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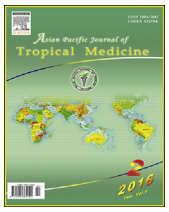

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Evaluation of anti-tubercular activity of linolenic acid and conjugated-linoleic acid as effective inhibitors against *Mycobacterium tuberculosis*

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ABSTRACT

Objective: To evaluate a new pharmacological activity/effect of linolenic acid (α- and γ-form) and conjugated-linoleic acid (CLA) causing antibacterial activity against *Mycobacterium tuberculosis* (Mtb).

Methods: The anti-Mtb activity/effect of linolenic acid and CLA were determined using different anti-Mtb indicator methods such as resazurin microtiter assay (REMA) and MGIT 960 system assay. The Mtb was incubated with various concentrations (12.5–200 μg/mL) of the compounds and anti-Mtb first-line drugs for 5 d in the REMA, and for 3 wk in MGIT 960 system assay.

Results: Linolenic acid and CLA obviously indicated their anti-Mtb activity/effect by strongly inhibiting the growth/proliferation of Mtb in a dose-dependent manner in the REMA and the MGIT 960 system assay. Interestingly, linolenic acid and CLA consistently induced anti-Mtb activity/effect by effectively inhibiting the growth/proliferation of Mtb in MGIT 960 system for 21 d with a single treatment, and their minimum inhibitory concentrations were measured as 200 μg/mL respectively.

Conclusions: These results demonstrate that linolenic acid and CLA not only have effective anti-Mtb activity/properties, but also induce the selective-anti-Mtb effects by strongly inhibiting and blocking the growth/proliferation of Mtb through a new pharmacological activity/action. Therefore, this study provides novel perspectives for the effective use of them and the potential that can be used as potent anti-Mtb candidate drugs, as well as suggests the advantage of reducing the cost and/or time for developing a new/substantive drug by effectively repurposing the existing drugs or compounds as one of new strategies for the global challenge of tuberculosis.

1. Introduction

The rapid emergence of tuberculosis (TB) is still causing a serious global challenge and difficulty including drug-resistant TB despite various global efforts such as hygiene education, environment improvement, and drug development. *Mycobacterium tuberculosis* (Mtb) is a major infectious factor causing the highest human mortality through the co-infection with HIV/AIDS as one of the most dangerous infectious bacteria globally. In 2013, 9.0 million people were estimated as new TB, 1.5 million died from the disease, and 360000 of them were HIV-positive. In addition, 1.1 million of the 9 million people diagnosed as new TB cases in 2013 were HIV-positive. Recently, TB cases co-infected with HIV/AIDS indicated the highest infectious rate in the African region compared with the other countries, particularly, in southern Africa. Furthermore, most of the estimated TB cases in 2013 occurred in Asia (56%) and the African region (29%), and the three countries of the largest number of TB cases were India (2.0

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million–2.3 million), China (0.9 million–1.1 million), and Nigeria (340 000–880 000) [1,2]. For these reasons, various studies for the treatment and/or the inhibition of TB that induces drug-resistance to existing agents have been carried out in the drug discovery field worldwide. Although various anti-TB drugs (from first-line drugs to third-line drugs) were developed for the treatment of TB patients, they have showed the limit in reducing the current TB patients. Recently, compounds such as PA 824, SQ 109, bedaquiline, and linezolid repurposed through chemical remodeling of the existing drugs as well as new compounds such as Q203, TMC 207, pyridomycin, and thiophenes are being tested in Phase I or Phase III trials as novel anti-TB drugs [1,3–7]. Until recently, in spite of the discovery of new targets and/or drugs for treating TB, the rapid emergence of TB, and multi-drug-resistant TB (MDR-TB) or extensively drug-resistant TB has still caused serious concerns in the public health field worldwide. For these reasons, various studies for developing the effective anti-TB drugs with novel mechanisms of action, low cytotoxicity and safety are urgently needed to block and/or to inhibit the TB. In this aspect, this study was carried out to evaluate anti-Mtb activity/effect/ability of linolenic acid (α- and γ-form) and conjugated-linoleic acid (CLA) that effectively act as bioactive substance, and to identify the potential for developing them as novel anti-tubercular drugs.

