Original Article

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Metabolic Regulation and Anti-Oxidative Effect of Ferula Asafoetida Ethanolic Extract on children with leukemia

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Abstract

Background: Childhood leukemia, the most common type of cancer in children and teens, is a cancer of the white blood cells. As antioxidants can promote immune system against all types of cancer, the aim of this study was to explore the metabolic regulation and anti-oxidative effect of sesquiterpen-rich extract of Ferula Asafoetida (FA) on children with leukemia.

Materials and Methods: In this study, 75 leukemic children with a mean age of 5 to 12 years old were treated with doses of 50 and 100 mg of FA extract daily. Then metabolic profile and oxidative biomarkers were assessed. ROS (Reactive oxygen species) and vasculature enzymes SOD (superoxide dismutase)/ CAT (catalase) in the action of extract were also examined.

Results: Receiving FA extract reduced glucose levels of plasma significantly from 105.42±45.22 to 81.40±41.74 mg/dL in 50 mg group and from 101.72±38.82 to 65.86±40.10 mg/dL in 100 mg group ($p < 0.05$). There was also a considerable decrease in the plasma level of TC. Mean value of TG level was also significantly reduced and level of LDL-C (low lipoprotein-C) significantly decreased too; however, level of HDL-C (high lipoprotein-C) increased. Moreover, the Sesquiterpene-rich extract significantly increased the activity of SOD/CAT and total antioxidant capacity (TAC) in patients ($P<0.05$). Notably, sesquiterpens significantly prevented protein and lipid peroxidation (PCO (Plasma protein carbonyls) / MDA (malondialdehyde) ($P<0.05$).

Conclusion: Treatment with Ferula Asafoetida ethanolic extract regulated the metabolic pathway. Sesquiterpens in the extract confers protection against oxidative stress-induced complications largely through interfering with ROS and PCO/MDA generation.

Key Words: Childhood leukemia, Ferula Asafoetida, Oxidative Stress, Sesquiterpen-rich Extraction

Introduction

Ferula Asafoetida (FA) is a traditional medicine originally grown in the hot area of Iran, on the desert of Yazd and mountains of Afghanistan. Locally, FA is employed as a treatment for some neoplastic disorders such as liver and breast cancer. The chemical composition of FA mainly includes sesquiterpens: namely α- longipinene and δ- terpinene, followed by Z- β-ocimene derivatives (1,2). Analyzing of ethanolic distillated-extracted products of FA by GC/MS has identified the sesquiterpen-rich composition constitutes mainly of α-longipinene (>29.6 %), δ- terpinene (>13.5 %), and and β Z- β-ocimene (~3.8%), as the major bioactive compounds among the 35 constituents characterized in the aerial part, and representing more than 68.2% of the total components detected in the ethanolicdistillated extraction (Table I) (3,4). Importantly, some sesquiterpenes from herbal medicine have been reported to exert anti-cancer as their anti oxidant properties. According to its chemical composition, the pharmacological activity and traditional uses of FA can be attributed to its sesquiterpene contents α-

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longipinene, δ- terpinene, and Z- β-ocimene. natural sesquiterpens chosed as an effective treatment for leukemia (5-11).
In regard to the fact that oxidative stress (OS) is the relevant factor in the pathogenesis of cancer, and some vascular complications, it is of significance to develop effective therapies against OS and impairment of vasculature system as well as apoptosis in patients with leukemia. For example, the extraction of ginger and turmeric contains a large variety of terpenoids, some of which are sesquiterpenes and pose antiproliferative and antioxidant properties (6, 12).
Childhood leukemia, the most common type of cancer in children and teens, is a cancer of the white blood cells. Each year approximately 13,000 children under the age of 20-years are diagnosed with cancer in the U.S (13). Almost all cases of childhood leukemia are acute and develop rapidly (14). Yet, there is no clinical study about the anti-proliferative and anti-oxidant effects of FA in humans (enzymatic and non-enzymatic effects). Herein, assayed the extent of OS in children with leukemia in terms of PCO/MDA and increased ESR, before and after consumption of FA sesquiterpene-rich extract. In this study, sesquiterpene-rich extract was also evaluated for their activity on SOD/CAT in children with leukemia. Oxidative modulation can regulate cell metabolism and would be a reason for scavenging of free radicals, which are the major reasons of altered physiological processes in cancer.
The study sample included 75 leukemic children. Patients received the plant extract at different doses (50 and 100 mg/day) for 45 days before lunch, while oxidative properties of plasma was measured.

