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Effects of Curcumin Plus Piperine on Serum Malondialdehyde Level, Catalase, and Superoxide Dismutase Enzymes in Patients with Non-alcoholic Fatty Liver Disease: A Randomized Double-blind Placebo-controlled Trial

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Key words:
Catalase, Malondialdehyde, Non-alcoholic fatty liver disease, Oxidative stress, Super-oxide dismutase

ABSTRACT

Aims Non-alcoholic fatty liver disease (NAFLD) stands as prevalent chronic liver condition on a global scale, with oxidative stress emerging as a pivotal factor in pathophysiological progression of this condition. The use of antioxidant agents for preventing and treating NAFLD has been suggested in several studies. Due to the antioxidant property of curcumin, this study investigated the effects of curcumin 500 mg plus piperine 5 mg on the serum level of malondialdehyde (MDA) as an oxidative stress marker and enzymes activity level of catalase (CAT) and superoxide dismutase (SOD).

Materials & Methods In this study, employing a double-blind, randomized, and placebo-controlled design, a total of 70 individuals diagnosed with NAFLD were chosen. They were then randomly assigned into two groups using a randomized block method: one group received a combination of curcumin (500 mg) and piperine (5 mg), while the other received a placebo. Levels of CAT and SOD markers were assessed through a photometric method, and the MDA marker was measured using a calorimetric method both before and after the intervention.

Findings Comparison of the changes in the serum level of MDA factor and the activity of CAT and SOD enzymes in the serum of the studied patients after the intervention showed that the serum activity of CAT enzyme increased significantly due to the consumption of curcumin 500 mg + piperine 5 mg (P=0.008), The SOD and MDA factors did not exhibit any notable differences between the two groups under investigation.

Conclusion The results indicated that the daily use of curcumin among NAFLD affected individuals could lead to an elevation in the serum activity of CAT. Based on the results of this study, the use of curcumin as a natural supplement may prove beneficial in mitigating the adverse impacts of oxidative stress in individuals diagnosed with NAFLD.

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مقاله پژوهشی

تاثیر ترکیب کورکومین با پیپرین بر مالون‌دی‌آلدهید و آنزیم های کاتالاز و سوپراکسیددیسموتاز در بیماران مبتلا به کبد چرب غیرالکلی: یک کارآزمایی بالینی دوسوکور تصادفی‌شده با دارونما

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چکیده

هدف بیماری کبد چرب غیرالکلی (Non-alcoholic Fatty Liver Disease (NAFLD، از آسیب‌های شایع و مزمن کبدی در جهان است و استرس اکسیداتیو یکی از عوامل مهم پاتوفیزیولوژی در ایجاد این بیماری است. در پژوهش‌های متعدد، استفاده از عوامل آنتی‌اکسیدان برای پیشگیری و درمان NAFLD پیشنهاد شده است. با توجه به عملکرد آنتی‌اکسیدانی کورکومین، در مطالعه‌ی حاضر، اثر کورکومین ۵۰۰ میلی‌گرم به همراه پیپرین ۵ میلی‌گرم بر سطح سرمی مارکر استرس اکسیداتیو مالون دی‌آلدهید (MDA) و میزان فعالیت آنزیم‌های کاتالاز (CAT) و سوپراکسید دیسموتاز (SOD) بررسی شده است.

مواد و روش ها در این مطالعه‌ی دوسویه‌کور تصادفی‌شده با کنترل دارونما، تعداد ۷۰ بیمار مبتلا به NAFLD انتخاب شدند و از طریق بلاک‌های تصادفی‌شده، در دو گروه تحت درمان با کورکومین دوز ۵۰۰ میلی‌گرم با پیپرین دوز ۵ میلی‌گرم و دارونما قرار گرفتند. مارکرهای CAT و SOD توسط روش فتومتریک و مارکر MDA توسط روش کالری‌متری به‌صورت قبل و بعد از مداخله اندازه‌گیری شد.

