in overweight subjects [22].

Then, some nutraceuticals seem to improve NAFLD, when prescribed on top of therapeutic lifestyle changes [23]. For instance, Silymarin, a mixture of flavolignans extracted from *Silybum marianum*, acting through different mechanisms and complex biological interactions has

largely shown to improve the prognosis of patients affected by chronic liver diseases [24]. The actions of berberine, curcumin and coenzyme q10 are related to the improvement of levels of indirect markers of hepatosteatosis (Hepatic Steatosis Index, Lipid Accumulation Product) for short-term supplements (2–4 months), even if each of these nutraceuticals showed any bioavailability problems [25-27]. In accordance with the parallel-hit hypothesis, stating that the development of liver disease involves a group of simultaneous alterations in the organism, combinations of different bioactive compounds that act against complementary targets have been examined [28]. Thus, most probably a combination of different nutritional interventions is needed to tackle the pathology [29].

The objective of the study is to examine whether patients with metabolic syndrome and NAFLD show reduced or normalized levels of hepatic functional parameters after taking a nutraceutical based on vitamins, minerals, amino acids and specific herbals.

## Materials and Methods

### Study design and study participants

This parallel arm, single-blind, placebo-controlled, randomized clinical trial was carried out in 80 adult subjects affected by metabolic syndrome and NAFLD, pharmacologically untreated, in primary prevention for cardiovascular diseases, consecutively enrolled in the ambulatory service of cardiovascular disease prevention in the Medical and Surgical Sciences Department of the University of Bologna. Metabolic syndrome was identified based on the ATPIII/IDF criteria [30]. The components were defined using the following ATPIII categorizations: 1) high blood pressure (BP  $\geq 130/85$  mmHg); 2) hypertriglyceridemia (TG  $\geq 150$  mg/dl), 3) low HDL-C ( $< 40$  mg/dl for men and  $< 50$  mg/dl for women); 4) hyperglycaemia (100 mg/dl  $>$  FPG), 5) waist circumference  $> 102$  cm for men and  $> 88$  cm for women (suggestive of high abdominal obesity). Subjects with at least three of the previous components were classified as having Metabolic syndrome. NAFLD was identified as having Fatty Liver Index (FLI)  $> 60$  or fatty liver confirmed by ultrasounds.

Exclusion criteria were:

- GOT, GPT or gGT  $> 3$  times ULN
- Alcoholism in active phase
- Diabetes mellitus with pharmacologic treatment
- Severe obesity (Body mass index  $> 35$  kg/m<sup>2</sup>)
- Inflammatory bowel disease
- Known individual intolerance to the components of the tested product

The study was fully conducted in accordance with the Declaration of Helsinki, and informed consent was obtained from all patients before the inclusion in the study.

At baseline, patients were given standard behavioral and qualitative (not quantitative) dietary suggestions to correct unhealthy habits. Standard diet advice was given by a dietitian and/or specialist doctor. Dietitian and/or specialist doctor periodically provided instruction on dietary

intake recording procedures as part of a behavior modification program and then later used the subject's food diaries for counseling. In particular subjects were instructed to follow general indication of a Mediterranean diet, avoiding excessive intake of dairy products and red meat derived products during the study, maintaining overall constant dietary habits. Individuals were also generically encouraged to increase their physical

activity by walking briskly for 20 to 30 minutes, 3 to 5 times per week, or by cycling.

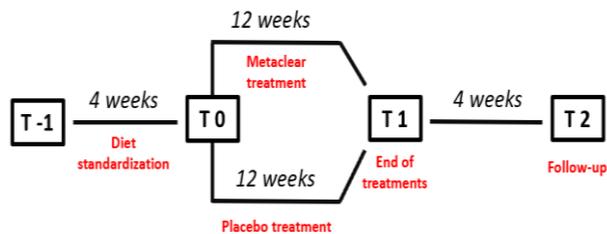
After 28 days, the subjects were randomized to be treated with a combined nutraceutical 2 tabs per day (Metaclear™, kindly provided by Metagenics Europe Ltd., Belgium) or Placebo 2 tabs per day for three months. The composition of the tested product is detailed in (Table 1).