Materials and Methods

In this study, 75 leukemic children with a mean age of 5 to 12 years old, referred to *AMIR* Oncology Hospital, Shiraz, Iran, were included in this study. Main clinical and laboratory characteristics of the patients are shown in Tables II and III.
The patients were divided randomly into three groups: 1) receiving placebo (n=25), 2) receiving capsules containing 50 mg (n=25), and 3) receiving 100 mg doses of FA ethanolic extract (n=25) (1, 15).
In this experimental study, the plant was collected from the desert of Yazd, Iran. The aerial part (flowers, leaves and stems) were dried. Following identification, drying, and powdering flowers, leaves and stems were soaked in 80% methanol for 48 hours at room temperature. After filtration, the residue was drained into the same flask. The solvent was dried by rotary evaporation under reduced pressure and at a temperature of maximally 45°C.

Biochemical analysis

Metabolic evaluation

Blood samples, before intervention and at the end of trial, after 45 days, were obtained from the subjects and placed in chilled tubes with 1mg/ml EDTA-K3 as anticoagulant (9, 10, 16).
The freshly drawn plasma was immediately processed for measurement of blood levels of the following biochemical parameters: FBS (fasting blood sugar), TG (triglyceride), TC (total cholesterol), LDL-C, HDL-C, urea, creatinine, ALT and AST. The extent of TAC of plasma and the ratio of TG to HDL-C concentration (TAG/HDL-C), as an important metabolic index in cancer patients were measured (14, 17, and 18).

Plasma SOD activity measurement

Total SOD activity was determined according to Mirsra and Fridovich method (17, 19). Briefly, 10 µL of plasma was added to 970 µL of carbonate buffer (0.05 M, pH 10. 2, 0.1 mM EDTA), at room temperature. Then, 20 µL of Epinephrine 30 mM (in 0.05% acetic acid) was added to the mixture, and OD was measured at 480 nm for 4 minutes against the blank. The phosphate buffer was used as a blank. One unit of SOD was defined as the

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amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 unit. The activity was expressed as units/mL of plasma/g protein.

Plasma CAT activity measurement

CAT activity of plasma was measured according to the Aebi method (17,18), at room temperature. 10 µL of plasma was added to 250 µL of H_2O_2 (66 mM H_2O_2 in phosphate buffer, pH 7.4), then a decrease in OD was measured at 240 nm for 1 min. The molar extinction coefficient of 43.6 M/cm was used to determine CAT activity. One unit of enzyme activity was equal to µM of H_2O_2 degraded/mL of plasma/g protein.

Plasma lipid peroxidation (MDA) measurement

The increase in plasma levels of MDA is a direct reflection of OS of vital organs/tissues (17-20). This assay was used to determine MDA level as described by the reference (19).

Protein oxidation biomarker (PCO) measurement

This assay was used to determine protein oxidation level of plasma as described by the reference (20). The plasma protein carbonyl (PCO) groups reacted with chromogene 2, 4-dinitrophenylhydrazine (DNPH) to generate chromatic dinitrophenylhydrazones.

Erythrocyte sedimentation rate (ESR)/TAC index measurement

ESR was determined by the Westergren method using EDTA-containing whole blood sample. ESR/TAC ratio vs. ESR/MDA may provide a global index for protein/lipid oxidations which are induced by oxidant stimuli (17, 18). A positive correlation between ESR with MDA/PCO concentrations, vs. TAC in patients with OS condition had been proved (20).

Statistical analysis

Results were expressed as mean ± standard deviation (SD). The student paired t-test was performed to evaluate the difference between the baseline and washout values of study outcomes. For the comparison of groups, variance analysis (one-way ANOVA) and the Pearson correlation test were used. $P< 0.05$ was regarded as statistically significant (14,19, 20).