یافته‌ها: مقایسه‌ی تغییرات سطح سرمی فاکتور MDA و فعالیت آنزیم‌های CAT و SOD در سرم بیماران مورد مطالعه بعد از مداخله نشان داد که در اثر مصرف ترکیب کورکومین ۵۰۰ میلی‌گرم و پیپرین ۵ میلی‌گرم، فعالیت سرمی آنزیم کاتالاز به‌طور درخور ملاحظه‌ای افزایش می‌یابد (P =۰/۰۰۸)، درحالی‌که فاکتورهای SOD و MDA در دو گروه مورد مطالعه تفاوت معناداری نشان ندادند (P>۰/۰۵).

نتیجه گیری: نتایج حاکی از آن است که استفاده‌ی روزانه از ترکیب کورکومین ۵۰۰ میلی‌گرم و پیپرین ۵ میلی‌گرم در بیماران مبتلا به NAFLD، می‌تواند سبب افزایش فعالیت سرمی آنزیم کاتالاز شود. طبق نتایج این مطالعه، می‌توان گفت که استفاده از کورکومین ۵۰۰ میلی‌گرم به همراه پیپرین ۵ میلی‌گرم می‌تواند به‌عنوان مکمل طبیعی در کاهش اثرهای منفی استرس اکسیداتیو در بیماران NAFLD مؤثر باشد.

کلیدواژه‌ها:
استرس اکسیداتیو
سوپراکسید دیسموتاز
کاتالاز
کبد چرب غیرالکلی
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Introduction

NAFLD, a potentially severe liver condition, poses substantial healthcare costs and economic burdens while diminishing the overall health-related quality of life on a societal scale. It is a heterogeneous disease from biological and clinical aspects, covering a wide range of histological conditions, contributing to a higher morbidity in both hepatic and non-hepatic contexts [1]. The disease is broadly classified into simple fatty liver and non-alcoholic steatohepatitis (NASH). Simple fatty liver typically does not induce liver damage or complications. However, NASH, beyond liver fat accumulation, can progress to hepatitis, kidney inflammation, liver cell damage, and ultimately, liver cancer [2].

The worldwide occurrence of NAFLD within the general population has been documented to range between 20% and 30% (approximately 25% for NAFLD and 5% for NASH) [3]. In Iran, the estimates made in a meta-analysis study by Moghadasifar et al. indicate a prevalence of 34% in the general population [4]. It is predicted that NAFLD along with obesity may be the most important causes of death due to liver diseases by 2030. Various mechanisms are effective in the pathophysiology of NAFLD, one of the most crucial of which is oxidative stress (OS) [5, 6]. Oxidative stress emerges as a result of the generation of reactive oxygen species (ROS), which specifically target the double bonds present in polyunsaturated fatty acids, initiating the process of lipid peroxidation. This sequence leads to the creation of highly reactive aldehyde components, such as malondialdehyde (MDA), ultimately inducing intracellular damage. Several OS biomarkers are involved in the lipid peroxidation cycle in NAFLD/NASH patients, whose high concentrations are associated with the severity of liver disease. An increase in oxidant markers would lead to a significant decrease in antioxidant compounds, such as catalase (CAT) and superoxide dismutase (SOD) [7].

Curcumin is a well-known pigment found in turmeric that benefits from numerous therapeutic properties, encompassing but not limited to anti-cancer, anti-inflammatory, and antioxidant. Curcumin protects biological membranes against peroxidative damage. Peroxidation of lipids, in the form of a chain reaction generating free radicals, causes damage to cell membranes. Curcumin, with its antioxidant properties, inhibits free radicals resulting from peroxidation [8]. The healthful impacts of curcumin and turmeric on fatty liver and serum aminotransferases have been shown in several animal studies. Finding from other researchers' publications have revealed the effect of curcumin alone on reducing gamma-glutamyl transpeptidase, ROS factors, liver function tests (aspartate transaminase, alkaline phosphatase, and alanine transaminase), steatosis, and inflammation in NAFLD patients. However, the examination of the effect of turmeric alone on liver enzymes and the grade of fatty liver in patients with NAFLD did not reveal any significant changes. The reason for these differences can be attributed to the type and dose of curcumin supplement consumed [9, 10]. Several studies have reported that simultaneous consumption of curcumin and piperine increases the oral bioavailability of curcuminoids to 20 times [11]. Considering the effect and performance of curcumin 500 mg and piperine 5 mg on pathways and markers of OS, the current investigation aimed to evaluate the impact of administering curcumin (500 mg) and piperine (5 mg) on the markers CAT, SOD, and MDA in individuals diagnosed with NAFLD.