**Table 1:** Description of the tested product

<b>INGREDIENTS PER 2 TABLETS:</b>		
		%RI*
Vitamin A (retinyl palmitate)	125 µg	16%
Vitamin B1 (thiamine monohydrate)	2,1 mg	191%
Vitamin B2 (riboflavin)	2,4 mg	171%
Vitamin B3 (niacinamide, niacin)	11,7 mg	73%
Vitamine B5 (calcium pantothenate)	9,0 mg	150%
Vitamine B6 (pyridoxal-5-phosphate)	3,0 mg	214%
Vitamine B12 (methylcobalamine)	25 µg	100%
Vitamine C (magnesium ascorbate)	66,67 mg	83%
Vitamine D (cholecalciferol)	25 µg	500%
Vitamine E (D-alpha tocopheryl acetate)	17,01 mg	142%
Biotin	33,30 µg	67%
Folate (calcium-L-methylfolate)	134 µg	67%
Copper (copper citrate)	333 µg	33%
Manganese (manganese citrate)	0,83 mg	42%
Molybdenum (sodium molybdate)	33,3 µg	67%
Selenium (selenium methionine)	25 µg	45%
Zinc (zinc citrate)	3,3 mg	33%
Artichoke plant extract (Cynara scolymus)	75 mg	
	Contains cynarine	3,75 mg
Green tea leaf extract (Camellia sinensis)	50 mg	
Contains: polyphenols	Min 25 mg	
Catechins	10-15 mg	
epigallocatechin-3-gallate	3,5-6,5 mg	
Caffein	2-3 mg	
Milk thistle seed extract (Silybum marianum)	100 mg	
	Contains silymarine	80 mg
Pomegranateextract (Punica granatum)	100 mg	
	Contains ellagic acid	40 mg
Watercress leaf extract (Nasturtium officinale)	100 mg	
N-acetyl-L-cysteine	150 mg	
Choline	82,5 mg	
Alpha-lipoic acid	50 mg	
L-cysteine HCl	5 mg	
L-glutathione	10 mg	
L-glycine	400 mg	
L-lysine HCl	35 mg	
L-threonine	35 mg	
Potassium citrate	214 mg	
Taurin	100 mg	
Sodium sulfate	50 mg	

\*RI = Reference Intake

Clinical and laboratory data have been obtained at the baseline, after three months, and four weeks after the last drug assumption (follow-up visit) (Figure 1).



**Figure 1:** Study design

Randomization was done using a drawing of envelopes containing randomization codes prepared by an independent statistician and specific software. The envelopes were then further mixed and distributed to the investigators who assigned the randomization code in a progressive way to the enrolled subjects. A copy of the code was provided only to the person responsible of performing the statistical analysis. Throughout the study, we instructed patients to take the new product first dose on the day after they were given the study product in a blinded box. At the same time, all unused products were retrieved for inventory. Product compliance was assessed by counting the number of product doses returned at the time of specified clinic visits. At the end of the study, patients were asked to evaluate the treatment acceptability in term of “very low”, “low”, “good”, or “very good”.

### Assessments

Patients’ personal history was evaluated, paying attention to cardiovascular disease and other diseases, dietary and smoking habits, physical activity, and pharmacological treatment. At each visit body weight and waist circumference were collected. Body mass index (BMI) was calculated as weight in kilograms (kg) divided by height in meters squared (m<sup>2</sup>). Height and weight were measured by standard procedures to the nearest 0.1 cm and 0.1 kg respectively, with subjects standing erect with eyes directed straight ahead, wearing light clothes, and with bare feet. Waist circumference (WC) was measured at the end of a normal expiration, in a horizontal plane at the midpoint between the inferior margin of the last rib and the superior iliac crest. Office blood pressure was also measured by standardized methodology [31].

All plasma parameters were obtained after a 12-hour overnight fast. Venous blood samples were drawn by a nurse in all patients between 8:00 a.m. and 9:00 a.m. Plasma used was obtained by addition of Na<sub>2</sub>EDTA (1 mg/mL) and centrifuged at 3,000 g for 15 minutes at 48°C. Immediately after centrifugation plasma samples were frozen and stored at -80°C for no more than 3 months. The following parameters were evaluated via standardized methods: total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), triglycerides (TG), LDL-Cholesterol (LDL-C), glucose, liver transaminases, and gamma-Glutamyl Transferase (gamma-GT) TC and LDL-C were measured at the baseline only [32].