Results

FA sesquiterpene-rich extract improved considerably metabolic statues of plasma in leukemic patients

All patients in the three groups were similar in age, sex, height, body weight, and body mass index (BMI), as shown in Table II. The mean values (±SD) of the biochemical parameters in the pre and post-intervention phases for the control and treated patients are shown in table II and 3. Daily receiving *FA* extract especially in 100 mg dose reduced significantly glucose levels of plasma .The mean value of FBS decreased from 105.42±45.22 to 81.40±41.74 mg/dL in 50 mg group and from 101.72±38.82 to 65.86±40.10 mg/dL in 100 mg group($p < 0.05$) (Figure 1A). There was also a considerable decrease in the plasma level of TC, after *FA* receiving. The blood profile of lipids were significantly decreased (from 162.30±33.81 to 135.90±23.29 mg/dL, and from 164.82±34.84 to 117.86±26.39 in in 50 and 100 mg doses, respectively) ($P<0.01$) (Figure 1B).

The mean value of TG level was also significantly reduced (from 184.74±30.68 to 163.00±27.38 mg/dL in 50 mg group and even more from 187.36±32.00 to 150.68±27.29 mg/dL in 100 mg dose, $p < 0.05$) (Figure 1C).

Considering, dramatically decreased level of LDL-C (from 148.2±25.5 to 128.34±33.4 mg/dL in 50 mg, and from 148.6±26 to 96.2±36.2 mg/dL in 100 mg dose, $P<0.05$), and an increased level of HDL-C (from 42.2±8.1 to 43.4±5.4 mg/dL in 50 mg, and from 41.8±5.1 to 46.7±6.7 mg/dL in group 500 mg, $P<0.05$) was observed, after 45-day

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receiving *FA* extract. Generally, a TG/TC/LDL-C lowering post-treatment effect was demonstrated in all individuals who received *FA* extract, but this reduction was more obvious in the 100 mg group.

FA sesquiterpene-rich extract increased ROS scavenging activity of plasma (SOD/CAT activity & TAC)

In human being, when suffered from free oxygen radicals, a complex defense system is activated (e.g. SOD & CAT) (18-20). In Table III, SOD and CAT activity, and TAC of plasma in children with leukemia are represented, before and after 45-day treatment with placebo, 50 and 100 mg doses of *FA* extract. As shown in Figure 3, SOD significantly elevated from 1324.82±135.81 to 1360.25±128.88 U/g protein in 50mg dose group, and from 1316.38±134.31 to 1430.42±140.12 U/g protein, in 100 mg dose group (Figure 2A). CAT activities elevated from 80.26±9.13 to 84.24±9.11 kU/g protein in 50 mg dose group and from 81.20±8.96 to 89.93±10.01 kU/g protein in 100 mg dose group (Figure 2B)($P<0.05$).

However, there was a significant difference in SOD activity in 50 mg treated group with received 100 mg dose ($P<0.05$).

The plasma levels of TAC were also found to be elevated in the groups receiving *FA* (from 0.55±0.011 to 0.56±0.022 and from 0.55±0.031 to 0.66±0.002 mM in 50 and 100 mg groups, respectively $p<0.05$) (Figure 2C).

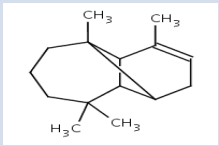
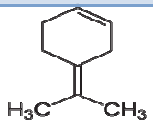
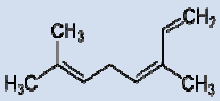
FA extract reduced the oxidative biomarkers of plasma in leukemic patients

Current study showed a significant reduction in the level of LPO and protein oxidation, in leukemic patients receiving *FA* extract. The MDA concentration in plasma of patients was considerably reduced from 0.57±0.07 to 0.39±0.01 µM in 50 mg and from 0.60±0.04 to 0.26±0.02 µM in 100 mg group, in comparison to control group (0.59±0.03 µM) ($p<0.05$) (Figure 3A). Similarly, a significant decreased level of PCO was observed (from 1±0.004 to 0.98±0.004 nM in 50 mg and from 1.01±0.006 to 0.97±0.004 nM in 100 mg group), in whom receiving *FA*, in comparison to control group (from 0.98±0.009 to 0.97±0.003 nM) ($p<0.05$) (Figure 3B).