Materials and Methods

This randomized, double-blind, placebo-controlled clinical trial was conducted over 2 months. Samples, methods, study design, and inclusion and exclusion criteria were based on previous research performed by Mirhafez et al. [12]. The sample size was determined at 38 individuals in each group according to the formula for determining the sample size in clinical trial studies and the information from previous studies, as well as taking into account the type 1 error of 0.05 and the power of the test 0.80 [13].

Participants were selected in accordance with specific inclusion and exclusion criteria at the Specialized Clinic of 22 Bahman Hospital in Neishabour during the year 2017. For this purpose, people were informed via a general call, and patients with fatty liver referred to the special clinic of this hospital. In the following, the individuals who met the inclusion criteria were divided into two groups, namely curcumin and placebo, using the block of four randomization method. Patients in each block received either treatment A (curcumin) or treatment B (placebo). This process continued until it reached the target sample size. To blind the study and prevent the predictability of the randomization process, one person (the secretary) performed this allocation, so that neither the patient nor the physician were aware of the size of the blocks and the type of treatment received. Sixty curcumin and 60 placebo treatments were put in similar packages. Letter A (curcumin) or letter B (placebo) was written on each drug package, and only the researcher was aware of the type of drug received.

A number of 80 patients of both genders with three grades of fatty liver were randomly selected. They had been diagnosed with fatty liver by laboratory and

ultrasound results. Sampling and separation of patients' blood serum were both performed at 22 Bahman Hospital. Inclusion criteria for participants encompassed individuals aged 18 to 65 years, with a confirmed diagnosis of fatty liver disease verified through ultrasound examination. and a specialist doctor, and the informed consent to enter the study. On the other hand, pregnant/lactating women; patients with alcoholic fatty liver, chronic cardiac or pulmonary disorders, and acute or chronic liver disorders; and patients taking anti-inflammatory drugs were excluded from the study. by the end of the study, 10 samples were lost to follow-up due to some reasons, including serum deficiency and device measurement errors. The drug was administered orally, one capsule daily after dinner. Necessary follow-ups were done through phone calls to ensure the adherence to medication schedule.

Curcumin capsules contained 500 mg of curcumin and 5 mg of piperine adjuvant (C3 complex^{TM} + Bioperine^{TM}) extract obtained from the fruits of black pepper (nigrumpiper), which was standardized with at least 95% piperine [14]. The placebo capsules were similar to the curcumin capsules in shape and size; however, they contained lactose due to its low side effects and insignificant effect on the development of NAFLD. The drug intervention was expected to last for 2 months.

Acknowledging the potential impact of varying patient diets on the studied outcomes, a nutritionist visited all patients to ensure uniform dietary guidelines and physical activity instructions. Consequently, patients were instructed to adhere to a balanced diet in line with medical recommendations from the National Institute of Health and the North American Association for the diagnosis, evaluation, and treatment of adults with overweight and obesity. Based on the mentioned guide, the recommended diet for patients included ≤ 30% fat (1.3 saturated fatty acids and 2.3 unsaturated fatty acids), 52-53% carbohydrates, 20-30 g/day fiber, ≤ 300 mg/dl cholesterol, and 15-18% protein. All patients were advised to do at least 30 minutes of physical activity three times a week.

Prior to sample collection, patients provided written consent affirming their willingness to participate in the sampling process, conducting additional tests, and publishing the results while maintaining the ethical principles of personal information. At the baseline and immediately after the intervention, necessary tests and investigations, including fasting blood collection, liver ultrasound, and a visit to a specialist doctor, were performed. Fasting whole blood samples - before and after the intervention - were taken from each patient, and to separate the blood serum, the samples were centrifuged for 10 min at 3,000 rpm.