Lipid accumulation product (LAP) was calculated as  $(WC - 65) \times TG$  (expressed in mmol/L) for men and  $(WC - 58) \times TG$  (expressed in mmol/L) for women, and hepatic steatosis index (HSI) resulted from

$8 \times ALT/AST \text{ ratio} + BMI (+2 \text{ for women})$  [33, 34]. FLI was calculated as follows:  $[e^{0.953 \times \ln(TG) + 0.139 \times BMI + 0.718 \times \ln(GGT) + 0.053 \times WC - 15.745} / (1 + e^{0.953 \times \ln(TG) + 0.139 \times BMI + 0.718 \times \ln(GGT) + 0.053 \times WC - 15.745})] \times 100$  [35].

### Statistical Analysis

The parameters to assess efficacy of the tested combined nutraceutical vs. placebo were the change in FPG, AST, ALT, gamma-GT and HSI, after 84 (end of treatment) and 114 (follow-up) days, compared to baseline. Efficacy endpoints were analysed for the ITT population. Continuous data were summarized by their mean, standard deviation (SD), median, minimum and maximum. Categorical data were summarized by frequencies and percentages. Mean absolute changes in plasma levels of FPG, AST, ALT and gamma-GT and the index HSI from baseline (T0) at the end of treatment (T84) and at follow-up (T114) between the tested combined nutraceutical and placebo were evaluated with linear mixed-effects models, using the lme4 package in the R environment [36, 37]. Patients were specified as a random factor (i.e. random intercept model). The assumptions underlying the analysis were verified using diagnostic plots (QQ-plots for the normality of the error term). All descriptive and statistical analyses were performed in R version 3.3.1. (R development core team, 2017). A p-value <0.05 was considered as statistically significant. No imputation of missing data is performed. The amount of missing data is presented in the tables wherever appropriate.

### Results

All patients completed the trial: no patient dropped out from the study because of adverse events. One subject treated with the combined nutraceutical and one treated with placebo claimed mild and transient abdominal discomfort. Compliance was high, being 92% in both treatment groups. The overall acceptability of the proposed treatment was defined as good-very good by the 90% of the nutraceutical treated patients and by the 86% of the placebo treated ones. At the baseline, enrolled patients were age- and sex- matched (M: 65% in the placebo group, 72.5% in the combined nutraceutical one). The baseline characteristics of patients assigned to the different treatments were similar, and no significant differences were observed regarding the studied parameters (Table 2). Liver parameters are reported in (Table 3).

From the randomization visit to the end of the study, the enrolled subjects maintained overall a similar dietary pattern, without significant change in total energy, total cholesterol and total saturated fatty acid intake. Mean waist circumference and BMI were very similar at baseline, the end of treatment and follow-up period for both the combined nutraceutical and the placebo treated group. In a generalized linear mixed model with TG as dependent variable, period (F-value= 35.3, p< 0.001) was significant but there was no evidence of a treatment effect (F-value= 0.08, p= 0.775). TG reduced with on average 36.9 units and 42.7 units at the end of treatment and at the follow-up visit compared to baseline, respectively. At the same time, in a generalized linear mixed model with HDL as dependent variable, period (F-value= 48.2, p<0.001) was significant but there was no evidence of a treatment effect (F-value= 0.95, p= 0.334). HDL increased on average 2.5 units and 2.1 units at the end of treatment and at the follow-up visit compared to baseline. FPG reduced with on average 3.4 units and 4.2 units at the end of treatment

and at the follow-up visit compared to baseline, respectively, but there was no evidence of a treatment effect (F-value = 2.9,  $p = 0.093$ ).

For the patients in the combined nutraceutical, AST reduced with on average 13.2 units and 12.4 units at the end of treatment and at the follow-up visit compared to baseline, respectively. For the patients in the placebo group, AST reduced with on average 3.5 units and 0.3 units at the end of treatment and at the follow-up visit compared to baseline, respectively. This difference in reduction between the treatments was significant. The reduction in AST from baseline at the end of treatment

and at follow-up was on average 9.7 units higher (95% CI: 6.7 - 12.8,  $p < 0.001$ ) and 12.1 units higher (95% CI: 9.0 - 15.2,  $p < 0.001$ ) for the combined nutraceutical group in comparison to the placebo group, respectively. This is also illustrated in the plots correspond with individual changes from baseline at end of treatment (T84) and at follow-up (T114) (figure 2). ALT reduced with on average 9.0 units and 9.2 units at the end of treatment and at the follow-up visit compared to baseline, respectively, even if there was no evidence of a treatment effect (F-value = 1.2,  $p = 0.271$ ).