2. Materials and methods

2.1. Materials

Various materials used in this study, rifampicin, isoniazid, linolenic acid (α- and γ-form), CLA [conjugated(9Z,11E)-linoleic acid, conjugated(9E,11E)-linoleic acid, and conjugated(10E,12Z)-linoleic acid], resazurin powder and DMSO, were purchased from Sigma–Aldrich Chemical, Co., Ltd. (St, Louis, MO, USA), and MGIT™ 960 system indicator 7 mL growth media tubes with BACTEC™ MGIT™ 960 supplement kit were purchased from Becton–Dickinson and Company (Sparks, MD, USA). All other chemicals and reagents were purchased from Merck Chemical Co., Ltd. (Darmstadt, Germany) and Sigma–Aldrich Chemical Co., Ltd. (St. Louis, MO, USA).

2.2. Preparation of anti-Mtb drugs

The anti-Mtb first-line drug, isoniazid, was dissolved in sterile distilled water, and rifampicin was dissolved in DMSO, to a concentration of 50 mg/mL according to the manufacturer's instruction. The anti-Mtb first-line drugs were used as reference standard drugs. All compounds were filtered using 0.2 μm membrane syringe filter (Roshi Kaisha, Ltd., Tokyo, Japan) before use and stored at −80 °C deep-freezer until use.

2.3. Preparation and growth conditions of Mtb

Mtb $H37R_V$ (ATCC 27294) used in this study was purchased from American Type Culture Collection (Manassas, VA, USA). Mtb $H37R_V$ was grown in Middlebrook 7H9 broth (Difco Laboratories, Detroit, MI, USA) supplemented with 10% (V/V) oleic acid/albumin/dextrose/catalase enrichment (Becton–Dickinson and Company, Sparks, MD, USA) and 0.05% (v/v) Tween 80 (Sigma–Aldrich Chemical, St, Louis, MO, USA) to the log phase at 37 °C for 4–5 wk on shaking incubation of 100 rpm.

2.4. Drug susceptibility testing of Mtb

The *in vitro* anti-Mtb activity of linolenic acid (α- and γ-form) and CLA [conjugated(9Z,11E)-linoleic acid, conjugated(9E,11E)-linoleic acid, and conjugated(10E,12Z)-linoleic acid] against Mtb was confirmed by resazurin microtiter assay (REMA) using a 96-well micro-plate. Briefly, Mtb was grown in fresh Middlebrook 7H9 broth supplemented with 10% (v/v) oleic acid/albumin/dextrose/catalase enrichment and 0.05% (v/v) Tween 80 until the culture reached a turbidity equal to that of 1.0 McFarland standard (3.0×10^8 CFU/mL) at 37 °C. The bacteria were adjusted to a density of 2×10^6 CFU/mL in fresh culture broth. Finally, the bacterial suspensions were inoculated into all wells of a 96 well microtiter plate containing final concentrations (12.5–200 μg/mL) of linolenic acid (α- and γ-form), CLA [conjugated(9Z,11E)-linoleic acid, conjugated(9E,11E)-linoleic acid, and conjugated(10E,12Z)-linoleic acid] and anti-TB first-line drugs (1.25–5 μg/mL), and Mtb growth controls containing no anti-Mtb first-line drugs and blank controls without inoculation were also included. The 96 well-plates, covered with lids, placed in a plastic bag, were incubated at 37 °C for 5 d. After incubation, 20 μL of freshly prepared 0.05% (w/v) resazurin solution was added to all wells of a 96 well microtiter plate, and the plates were re-incubated at 37 °C for 36 h. A change in color from blue to pink indicating bacterial growth was observed after 36 h of incubation. The minimum inhibitory concentration (MIC) was expressed as the lowest concentration of the drug that inhibited Mtb growth or prevented change in color of the resazurin from blue to pink based on a REMA.