The activity level of CAT and SOD enzymes and the serum level of MDA were measured using Teb Pazhouhan Razi kits (TPR, Tehran, Iran). According to the kit protocol, the activity of this enzyme was assessed by assessing the reaction of CAT and methanol in the presence of hydrogen peroxide and the formation of formaldehydes. The activity of SOD was assessed by the appearance of a yellow color resulting from the reaction of tetrazolium salt with superoxide anion. The evaluation of these two markers was done by photometric methods at 530-540 nm wavelength. MDA also reacted with thiobarbituric acid and produced an additional compound that could be measured by the fluorometric method (Ex/Em=532/553nm).

The investigated variables were characterized using mean ± standard deviation. The normality of the variables was assessed using the Kolmogorov-Smirnov test. For normally distributed data, a paired t-test was employed to compare variables before and after the intervention, while the Wilcoxon test was used for non-normally distributed data. To compare changes in variables throughout the intervention, an independent t-test was applied for normal variables and a Mann-Whitney U test for non-normal variables. Statistical significance was established at $p < 0.05$.

Results

In line with the CONSORT chart (Figure 1), the study initially enrolled 87 eligible patients. However, seven patients were found not to meet the inclusion criteria. Consequently, the remaining 80 patients were then allocated into either the curcumin or placebo groups using a randomized block design. During the 2-month follow-up of the intervention, 7 individuals were removed from the curcumin group due to the side effects of the drug (n=1) and non-use of the drug (n=6); likewise, in the placebo group, 3 people were excluded due to forgetting to take the medicine, travel, and not being available. Finally, the eligible patients with NAFLD who took part in this trial were placed into the placebo group (n=37) and curcumin 500 mg plus piperine 5 mg group (n=33).

Oxidative stress marker MDA and enzymes CAT and SOD were compared between the placebo and curcumin 500 mg and piperine 5 mg groups before the intervention (Table 1). As per the data presented in Table 1, the CAT enzyme exhibited a statistically significant difference between the placebo and intervention groups (P=0.006), with higher values observed in the placebo group compared to the intervention group.