**Table 2:** Patient characteristics at baseline (data reported as mean and standard deviation)

	Placebo	Combined nutraceutical		Placebo	Combined nutraceutical
Age (years)	57.4 (13.6)	59.6 (12.5)	Total Cholesterol (mg/dL)	224.4 (38.5)	221.3 (34.8)
Systolic blood pressure (mmHg)	143.3 (20.4)	141.5 (17.9)	Triglycerides (mg/dL)	189.1 (98)	199.3 (152)
Diastolic blood pressure (mmHg)	78 (9.4)	75 (7.7)	HDL-Cholesterol(mg/dL)	42.5 (11.5)	39.4 (11.9)
Waist circumference (cm)	103.4 (7.6)	100.5 (5.9)	LDL-Cholesterol (mg/dL)	143.8 (33.9)	148.2 (34.9)
Body Mass Index (kg/m <sup>2</sup> )	29.8 (2.9)	28.9 (2.4)	Fasting Plasma Glucose (mg/dL)	97.7 (11.6)	95.3 (11.8)

**Table 3:** Liver parameters at the baseline in both studied groups (data reported as mean and standard deviation)

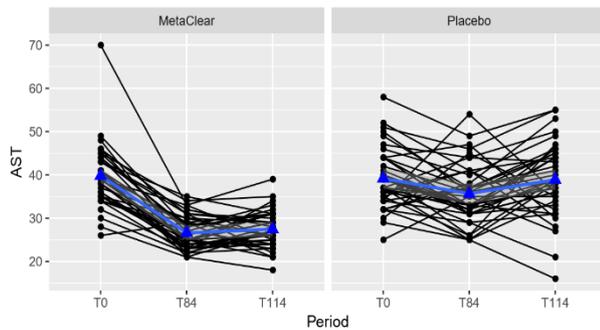
	Placebo	Combined nutraceutical		Placebo	Combined nutraceutical
Aspartate transaminase (AST)	39.2 (6.9)	39.8 (7.2)	Fatty Liver Index (FLI)	87.5 (11.1)	88 (8.9)
Alanine transaminase (ALT)	41.4 (9.7)	41.9 (10.3)	Hepatic Steatosis Index (HSI)	31.6 (2.8)	30.6 (3.1)
gamma-Glutamyl Transferase	44.2(13.6)	44.9 (12.9)	Lipid Accumulation Product (LAP)	84.1 (43.2)	78.5 (50.1)

For the patients in the combined nutraceutical, gamma-GT reduced with on average 14.6 units and 15.9 units at the end of treatment and at the follow-up visit compared to baseline, respectively. For the patients in the placebo group, gammaGT reduced with on average 9.3 units and 7.5 units at the end of treatment and at the follow-up visit compared to baseline, respectively. This difference in reduction between the treatments was significant. The reduction in gamma-GT from baseline at the end of treatment and at follow-up was on average 5.4 units higher (95% CI: 0.5 - 10.2,  $p = 0.030$ ) and 8.4 units higher (95% CI: 3.6 - 13.2,  $p = 0.001$ ) for the combined nutraceutical group in comparison to the placebo group, respectively. This is also illustrated in the plots correspond with individual changes from baseline at end of treatment (T84) and at follow-up (T114) (Figure 3). In the active group, FLI decreased with on average 11.9 units and 13.6 units at the end of treatment and at the follow-up visit compared to baseline, respectively. For the patients in the placebo group, FLI decreased with on average 6.0 units and 5.1 units at the end of treatment and at the follow-up visit compared to baseline, respectively. This difference in decrease between the treatments was significant. The decrease in FLI from baseline at the end of treatment and at follow-up was on average 5.9 units higher (95% CI: 3.3 - 8.4,  $p < 0.001$ ) and 8.6 units higher (95% CI: 6.0 - 11.1,  $p < 0.001$ ) for the combined nutraceutical group in comparison to the placebo

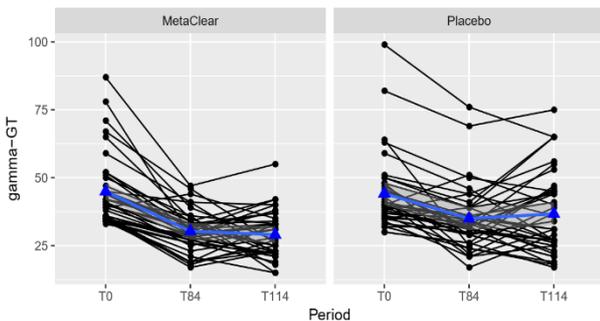
group, respectively. This is also illustrated in the plots correspond with individual changes from baseline at end of treatment (T84) and at follow-up (T114) (Figure 4).

