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Oxidative stress in liver of streptozotocin-induced diabetic mice fed a high-fat diet: A treatment role of *Artemisia annua* L.

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Objective. The aim of this study was the investigation of a treatment role of Artemisia annua L. (AA) on liver dysfunction and oxidative stress in high-fat diet/streptozotocin-induced diabetic (HFD/STZ) mice.

Methods. Sixty mice were divided into 12 groups including control, untreated diabetic, and treated diabetic ones with metformin (250 mg/kg), and doses of 100, 200, and 400 mg/kg of water (hot and cold) and alcoholic (methanol) extracts of AA. Type 2 diabetes mellitus (T2DM) was induced in mice by high-fat diet for 8 weeks and STZ injection in experimental animals. After treatment with doses of 100, 200 or 400 mg/kg of AA extracts in HFD/STZ diabetic mice for 4 weeks, oxidative stress markers such as malondialdehyde (MDA), glutathione (GSH), and free radicals (ROS) were determined in the liver tissue in all groups.

Results. Diabetic mice treated with metformin and AA extracts showed a significant decrease in ROS and MDA concentrations and a notable increase in GSH level in the liver. Effectiveness of higher doses of AA extracts (200 and 400 mg/kg), especially in hot-water and alcoholic ones, were similar to and/or even more effective than metformin.

Conclusion. Therapeutic effects of AA on liver dysfunction showed that antioxidant activity of hot-water and alcoholic AA extracts were similar or higher than of metformin.

Key words: *Artemisia annua* L., oxidative stress markers, type 2 diabetes, metformin

Nowadays, one of the most important causes of morbidity and mortality is type 2 diabetes mellitus (T2DM) that influences a large number of people worldwide (Komeili et al. 2019). Its prevalence in 2040 might reach around 642 million people (Bellou et al. 2018). T2DM impairs the glucose homeostasis and induces hyperglycemia via defecting the insulin action (Deayu Putri et al. 2020). Hyperglycemia and/or abnormal glucose metabolism produces oxidative stress. Oxidative stress plays an important role in the pathogenesis of T2DM and the development of its complications (Aouacheri et al. 2015). It occurs as a result of an imbalance between the production of free radicals and antioxidant defenses (Jabir and Shaker 2020).

Oxidative stress leads to an increase in reactive oxygen species (ROS) (Taile et al. 2020) or free radicals, malondialdehyde (MDA), and glutathione (GSH) in the liver (Guven et al. 2006; Mohamed et al. 2016; Deayu Putri et al. 2020; Eltayef et al. 2020).

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MDA is the end product of the membrane lipid peroxidation produced by action of free radicals (Shokrzadeh et al. 2015; Shaki et al. 2021). ROS are products of normal metabolism in body (Teow et al. 2007; Iqbal et al. 2012) that is excessively generated by a prolonged hyperglycemia (Taile et al. 2020). Glutathione is an important intracellular and not-enzymatic antioxidant present in the mammalian tissues (Kennedy et al. 2020; Shaki et al. 2021). It plays an important role in the cellular redox state regulation subsequently protecting the tissue cells from reactive oxygen damage, lipid peroxides, xenobiotic, and nitrogen species (Kennedy et al. 2020).

The most important factor for the liver damage in diabetic patients is oxidative stress that leads to disturbances in lipid, protein, and carbohydrate metabolism (Mohamed et al. 2016; Yazdi et al. 2019). These defects, in turn, lead to further oxidative stress and inflammation. Antioxidant activity is important for protecting the biological systems, because the ROS play a major role in proteins, lipids, and DNA oxidation in tissues, which may lead to a cell damage and developing of chronic diseases, especially diabetes (Messaili et al. 2020). Overproduction of ROS and MDA and low production of GSH are outcomes of the oxidative stress in T2DM (Aouacheri et al. 2015).