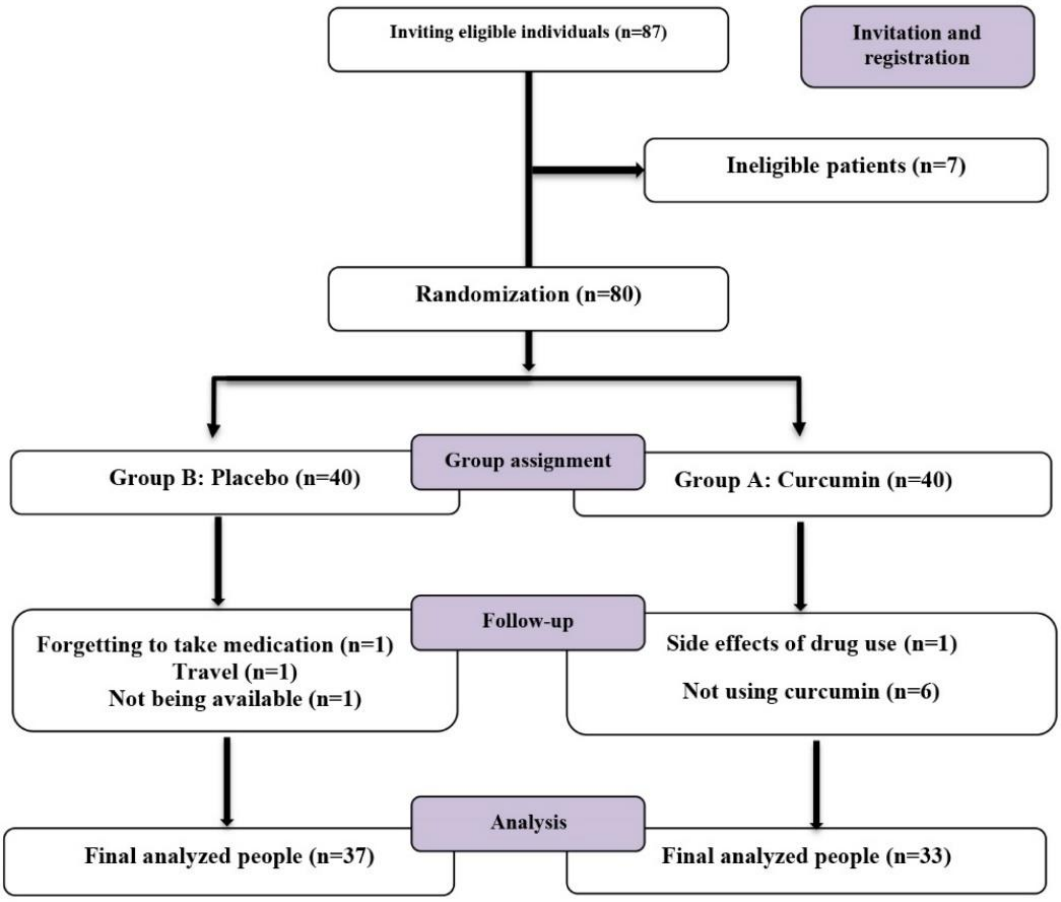

Figure 1. CONSORT diagram of participation in the study
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Table 1. Level of MDA, CAT, and SOD activity in patients with NAFLD

Factor	Placebo (n=37)	Curcumin 500 mg and piperine 5 mg (n=33)	P-value
Catalase (IU/l)	22.8±18.2	12.7±8.7	0.006
Super-oxide dismutase (IU/l)	13.5±2.8	13.3±3.6	0.782
Malondialdehyde (µmol/l)	26.7±11.6	24.6±10.6	0.413

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Data are shown as mean ± standard deviation.
The international unit (UI) of CAT activity is the amount of enzyme that breaks down 1 nmol of substrate in one minute. The international unit of SOD activity is the amount of enzyme required to inhibit 50% of superoxide ions. Independent t-test and Mann-Whitney U test were used for normal and abnormal variables, respectively.

When comparing the values of SOD and MDA before the intervention, it was noted that these two markers showed higher levels in the intervention group compared to the placebo group; however, this disparity did not reach statistical significance (P>0.05). Table 2 gives information about the comparison of the investigated variables in each group before and after the intervention. Based on the results of Table 2, the level of CAT activity increased after the intervention; nevertheless, this increase was statistically significant only after the consumption of curcumin 500 mg and piperine 5 mg (P<0.001). The intervention led to a decrease in the activity of SOD enzyme in both, which was statistically significant (P<0.001). MDA also increased in the two groups after the intervention, which was not statistically significant.

Table 3 presents the changes in the studied markers as a result of the intervention. Accordingly, due to the consumption of curcumin 500 mg and piperine 5 mg, CAT activity increased, which was significant compared to the placebo group. The changes in SOD after the intervention showed a decrease in activity in the two groups; however, the comparison of this decrease between the two groups was not statistically significant. The intervention increased the MDA marker, which was not statistically significant.

Table 2. Intragroup comparison of MDA, CAT, and SOD markers in two groups of placebo and curcumin 500+piperine 5

Factor	Before taking the placebo (n=37)	After taking placebo (n=37)	P-value	Before taking curcumin with piperine (n=33)	After taking curcumin with piperine (n=33)	P-value
Catalase (IU/l)	21.4±0.17	25.3±18.5	0.445	12.7±8.7	40.6±38.6	<0.001
Super-oxide dismutase (IU/l)	13.6±2.5	6.0±3.8	<0.001	13.5±3.7	6.7±3.8	<0.001
Malondialdehyde (µmol/l)	26.4±12.3	29.0±15.6	0.434	24.4±9.9	27.6±0.13	0.225

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Data are shown as mean ± standard deviation.
The international unit (IU) of CAT activity is defined as the quantity of enzyme capable of breaking down 1 nmol of substrate in one minute. Similarly, the international unit of SOD activity is the amount of enzyme needed to inhibit 50% of superoxide ions. Statistical analyses involved the use of an independent t-test for normal variables and a Mann-Whitney U test for abnormal variables.