Overall, LAP reduced with on average 17.8 units and 18.9 units at the end of treatment and at the follow-up visit compared to baseline, respectively, but there was no evidence of a treatment effect (F-value = 2.1,  $p = 0.148$ ). Finally, for HSI, no evidence of treatment effect (F-value = 0.2,  $p = 0.677$ ) or a period effect was found (F-value = 2.1,  $p = 0.085$ ).

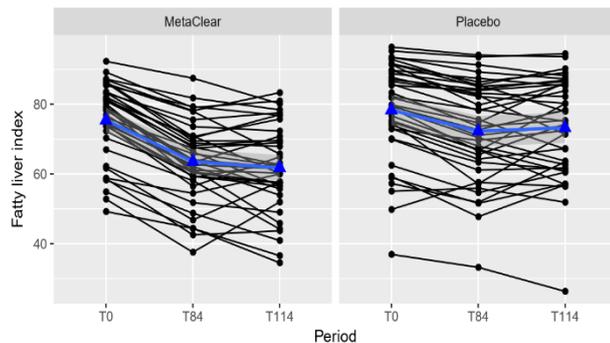
In summary, BMI, WC, and HSI did not change in function of period and no differences were observed between the treatments. TG, FPG, ALT and LAP reduced in comparison to baseline, however this reduction was similar for the placebo and combined nutraceutical group. HDL increased in comparison with baseline, however this increase was similar for the placebo and combined nutraceutical group. AST, gamma-GT, and FLI reduced at the end of treatment and at follow-up, this reduction was significantly higher for the group that received the combined nutraceutical.



**Figure 2:** Individual profile plots (black) and mean trend (blue) for AST.



**Figure 3:** Individual profile plots (black) and mean trend (blue) for gamma-GT.



**Figure 4:** Individual profile plots (black) and mean trend (blue) for FLI.

## Discussion

There is a growing interest in the study of nutraceuticals with hepatoprotective activities as confirmed in several randomized controlled clinical trials [38]. This trend could be explained to the large increase in the incidence of NAFLD; in fact, it is well known that this condition is the most common cause of chronic liver disease in Western countries [38]. Its clinical burden is not only confined to liver-related morbidity and mortality, but there is now growing evidence that NAFLD is a multisystem disease, affecting extra-hepatic organs and regulatory pathways. For example, NAFLD increases risk of type 2 diabetes, cardiovascular diseases, and chronic kidney disease [39]. Even if the main preventive/therapeutic tool of NAFLD is currently the improvement of lifestyle, most of the time is not enough and could be adjuvanted with nutraceuticals [40].

In our study, the tested association, induced a significant improvement in NAFLD biomarkers (FLI, gamma-GT, AST) compared to placebo, in

association with a standardized stabilization diet. Liver detoxification is an essential process in our lives as we are constantly exposed to toxic substances. These substances originate from air pollution, water pollution, alcohol, medicine intake, pesticides etc. [41]. Detoxification is a complicated process composed of three phases to make the apolar toxic molecule water soluble and ready for excretion. These three detoxification phases are functionalization, conjugation and elimination. During the functionalization phase, a functional group is placed on the apolar toxin via cytochrome P450 enzymes and free radicals are captured. In the next phase, this intermediate product is quickly converted - by conjugation with a molecule - to make it water soluble as this intermediate is often more reactive than the original molecule [42]. This polar end-product is afterwards excreted via urine or via bile in feces. The above liver detoxification process might be positively influenceable by providing the right vitamins, minerals and co-factors to the body via e.g. the intake of the tested product.

Choline is important to phospholipid after phosphorylation and donor of methyl-groups after oxidation, and it's shown that choline-deprived humans suffer from hepatosteatose and liver cell death as choline is an essential nutrient metabolized in the liver [43, 44]. Choline (via phospholipids synthesis) next to Vitamins B2, B3, B6 and B12, folic acid and omega-3 (alpha-lipoic acid) play an important role in phase I liver detoxification as well as Vitamins A, C and E, Selenium, copper, zinc, Manganese, flavonoids (from plant extracts) and Molybdenum [45, 46].