2.5. Evaluation of drug susceptibility of Mtb by MGIT 960 system assay

To evaluate anti-Mtb effects of linolenic acid (α- and γ-form) and CLA [conjugated(9Z,11E)-linoleic acid, conjugated(9E,11E)-linoleic acid, and conjugated(10E,12Z)-linoleic acid] against the growth/proliferation of Mtb, the drug susceptibility testing of the strain was performed using the BACTEC™ MGIT 960 system (Becton Dickinson and Company, Sparks, MD, USA.). In brief, 100 μL of a suspension of Mtb culture, adjusted to 9.6×10^6 CFU/mL, was inoculated in an MGIT growth media tube with BACTEC™ MGIT 960 growth supplement (Becton Dickinson and Company), which were incubated with different concentrations (12.5–200 μg/mL) of the tested compounds and anti-Mtb first-line drugs (10 μg/mL), and Mtb growth controls containing no anti-Mtb first-line drugs were also included. They were incubated into the BACTEC™ MGIT 960 system device for 3 wk for determination of Mtb drug susceptibility.

2.6. Statistical analysis

All results were expressed as mean ± standard deviation of three independent experiments. Statistical analysis of the data was performed using the Student's *t*-test and one-way analysis of variance.

3. Results

3.1. Evaluation of anti-Mtb activity of the compounds against Mtb

The anti-Mtb activity of linolenic acid (α- and γ-form) and CLA [conjugated(9Z,11E)-linoleic acid, conjugated(9E,11E)-linoleic acid, and conjugated(10E,12Z)-linoleic acid] were evaluated using the REMA. After the bacteria were incubated with various concentrations (12.5–200 μg/mL) of the compounds and anti-Mtb first-line drugs (rifampicin and isoniazid) for 5 d, the growth/proliferation of Mtb were markedly inhibited in a concentration-dependent manner (Table 1), and the MIC values of linolenic acid (α- and γ-form) and CLA against the viability of Mtb were determined as 75 μg/mL and 100 μg/mL, respectively (Table 2). Furthermore, all the compounds demonstrated their Mtb-inhibitory effects as well as anti-Mtb activity through the bacterial color change observed in the REMA. In the REMA, the superiority among the compounds regarding anti-Mtb activity/effect was confirmed that linolenic acid (α- and γ-form) was more effective compared with the CLA. These results demonstrate that the compounds not only induce anti-Mtb activity by strongly inhibiting the growth/proliferation of Mtb, but also have the potential that can be developed as potent anti-Mtb agents through their new pharmacological function/action.

3.2. Anti-Mtb effects of the compounds against the growth/proliferation of Mtb

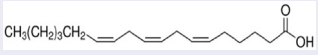
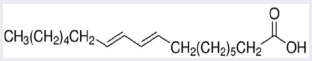
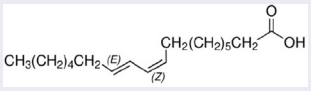
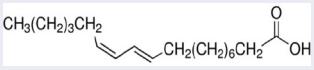
The anti-Mtb effects of the compounds against Mtb were further evaluated using the MGIT 960 system assay. The bacteria were incubated with various concentrations (12.5–200 μg/mL) of the compounds and anti-Mtb first-line drugs (10 μg/mL) in an MGIT growth media tube of the BACTEC™ MGIT 960 system device for drug susceptibility testing for 3 wk, and their growth units were markedly inhibited in a concentration-dependent manner. Particularly, when Mtb was incubated with 100 μg/mL of the CLA, their growth units were detected at about 8.3 d, whereas 100 μg/mL of linolenic acid (α- and γ-form) strongly inhibited the growth/proliferation of Mtb compared with the CLA, and the growth unit of Mtb was detected at 9.3 and 10.3 d, respectively (Figure 1). Furthermore, when Mtb was incubated with all the tested compounds (200 μg/mL), their growth units were not detected at 21 d. In this MGIT 960 system assay, linolenic acid (α- and γ-form) and CLA effectively inhibited the growth/proliferation of Mtb for 3 wk with a single treatment, which showed clearly that these compounds induced similar inhibitory effects against the growth/proliferation of Mtb. These results demonstrate that the compounds induce anti-Mtb activity/effect causing the inactivation of Mtb by effectively blocking or consistently inhibiting the growth/proliferation of Mtb, as well as have unique anti-mycobacterial properties that induce anti-Mtb activity in a concentration-dependent manner.