Interestingly, receiving *FA* doses also changed the ESR/MDA, ESR/TAC and MDA/TAC ratios, in diabetic patients (Figures 4) ($P<0.05$). In contrast to ESR/MDA index (Figure 4A) which was considerably elevated, two other indexes ESR/TAC (Figure 4B), and MDA/TAC (Figure 4C), were lowered significantly ($P<0.05$), especially in the group received dose of 100 mg. Decreases in two later ratios indicated that anti-oxidant activities of plasma were efficiently induced by *FA* extract. However, the results depicted a considerable increase in ESR/MDA ratio due to a potent negative correlation between elevated TAC vs. lowered MDA levels.

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Table I. The main chemical constituents found in hydrodistillation extract of aerial part of Ferula Asafoetida (FA), analyzed by GC and GC/MS, namely α- longipinene and δ- terpinene, followed Z- β-ocimene (1, 2).

Composition %	Biochemical Structure	Compound name
> 29.6%		α- longipinene, a sesquiterpene
>13.5%		δ- terpinene**,** a sesquiterpene
~ 3.8%		Z- β-ocimene, a sesquiterpene

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Table II. Demographic and biochemical characteristics of children with leukemia

P value	Control (n=25)	50 mg (n=25)	100 mg (n=25)	Parameters
> 0.05	9.12±3.24	8.31±4.73	8.40±4.33	**Age (years)**
> 0.05	25	25	25	**Sample size**
				FBS (mg/dL)
> 0.05	101.44±49.34	105.42±45.22	101.72±38.82	Before treatment
< 0.05	99.04±38.37	81.40±41.74*	65.86±40.10**	After treatment
	> 0.05	< 0.01	< 0.01	**P value**
				TC (mg/dL)
> 0.05	171.16±31.97	162.30±33.81	164.82±34.84	Before treat
< 0.05	168.64±37.66	135.90±23.29*	117.86±26.39**	After treat
	> 0.05	< 0.01	< 0.01	**P value**
				TG (mg/dL)
> 0.05	180.02±33.16	184.74±30.68	187.36±32.00	Before treatment
< 0.05	174.02±41.77	163.00±27.38*	150.68±27.29**	After treatment
	> 0.05	< 0.01	< 0.01	**P value**
				LDL-C (mg/dL)
> 0.05	147.9± 25.6	148.9± 25.5	148.6± 24	Before treatment
< 0.05	154.1± 24.5	128.1± 23.4*	96.1± 36.2**	After treatment
	> 0.05	< 0.01	< 0.01	**P value**
				HDL-C (mg/dL)
> 0.05	42.4± 5.4	42.9± 5.1	40.2± 5.7	Before treatment
> 0.05	43.2± 8.1	42.7± 6.7	41.4± 1.25	After treatment
	> 0.05	>0.01	> 0.01	**P value**

FBS: fasting blood sugar; TC: total cholesterol; TG: triglycerides; LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol.
Values are mean±SD of 25 patients. *$P < 0.05$ vs. control group and pre-treatment values; **$P < 0.01$ vs. 50 mg group and control group and pre-treatment values. For a comparison of pre- and post-treatment values in the respective groups we used paired t-test, while differences between groups were analyzed by one-way ANOVA. Any P-value <0.05 was considered statistically significant.

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Table III. Anti-oxidant status of the patients before and after the use of Ferula Asafoetida (FA) extract.

P value	Control (n=25) mean±SD	50 mg (n=25) mean±SD	100 mg (n=25) mean±SD	Parameters
				SOD (U/g protein)
> 0.05	1341.29±144.44	1324.82±135.81	1316.38±134.31	Before treatment
< 0.05	1338.11±132.12	1360.25±128.88*	1430.42±140.12**	After treatment
	> 0.05	< 0.05	< 0.05	***P* value**
				CAT (kU/g protein)
> 0.05	81.25±9.11	80.26±9.13	81.20±8.96	Before treatment
< 0.05	82.21±8.98	84.24±9.11*	89.93±10.01*	After treatment
	> 0.05	< 0.05	< 0.05	***P* value**
				TAC (mM)
> 0.05	0.58±0.01	0.55±0.011	0.55±0.031	Before treatment
< 0.05	0.57±0.04	0.56±0.022	0.66±0.02**	After treatment
	> 0.05	> 0.05	< 0.05	***P* value**