Table 3. Comparison of intergroup changes of MDA, CAT, and SOD markers in two groups of placebo and curcumin 500+piperine 5

Factor	Placebo (n=37)	Curcumin 500 mg and piperine 5 mg (n=33)	P-value
Catalase (IU/l)	3.9±27.4	39.7±27.8	0.008
Super-oxide dismutase (IU/l)	-7.7±5.3	-6.8±5.6	0.522
Malondialdehyde (µmol/l)	2.6±17.7	3.2±14.5	0.882

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Data are shown as mean ± standard deviation.
The international unit (IU) of CAT activity is defined as the quantity of enzyme capable of breaking down 1 nmol of substrate in one minute. Correspondingly, the international unit for SOD activity represents the amount of enzyme required to inhibit 50% of superoxide ions. To analyze normal and abnormal variables, an independent t-test and a Mann-Whitney U test were employed, respectively.

Discussion

Due to the global spread of NAFLD and the lack of definitive treatment, the need to design novel and efficient treatment methods is one of the most challenging issues in public health. Considering the importance of the OS process in the pathophysiology of NAFLD, compounds with an antioxidant nature can be effective in reducing the adverse effects caused by OS. The natural pigment in curcumin has anti-inflammatory and antioxidant properties, which can make it a suitable option for the treatment of NAFLD. In this regard, this study investigated the antioxidant impact of curcumin 500 mg and piperine 5 mg on the OS marker MDA and the CAT and SOD enzymes. The focus of numerous studies has been on curcumin as an effective compound in preventing and treating inflammatory diseases affected by OS, including NAFLD [15]. the presence of phenoxy structure and conjugated double bond, which is able to remove free radicals are what give this compound its significant Anti-oxidant property [16]. The findings of studies demonstrate that pure curcumin increases the total antioxidant capacity by improving the activity of CAT and SOD and reducing the concentration of MDA [17].

In the current study, the effect of curcumin 500 mg and piperine 5 mg was examined on the OS variables affecting NAFLD, the results of which revealed a significant difference in the increase in the activity level of CAT enzyme after the intervention, while no significant changes were observed in SOD and MDA. It was also found that in the placebo group, the level of CAT activity before and after the intervention was associated with a slight increase, while this increase was significant in the curcumin 500 mg and piperine 5 mg group.

These findings were relatively consistent with those reported by Singh et al., showing that curcumin could be a strong protective agent against lindane, a pesticide that can cause hepatotoxicity in male Wistar rats [18]. According to their results, after the induction of hepatotoxicity due to the use of pesticides, the activity of both target enzymes (i.e., CAT and SOD) increased by curcumin treatment with doses of 100 and 200 mg/kg body weight of rats. In contrast, in the present study, only the level of CAT activity was associated with a significant increase, whereas SOD enzyme activity decreased with or without curcumin administration. Although this reduction was significant in the intra-group comparison of both the placebo and curcumin 500 mg and piperine 5 mg groups, the inter-group comparison of these two groups showed the absence of a significant relationship in SOD activity in both groups. Decreased curcumin-independent activity of SOD seems to be part of the progression process of NAFLD; in which, as a consequence of increased ROS, there is a rise in superoxide free radical anion, resulting

in a significant utilization of SOD to counteract this oxidant anion.

This discrepancy can be attributed to the different nature of these two studies. The study by Singh et al. was conducted on 42 Wistar rats of one gender: male; since the samples were non-human, this study cannot be classified as a randomized clinical trial. In addition, due to the lack of use of a placebo in the above-mentioned research and the lack of attention to the double-blind randomization of the samples, the findings of the present study seem to be more valid and generalizable owing to the investigation of human samples and the use of the precise method of double-blind, randomized, placebo-controlled clinical trial. To elaborate on this, the findings of the present study showed a significant decrease in the activity of SOD enzyme in the two groups of curcumin 500 mg and piperine 5 mg and placebo before and after the intervention. As mentioned, this can be seen as a result of the natural progression of NAFLD and the lack of the effect of curcumin 500 mg and piperine 5 mg on SOD activity. This procedure can be affected by the positive or negative psychological effects of using drugs or other interfering factors [19, 20].