Table 2
MICs of linolenic acid (α- and γ-form) and CLA against the growth/proliferation of Mtb determined by different anti-Mtb assays.

Tested compounds	MIC (μg/mL) of the compounds determined by different anti-Mtb assays	
	REMA (incubation days: 5)	MGIT 960 system (incubation days: 21)
α-Linolenic acid	75	200
γ-Linolenic acid	75	200
CLA Conjugated(9E,11E)-linoleic acid	100	200
Conjugated(9Z,11E)-linoleic acid	100	200
Conjugated(10E,12Z)-linoleic acid	100	200

The anti-Mtb activity/effect of the compounds was measured by the REMA and the MGIT 960 system assay. Mtb H37Rv was incubated with different concentrations (12.5–200 μg/mL) of the compounds at 37 °C, and its susceptibility was confirmed by different anti-Mtb indicator assays. The results of the REMA and the MGIT 960 system assay were evaluated after 5 d and 21 d of incubation, respectively.

Table 1
Anti-Mtb activity of linolenic acid (α- and γ-form) and CLA against Mtb growth/proliferation determined by the REMA assay.

Tested compounds	Structure	Mol. weight (g/M)	Concentrations (μg/mL)			
			200	100	50	25
α-Linolenic acid		278.43	+	+	±	–
γ-Linolenic acid		278.43	+	+	±	–
Conjugated(9E,11E)-linoleic acid		280.45	+	+	±	–
Conjugated(9Z,11E)-linoleic acid		280.45	+	+	±	–
Conjugated(10E,12Z)-linoleic acid		280.45	+	+	±	–

The anti-Mtb activity of the compounds against the growth/proliferation of Mtb H37Rv was measured using the REMA assay. The Mtb was incubated with different concentrations (12.5–200 μg/mL) of the compounds at 37 °C for 5 d. The '+' sign indicates anti-Mtb activity of the compounds, and '±' sign shows 60%–75% inhibitory rates of the compounds against the growth/proliferation of Mtb.

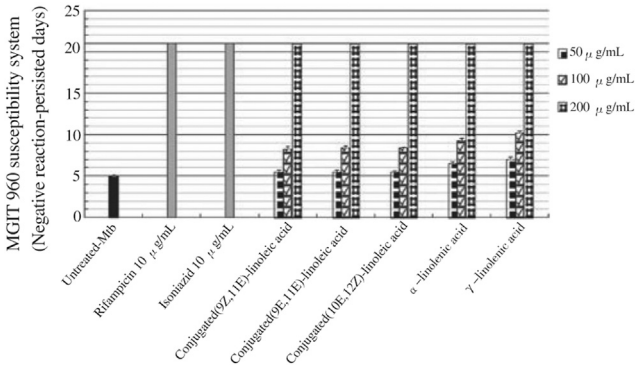


Figure 1. Anti-Mtb activity/effect of linolenic acid (α- and γ-form) and CLA measured using the MGIT 960 system assay.
The Mtb was incubated with different concentrations (12.5–200 μg/mL) of the compounds in the MGIT 960 system device at 37 °C for 3 wk.