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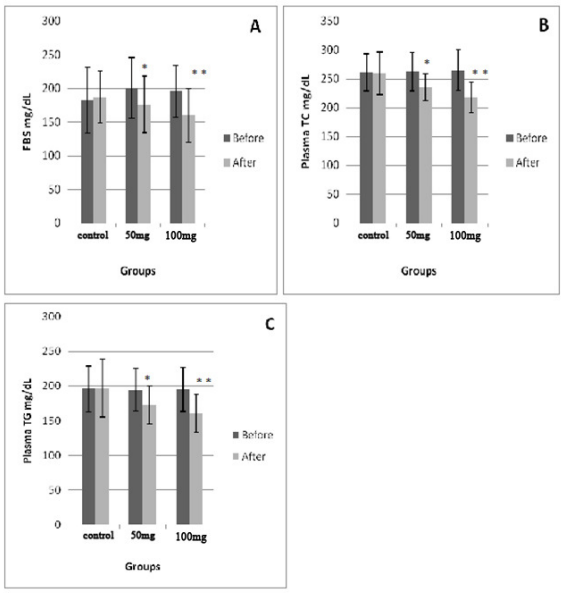


Figure 1. Plasma levels of glucose (FBS)(A), total cholesterol (TC)(B) and triglycerides (TG)(C), in children with leukemia, before and after 45 days received capsules containing 50 or 100 mg doses of DA extract, daily before lunch. The controls received only placebo capsules.
Values are means ± SD of 25 patients, *$P < 0.05$ vs. control group and pre-treatment; **$P < 0.01$ vs. 50 mg and control group and pre-treatment. For a comparison of pre- and post-treatment values in the respective groups used paired t-test, while differences between groups were compared by one-way ANOVA, P-value <0.05 was considered statistically significant.

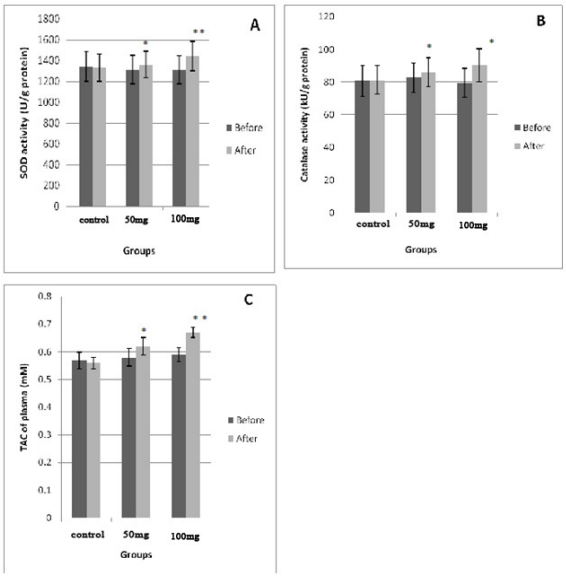


Figure 2. Superoxide dismutase (SOD)(A), catalase (CAT)(B) activities and total anti-oxidant capacity (TAC) of plasma in children with leukemia, before and after 45 days received capsules containing placebo (as control group), 50 and 100 mg doses (50 mg & 100 mg, respectively) of *FA* extract, daily before lunch. Values are means ± SD of 25 patients, *$P < 0.05$ vs. control group and pre-treatment; **$P < 0.01$ vs. 50 mg and control group and pre-treatment. For a comparison of pre- and post-treatment values in the respective groups used paired t-test, while differences between groups were compared by one-way ANOVA, P-value <0.05 was considered statistically significant.