N-Acetyl-L-Cysteine (L-NAC) acts outside the cell to reduce cystine to cysteine which is 10 times faster transported in the cell and which can be used for the biosynthesis of glutathione (GSH) [47]. In the biosynthesis of GSH, NAC plays an indirect anti-oxidative role by enhancing the Glutathion-S-transferase activity and by delivering GSH for the glutathionperoxidase catalyzed detoxification of peroxides. NAC can also capture free radicals and reacts strongly with HOCL. It is also able of reducing HO and H<sub>2</sub>O<sub>2</sub> [48]. This anti-oxidative quality is very useful after Phase I of liver detoxification.

In phase II, characterized of the conjugation of the intermediate with a molecule (glucuronidation, sulfation, methylation, amino acid conjugation, glutathione conjugation, and acetylation), methylation is supported by nutrient cofactors and methyl donors such as folate and vitamin B12 [49]. Other components supporting conjugation are glutathione and NAC for glutathione conjugation, sulfur for sulfation and co-factors glycine, glutamine and taurine [50].

For both Phase I and Phase II, selective induction or modification of metabolic enzymes is supported by watercress, catechins from green tea and Ellagic acid from pomegranate. They inhibit the overproduction of phase I enzymes and stimulate the production of phase II enzymes in order to quickly convert the active metabolite formed after phase I [51-53]. Next to this, catechins and polyphenols from green tea, Cynarine from artichoke leaves and phytochemicals from pomegranate have also an anti-oxidative effect [54, 55]. Silymarin from the milk thistle has a double effect on this pathway. On one hand, it increases serum glutathion and glutathione peroxidase stimulating phase II and on the other hand, silymarin glucosides have strong antioxidant characteristics [56]. Furthermore, Artichoke is known as hepatoprotectant, antioxidant and reducer of glutathione loss [57].

Phase III – excretion - is supported on the urine and on the bile level. On the urine level, Caffeine from green tea acts as a diuretic (20) and urinary PH is elevated with potassium citrate [58]. On the bile level, bile production is stimulated with cholagogue and choleric plant extracts from artichoke [29].

Next to the support of liver detoxification and the anti-oxidative characteristics of many substances, others support mainly general liver function. Lysine and threonine are essential amino acids needed for the metabolism of fatty acids in the liver as deficiencies accompany the development of a fatty liver [60]. Next to this, Lysine acetylation is used for regulation of different processes in the body e.g. the regulation of the glycolysis, gluconeogenesis, Citric acid cycle, urea cycle, glycogen and fatty acid metabolism influencing the health status of the liver [61]. The glucose and lipid metabolism are also influenced by biotin. Biotin reduces hypertriglyceridemia, triacylglycerol and VLDL, especially when lipid levels are elevated, which reduces risk of development of fatty liver disease and attenuated hepatotoxicity [62]. Vitamin D plays a role as regulator of glucose and lipid metabolism, inflammation, cellular proliferation, differentiation and apoptosis in the liver, as well. Studies have shown the link between vitamin D deficiency and chronic liver disease as well as the improvement in hepatic inflammation and fibrosis via vitamin D in Non-alcoholic Fatty liver disease [63]. This is also modified by pantethine, a derivate of vitamin B5 and the active site of CoA. Pantethine pools triglycerides in the body as hepato-visceral and subcutaneous fat instead of storing it in the liver while CoA is used for acetylating and amino acid conjugation in phase II of liver detoxification [64]. In addition, Vitamin B1 or Thiamine deficiencies are commonly found in patients with chronic liver disease [65]. Thiamine is converted in the body to Thiamine pyrophosphate, a co-factor in intermediary metabolism but can also be used as essential co-enzyme for glucose utilization, both beneficial for the liver [66].

As deficiencies of most of above-mentioned nutrients are commonly seen in patients with NAFLD, it is important to provide them with a full spectrum supplement not only supporting the detoxification of the liver but also the general liver function in order to overall sustain the liver function.

This study has some limitations that have to be considered in the evaluation of the proposed results. Firstly, the sample size was relatively small, even though sufficiently powered for the aim of the study. Secondly, the duration of exposure was relatively short, but still useful to evaluate the positive effects of the tested nutraceutical on NAFLD biomarkers and its excellent tolerability in the short term. In addition, due to the high complexity of the tested formulation for to the large number of molecules present in it, it is difficult to separate and understand the efficacy of each single molecule as well as the related pharmacokinetic and pharmacodynamic profiles that might be altered due to interference with other substances contained in the tested product.

In conclusion, the tested nutraceutical association has proven to effectively and safely improve liver steatosis markers in the short-middle term. This result should be confirmed in long-term larger studies.

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