4. Discussion

The recent infectious pathogens have enhanced their infectivity and viability through genetic mutation or adaptability to environmental variation, which affects the treatment or prevention of the disease as well as the novel drug discovery and development. Recently serious infectious diseases such as MERS, SARS, Influenza and Ebola have been occurred in various countries worldwide, particularly, in Asia, Middle East and Africa. These diseases have typically caused more serious risk to young children whose immune function is low and the elderly who have other underlying diseases compared with normal adults. In these aspects, TB is a contagious/infectious disease to cause infection through droplets, which has caused comparatively high prevalence and mortality rates worldwide. In addition, TB is the most dangerous factor among various infectious diseases, which accounted for the second leading cause of death among infectious diseases of the world's population in 2013 and 2014 [1,2]. In particular, the increase of HIV-co-infection and the rapid appearance of MDR-TB strains strongly suggest an urgent need to develop effective/safe drugs for consistently treating TB as well as induce serious concerns regarding drug-resistance in the public health fields. Recently, in spite of various efforts to discover new anti-Mtb drugs, the development for effective/safe anti-TB drugs has been still faced with a global difficulty, including the cost & time and the indifference of global pharmaceutical companies. Until recently, novel antibiotics derived from diverse biological resources and synthetic compounds have been developed to treat infectious diseases or to inhibit pathogenic microorganisms. However, the overuse of antibiotics and antibacterial drugs has rapidly increased the incidence of multi-drug-resistant bacteria, so that many medical doctors compared with the past were confronted with more difficulty to treat patients with serious bacterial infections such as MDR-TB and extensively drug-resistant TB. In addition, the current anti-TB drugs have been causing serious side effects such as hepatotoxicity, ototoxicity, and nephrotoxicity. For these reasons, as one of feasible solutions for overcoming limitations of the drug's side effects, a number of researches regarding anti-Mtb drugs were reported from active substances derived from diverse medicinal plants and biological resources, and some of natural products have been studied or developed as potent candidate substances for treating TB [8–12]. However, despite various efforts for developing novel anti-TB drugs, effective/safe next-generation anti-Mtb agents that can be used as a potential anti-TB drug, the extracts, natural products, semi-synthetic compounds and/or biomedicine, have not yet been reported or released in pharmaceutical market. Recently, innovative and creative alternatives for developing effective/safe drugs through substantive solutions between scientists and pharmaceuticals are being required globally.

In this aspect, the new pharmacological activity/effect of existing drugs/compounds used for treating different diseases can provide the advantages of both the safety and efficacy compared with newly developed drugs, which may be used or utilized as one of effective strategies for developing novel drugs and as one of feasible alternatives for developing effective/safe next-generation drugs. Moreover, the compounds derived from the repurposing of existing drugs may increase the potential for developing new-next-generation drugs as a useful resource for developing new substances in a medical field. For these reasons, this study was focused on major points for the discovery of new candidate substances as one of various strategies for developing anti-Mtb drugs, which were the minimization of side effects, the safety of drugs, and the finding of novel pharmacological activity/effect/function/action of existing drugs or known compounds. In these perspectives, CLA and linolenic acid (α- and γ-form) are being used as adjuvant for both medical use and healthcare as bioactive substances derived from various biological resources, including medicinal plants and marine resources. Recently, new pharmacological activity/effect/actions of CLA and linolenic acid (α- and γ-form) have been variously reported as follows: 1) Anti-adipogenic effect of α-linolenic acid through AMP-activated protein kinase [13], 2) γ-linolenic acid increases the therapeutic efficacy of irradiation in the treatment of gliomas [14], 3) The modulatory effects of CLA against both obesity and inflammatory bowel disease through peroxisome proliferator-activated receptor gamma pathway [15], 4) α-linolenic acid protects the heart from myocardial fibrosis and cardiac injury in the rat [16], 5) Anti-allergic effect of α-linolenic acid in the gut of murine food allergy model [17], 6) The effect of omega-3 polyunsaturated fatty acid including α-linolenic acid in treating patients with non-alcoholic steatohepatitis [18].