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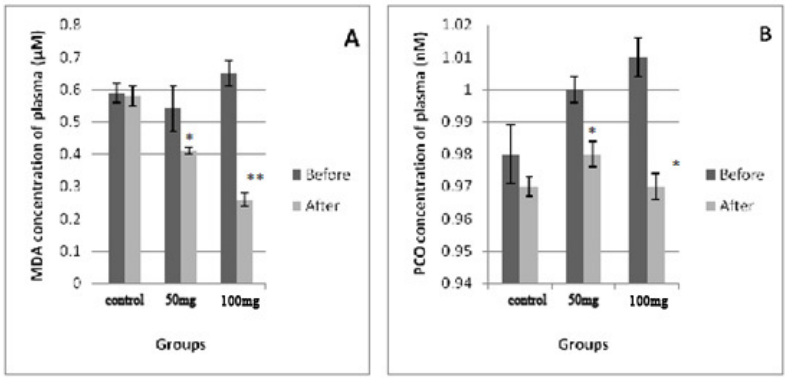


Figure 3. Plasma lipid peroxidation (A) and protein carbonyls (PCO)(B) concentrations in children with leukemia received capsules containing placebo (as control group), 10 nd 20 mg doses (50 mg and 100 mg groups) of FA extract, for 45 days, daily before lunch. Concentration of MDA is expressed as µmol/L and for PCO is expressed as nmol/L of plasma. Values are means ± SD of 25 patients, **P* < 0.01 vs. control group and pre-treatment; ***P* < 0.001 vs. 50 mg and control group and pre-treatment. For a comparison of pre- and post-treatment values in the respective groups used paired t-test, while differences between groups were compared by one-way ANOVA, *P*-value <0.01 was considered statistically significant.

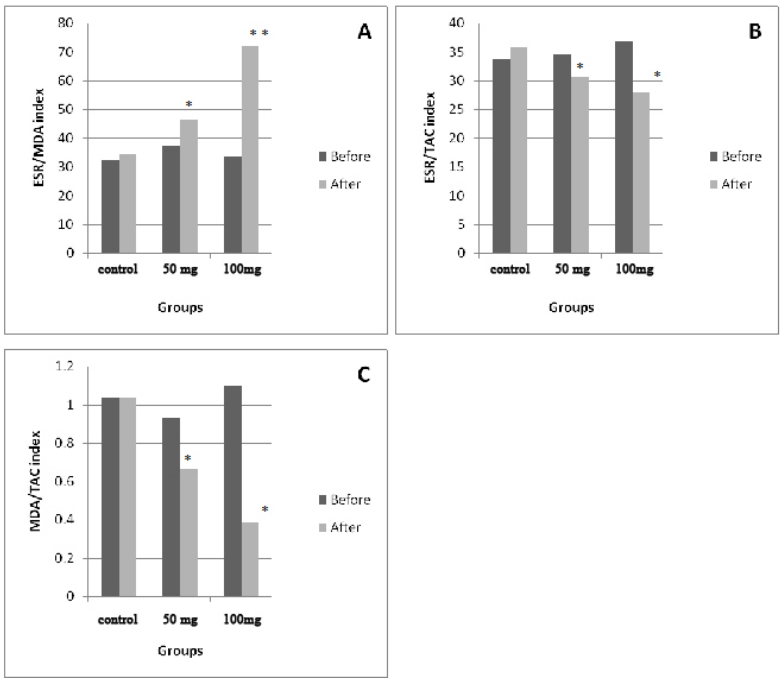


Figure 4. Increase in the total anti-oxidant activity of plasma causes a significant change in the values of ESR/MDA, ESR/TAC and MDA/TAC index. There is a positive correlation between erythrocyte sedimentation rate (ESR) value vs. malondialdehyde (MDA) concentration, and a negative correlation between total antioxidant capacity (TAC) vs. ESR value in children with leukemia. Values are means of 25 patients, **P* < 0.05 vs. control group and pre-treatment; ***P* < 0.001 vs. 50 mg and control group and pre-treatment. For a comparison between the respective groups used one-way ANOVA, *P*-value <0.05 was considered statistically significant.

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Discussion

Ferula Asafoetida (FA) plant belongs to the *Apiacea* family, the *Ferula* genus. In animal models, some biological activities of *FA* such as antihyperlipidaemic, antihypercholesterolaemic, epatoprotective and anti-thrombotic effects have been shown (1-4). In vivo, studies have shown that *FA* extract has protective effects in thyroid disorders as well as the ability to regulate activity of Aromatase and 5-α Reductase in the gonads of animal model (21, 22).