However, these results are not in line with those of previous studies, in which an increase was reported in the SOD activity following the administration of curcumin [20]. An example of such a contradiction can be found in the comparison of a meta-analysis study by Panahi et al. with the present research. This study investigated the effect of the curcuminoid-piperine combination (with a ratio of 1,000 to 10 mg/day) over 8 weeks on the serum level of liver factors SOD, MDA, and CRP, the results of which indicated that curcumin increased the serum level of SOD enzyme. This was in clear contradiction with the findings of the present research, showing that no increase was observed in the level of SOD activity, rather, a significant decrease was found in the activity of this enzyme in both studied groups. These discrepancies in the results can be attributed to the use of a reduced concentration of curcumin combined with piperine (with a ratio of 500 to 5 mg/day) in our study. It is also noteworthy that the study by Panahi et al. was conducted on a larger population (109 samples vs 70 samples in the present study) [21].

The latest meta-analysis and systematic review by Mousavian et al. has highlighted the potential of SOD enzyme activation by curcumin. The consequence of this increase in activity is an enhanced ability to neutralize various free radicals [22]. Current results are more in line with those of previously mentioned publications. The comparison of these two studies regarding the oxidant product MDA also revealed different results. An increase was observed in MDA values after the intervention of curcumin in the work by Panahi et al. and the present study. On the other hand, the findings of a case study showed a decline in MDA following the use of curcumin against OS caused by the organophosphate insecticide malathion [23]. These findings were inconsistent with those of the current trial study, in which MDA levels did not show any significant difference in any of the two groups.

Despite the contradictions mentioned in the research records with the present study regarding the antioxidant effects of curcumin, the consistency of the results of this study with similar studies can be justified through two proposed mechanisms. First, the antioxidant property of curcumin can be attributed to the presence of various functional groups, including methoxy, phenoxy, and carbon-carbon double bonds in their structure [24]. Second, the antioxidant function of curcuminoids is related to an increase in the activity of CAT, SOD, glutathione peroxidase, and active enzymes in neutralizing free radicals [25].

Regarding the use of piperine adjuvant with curcumin, it should be kept in mind that various strategies have been explored to enhance the bioavailability of curcuminoids, which include the blockage of metabolic pathways by combining other substances to administer, structural modifications, nanofabrication, combination with phospholipids, and encapsulation. Among these, the simultaneous use of piperine with curcuminoids has been considered in numerous RCR studies. Piperine recognized for enhancing the pharmacokinetics of diverse compounds, including curcuminoids [26]. Its mechanisms involve inhibiting liver drug metabolizing enzymes, fortifying intestinal perfusion, slowing gastric emptying, and impeding drug efflux pumps (P-glycoproteins) [27]. Studies have demonstrated a 20-fold increase in curcuminoid oral bioavailability when used concurrently with piperine [28].

Conclusion

In the current trial, it was found that curcumin 500 mg and piperine 5 mg had modulating effects on maintaining the pro-oxidant-antioxidant balance in patients with NAFLD, led to an increase in CAT enzyme activity, and could improve the severity of fatty liver disease because of curcumin consumption. Therefore, curcumin as a natural supplement shows potential as an effective candidate for NAFLD treatment in future studies.

The results obtained in this study indicated that the consumption of curcumin 500 mg and piperine 5 mg caused a significant increase in CAT activity among the three measured markers involved in OS (i.e., CAT, SOD, and MDA), while no significant changes were observed in SOD and MDA markers, in contrast to the hypothesis.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethical Review Board of Neyshabur University of Medical Sciences (IR.NUMS.REC.1394.18) and registered in the Iranian Registry of Clinical Trials (IRCT2015052322381N1).

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Authors' contributions

S.R.M., A.S. and J.M. designed and directed the project; S.A.H, J.S. and R.H performed the experiments and data collection. S.R.M. performed data analysis and S.A.H wrote the article.

Conflicts of interest

The authors declare that there is no conflict of interest.

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