Taken together, these studies indicate substantial and significant evidence that linolenic acid (α- and γ-form) and CLA have not only various pharmacological activities/effects/actions in both '*in vitro*' and '*in vivo*' but also the potential that can be used as therapeutic agents. However, in spite of the pharmacological activities/effects/actions of them provided through these studies, their anti-TB activity/effects against Mtb have not yet been reported in both '*in vitro*' and '*in vivo*'. In these aspects, this study has begun from hypothesis that linolenic acid (α- and γ-form) and CLA may effectively inhibit or interrupt and modulate the growth/proliferation of Mtb causing TB, which can improve the safety and increase or induce the minimization of the side effects that are caused by drug treatment. As mentioned above, the results of this study showed anti-tubercular activity/effect/ability of CLA and linolenic acid (α- and γ-form) by strongly inhibiting or blocking the growth/proliferation of Mtb through their novel pharmacological activity/properties. Particularly, anti-Mtb activity/effect of linolenic acid (α- and γ-form) was effectively demonstrated through the selective Mtb susceptibility-indicator assays such as the REMA and MGIT 960 system assay. All the tested compounds consistently indicated anti-Mtb activity/effect by strongly inhibiting or blocking

the growth/proliferation of Mtb for 21 d with a single treatment (200 μg/mL) in MGIT 960 system assay. In addition, the differences in anti-Mtb activity/effect of them were obviously confirmed in the MGIT 960 system assay and the REMA assay; Linolenic acid (γ-form) more effectively inhibited the growth/proliferation of Mtb compared with those of linolenic acid (α-form) and CLA. The important aspect that can be considered at this point is that all the tested compounds are unsaturated fatty acid. This suggests that when the compounds are bound with Mtb in the growth stages of Mtb, chemical-binding effects between the compound and the bacteria can be increased owing to its chemical properties such as chemical affinity, absorbability, melting point, and liposolubility. Furthermore, it implies that the biochemical reactions and/or functions of the cell wall of Mtb may be blocked or inhibited by the binding of the compounds, and suggests that a cascading intracellular signaling-pathway of replication as well as cell cycle-key proteins in cytoplasm may be deactivated by the compound at check points that accelerate the growth/proliferation of Mtb.

In summary, linolenic acid (α- and γ-form) and CLA strongly inhibited the growth/proliferation of Mtb that can induce TB in those with weakened immune systems through their novel pharmacological activity/effect/function, and their anti-Mtb activity/effect and action were demonstrated through different anti-Mtb indicator assays such as the MGIT 960 system assay and the REMA. In particular, linolenic acid (γ-form) more effectively inhibited the growth/proliferation of Mtb compared with other compounds used in this study. These results showed not only the potential for the effective use of linolenic acid (α- and γ-form) and CLA as one of feasible solutions for inhibiting or blocking TB that causes a serious threat in public health fields, but also a new pharmacological function/action concerning anti-Mtb activity/effect of them against Mtb. Therefore, this study provides significant results that linolenic acid (α- and γ-form) and CLA can be effectively used or utilized as potent candidate drugs for developing new anti-Mtb drugs of the effective/safe next-generation in the near future. In addition, this study suggests that the compounds have the potential which may decrease the cost and/or time in clinical trials through their repurposing.

Conflict of interest statement

The author declares that there is no conflict of interest.

References

[1] World Health Organization. *Global tuberculosis report 2013*. Geneva: World Health Organization Press; 2013.

[2] World Health Organization. *Global tuberculosis report 2014*. Geneva: World Health Organization Press; 2014.

[3] Koul A, Arnoult E, Lounis N, Guillemont J, Andries K. The challenge of new drug discovery for tuberculosis. *Nature* 2011; **469**(7331): 483-490.

[4] Hartkoorn RC, Sala C, Neres J, Pojer F, Magnet S, Mukherjee R, et al. Towards a new tuberculosis drug: pyridomycin – nature's isoniazid. *EMBO Mol Med* 2012; **4**(10): 1032-1042.