Studies on the extract from aerial parts of *FA* have exhibited a large group of terpenoids mainly sesquiterpene compounds α- longipinene (>29.6%), δ-terpinene (13.5 %) and Z- β-ocimene with potent antioxidant activities (1-4, 23) (Table I).

Therefore, pharmacological activity of *FA* as being effective in the treatment of leukemia, can be attributed to its sesquiterpene contents. There are references about these contents regarded as an effective treatment for cancer. For example, the extraction of ginger and turmeric contains a large variety of terpenoids, some of which are sesquiterpenes (longipinene and terpinene), and possess antidiabetic and antioxidant properties (5, 6,11).

Indeed, sesquiterpenes of the plant are believed to be the main constituent contributing to control of metabolism statues in the body especially sugar and lipid metabolisms and because of their antioxidant properties, it may be especially useful in the treatment of some neoplastic disorders such as breast cancer and metabolism disorders (1, 4, 15, 23). Sesquiterpens exhibit cellular effects, possibly through the inhibition of protein tyrosine phosphatases, with more specific inhibition against PTP1B (9).

It was reported that herbal natural sesquiterpens have potential therapeutic effects against cancer. Pharmacological inhibition of p38 and JNK by sesquiterpenes reverses apoptotic effect (10).

Both ginger and turmeric sesquiterpenoid extractions exhibit hypoglycemic effects via peroxisome proliferator-activated receptor-γ (PPAR-γ) activation, and suppress an increase in blood glucose levels in cancer patients. The effect is synergistic when both sesquiterpenoids are applied together (2, 5, 6).

Activation of PPAR family especially PPAR-γ is the most important metabolic regulation effect of sesquiterpenes which subsequently may result in transactivating of PPAR-associated genes. For example, PPAR-γ activators can transactivate ABCA1 promoter and enhance apoA-I and apoA-II production, whereas it can reduce apoC-III production significantly. PPAR-γ ligands cause a significant increase in HDL cholesterol levels. PPARs are ligand-activated transcription factors that belong to a nuclear receptor superfamily and are involved in the regulation of cells metabolism. Especially, PPAR agonists can regulate the atherosclerosis index represented by total cholesterol/HDL ratio (12, 24).

Data also demonstrated that *FA* sesquiterpenes are able to regulate the expression or biosynthesis of some factors involved in the treatment of hyperlipidemia and control the atherogenicity index. *FA* treatment could effectively elevate HDL levels and lower triglyceride levels in cancer patients which it might be through regulating a series of genes associated with nuclear receptor family of PPARs.

PPARs had important anti-inflammatory, vasoprotective actions in addition to antiglycemic and/or antidyslipidemic activities. Herbal sesquiterpens induced PPAR-γ transactivation activity by directly binding to PPAR-γ ligand binding domain. Additionally, these results highly indicated

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that sesquiterpens were active components of herbal medicine to exert anti-diabetic effect through PPAR-γ pathway. The sesquiterpenes have been observed to behave as PPAR-α/γ dual agonists so they might be useful as the potential herbal treatment for children with all types of leukemia (7, 8, 11, 25-27).

It is now well established that hyperglycemia is associated with OS which provides a potential 'link' between cancer, diabetes and vascular complications (11, 28-31). OS is a relevant factor in the pathogenesis of diabetes complications. Previous studies also implicate that sesquiterpenes have the potential to exhibit anti-oxidative and anti-inflammatory effects in cancer patients (5-8, 12).

In cancer patients, ROS production increase (31). This increase in ROS production is an important cause of NADPH oxidase mRNA expressions, an enzyme complex which generates ROS, and also cause of decrease in mRNA expressions and activities of SOD/CAT. In vasculature system, SOD and CAT are the key enzymes in the metabolism and naturalization of ROS (29-31). Whereas, over-expression of anti-oxidant enzymes SOD/CAT would prevent the development of TNF-α -induced insulin resistance in glucose-metabolism related tissues, it can down regulate the severity of leukemia in children (1, 25-31).