[5] Lee M, Lee J, Carroll MW, Choi H, Min S, Song T, et al. Linezolid for treatment of chronic extensively drug-resistant tuberculosis. *N Engl J Med* 2012; **367**(16): 1508-1518.

[6] Pethe K, Bifani P, Jang J, Kang S, Park S, Ahn S, et al. Discovery of Q203, a potent clinical candidate for the treatment of tuberculosis. *Nat Med* 2013; **19**(9): 1157-1160.

[7] Wilson R, Kumar P, Parashar V, Vilchèze C, Veyron-Churlet R, Freundlich JS, et al. Antituberculosis thiophenes define a requirement for Pks13 in mycolic acid biosynthesis. *Nat Chem Biol* 2013; **9**(8): 499-506.

[8] Copp BR, Pearce AN. Natural products growth inhibitors of *Mycobacterium tuberculosis*. *Nat Prod Rep* 2007; **24**(2): 278-297.

[9] Tandon R, Ponnan P, Aggarwal N, Pathak R, Baghel AS, Gupta G, et al. Characterization of 7-amino-4-methylcoumarin as an effective antitubercular agent: structure-activity relationships. *J Antimicrob Chemother* 2011; **66**(11): 2543-2555.

[10] Saikia D, Parveen S, Gupta VK, Luqman S. Anti-tuberculosis activity of Indian grass KHUS (*Vetiveria zizanioides* L. Nash). *Complement Ther Med* 2012; **20**(6): 434-436.

[11] Gemechu A, Giday M, Worku A, Ameni G. *In vitro* anti-mycobacterial activity of selected medicinal plants against *Mycobacterium tuberculosis* and *Mycobacterium bovis* strains. *BMC Complement Altern Med* 2013; **13**: 291; http://dx.doi.org/10.1186/1472-6882-13-291.

[12] Leitao F, Leitao SG, de Almeida MZ, Cantos J, Coelho T, da Silva PE. Medicinal plants from open-air markets in the State of Rio de Janeiro, Brazil as a potential source of new anti-mycobacterial agents. *J Ethnopharmacol* 2013; **149**(2): 513-521.

[13] Zhou X, Wu W, Chen J, Wang X, Wang Y. AMP-activated protein kinase is required for the anti-adipogenic effects of alpha-linolenic acid. *Nutr Metab (Lond)* 2015; **12**(1): 10; http://dx.doi.org/10.1186/s12986-015-0006-5.

[14] Antal O, Peter M, Hackler L Jr, Man I, Szebeni G, Ayaydin F, et al. Lipidomic analysis reveals a radiosensitizing role of gamma-linolenic acid in glioma cells. *Biochim Biophys Acta* 2015; **1851**(9): 1271-1282.

[15] Yuan G, Chen X, Li D. Modulation of peroxisome proliferator-activated receptor gamma (PPAR γ) by conjugated fatty acid in obesity and inflammatory bowel disease. *J Agric Food Chem* 2015; **63**(7): 1883-1895.

[16] Folino A, Sprio AE, Di Scipio F, Berta GN, Rastaldo R. Alpha-linolenic acid protects against cardiac injury and remodelling induced by beta-adrenergic overstimulation. *Food Funct* 2015; **6**(7): 2231-2239.

[17] Kunisawa J, Arita M, Hayasaka T, Harada T, Iwamoto R, Nagasawa R, et al. Dietary ω3 fatty acid exerts anti-allergic effect through the conversion to 17, 18-epoxyeicosatetraenoic acid in the gut. *Sci Rep* 2015; **5**: 9750; http://dx.doi.org/10.1038/srep09750.

[18] Nogueira MA, Oliveira CP, Ferreira Alves VA, Stefano JT, Rodrigues LS, Torrinhas RS, et al. Omega-3 polyunsaturated fatty acids in treating non-alcoholic steatohepatitis: a randomized, double-blind, placebo-controlled trial. *Clin Nutr* 2015; http://dx.doi.org/10.1016/j.clnu.2015.05.001.