Several studies have reported the effects of PPAR-γ agonists on OS and expression of SOD/CAT in human. Treatment with PPAR-γ agonists would reduce the generation of ROS and lipid peroxidation in cancer patients. Recent studies suggest the potential of sesquiterpens as natural products to act as ligands/activators for PPARs and to increase SOD/CAT gene expression and protein levels in human vasculature system (25, 29-32). Oxidative modulations of plasma in cancer patients could regulate cell metabolism and may increased scavenge of free radicals in plasma which are the major reasons of altered physiological processes in cancer (13, 18, 29, 30).

Sesquiterpenes are known for their molecular activity against PPARs transactivation to exert anti-diabetic effect in cancer patient through PPAR pathway (7). Moreover, sesquiterpenes have been reported to behave as PPAR-α/γ dual agonists so they might be useful as the potential herbal treatment for cancer (7, 26, and 27). The plant compounds enhance PPAR transactivation activity by directly binding to PPAR ligand binding domain and inducing their interactions with their promoters including PPRE (PPAR-response element), SHP, and ABCA1 gene promoters in dose-dependent manner (7, 8).

Activated by their ligands, PPARs promote their target genes. Importantly, the PPAR element is located in the sequence of the SOD/CAT genes, which may contribute to the antiatherosclerotic effects of PPARs. Proteins and lipids are the major targets of OS (17-19). OS might involve oxidation of proteins and rapids (13, 20).

Previous studies revealed that antioxidant defense system (antioxidant enzymes SOD, CAT) is unable to compromise the increased concentrations of PCO (the most potent marker of oxidative stress) and decreased levels of TAC in cancer patients (20, 28, and 29).

It is also demonstrated that under OS, the erythrocyte membrane is prone to MDA and PCO formation at their double bonds which shows a significant positive correlation between ESR and the erythrocyte MDA/PCO level. ESR, a serologic marker of inflammation, shows a negative correlation to TAC vs. MDA levels of plasma. The correlation between ESR vs. TAC and ESR vs. MDA in plasma of patients is useful in evaluating disease activity (20, 17).

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Nowadays, herbal medicine *FA* has been found to include particular medicinal properties including anti-inflamatory and anti- atherosclerotic effects in animal models of Breast cancer (1, 15, and 23).

Herein from these results, it is indicated that natural sesquiterpens in *FA* restored to both SOD and CAT activity through inducing the PPAR-associated pathway, whereas inhibited ROS generation. Importantly, SOD/CAT activity was positively correlated with the TAC of plasma, whereas was negatively correlated with PCO/MDA production and ESR value of the blood, both of which are positively correlated with aortic stiffness (25). *S*esquiterpenes may play a role not only in preventing against oxidative damage as an exogenous antioxidant in cancer patients by scavenging free radicals and superoxide but also in modulating the expression of the endogenous antioxidant enzymes as a gene regulator through PPAR-γ and NF-κB in the vasculature system (6-8, 12, 31, 32). These findings are expected to expand the knowledge about biological activities of herbal sesquiterpenes, while revealed that these compounds could be as potential lead compounds in the development of an alternative adjuvant in cancer treatment.

PPAR activity with these drugs inhibits also NAD (P)H oxidase activity in the aorta. Vascular SOD and PPAR play a crucial role in the anti-atherogenic effects of anti-hyperlipidemic drugs and in hypercholesterolemia in vivo (27).

Conclusion

The result of this stndy indicated that *FA* ethanolic extract which is rich in sesquiterpens has a positive impact on metabolism and oxidative statues of plasma in child with leukemia, especially in dose of 100 mg or more.

Finally, these findings were in the consistence with the other studies which characterized anti-hyperlipidemic, anti-hypercholesterolemic, hepatoprotective and antioxidant effects of *FA* extract in animal models. This trial provided an evidence to support the use of *FA* extract or its sesquiterpen constitutes for the prevention of cancer/cardiovascular complications in the population with metabolic disorders.

It can be concluded that *FA* extract in longer periods and in higher doses would have more positive effects on leukemic children.

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Conflict of interest statement

We also confirm that there is no conflict of interest to disclosure.

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