Many alternative therapies are available today for T2DM, including medicinal plants. Alternative treatment with natural antioxidants is more safe, economical, and effective than synthetic antioxidants (Iqbal et al. 2012). Many medicinal herbs contain antioxidant activity due to the presence of the antioxidant compounds (Iqbal et al. 2012). In various studies, the protective and/or therapeutic action of herbal medications have been reported in scavenging ROS and, in turn, recovering liver dysfunction in various diabetes experimental models (Yazdi et al. 2019). Genus Artemisia, as the largest and most important genera in the Asteraceae (Compositae) family, comprises over 500 species, whereas most of them are medicinal herbs and have been traditionally used throughout the world (Das 2012; Yazdi et al. 2019).

Artemisia annua L. (AA), commonly known as sweet wormwood, sweet Annie, sweet sagewort, and annual wormwood (Chinese: qngho), is widely used as a medicinal plant for treatment of many diseases, such as diabetes mellitus, in the East Asia and Korea. AA possesses several biological activities, especially antidiabetic effects (Jung et al. 2007; Kang et al. 2008; Nofal et al. 2009; Ghazanfar et al. 2014; Ogbonna et al. 2017). AA antioxidant property has been proven in several studies. However, it is less investigated in T2DM induced by high-fat diet and streptozotocin (STZ). This study was conducted to determine whether hot-water, cold-water, and alcoholic AA extracts could improve the oxidative stress-induced liver dysfunction in a high-fat diet/STZ-induced T2DM (HFD/STZ-induced T2D) mice.

Material and Methods

Animals. Male albino mice (8–10 weeks of age with weight of 30–35 g,) were maintained under standard conditions (12 h light/dark, temperature 22±2 °C) with food and tap water *ad libitum*. All procedures in the present study were performed in accordance with the local Ethics Committee of Mazandaran University of Medical Sciences, Sari, Iran (IR.MAZUMS.IMAMHOSPITAL.REC.1398.3273).

Control (CON) group: the animals were injected intraperitoneally (i.p.) with saline; type 2 diabetes mellitus (T2D) group: T2DM was induced by a high-fat diet and streptozotocin (STZ) injection in mice; metformin (MTF) group: T2D mice were treated with metformin (250 mg/kg) (as a standard drug in treating T2DM) i.p. daily for 4 weeks; T2D+hot-water groups: T2DM mice treated with hot-water AA extract at doses of 100, 200 or 400 mg/kg via gavage, daily for 4 weeks; T2D+cold-water groups: T2DM mice treated with cold-water AA extract at doses of 100, 200 or 400 mg/kg via gavage, daily for 4 weeks; and T2D+alcoholic groups: T2DM mice treated with alcoholic AA extract at doses of 100, 200 or 400 mg/kg via gavage, daily for 4 weeks.

In all animal groups, the body weight was measured at the end of each week until the experiment lasting. At the end of the experiment period, all mice fasted for 16 h and blood glucose was detected by glucometer. After a deep anesthesia, the liver tissues of all groups were isolated for the oxidative stress factors measurements.

Induction of T2DM. For the induction of hyperglycemia, the mice were fed with a high-fat diet for 8 consecutive weeks (Gao et al. 2019). Then, the mice were injected i.p. with a single dose of STZ (65 mg/kg body weight) (Sigma-Aldrich, USA) to induce a sustained hyperglycemia. Blood glucose was detected by glucometer after 10 days. Blood glucose level ≥200 mg/dl was considered as diabetic and included into the experiment (Gao et al. 2019). Standard pellet (Behparvar production and distribution of animal feed company, Iran) consisted of carbohydrates (72.1%), proteins (22.1%), and lipids (7.5%) with a total caloric value of ~2900 kcal/kg. The

content of high-fat diet consisted of carbohydrates (27.5%), proteins (14.5%), and lipids (58.5%) with a total caloric value of ~4700 kcal/kg.

Plant material. Aerial parts of *Artemisia annua* L. (AA) were gain from the north of Iran in May and June 2018. Genus and scientific species of plant were bio-technically identified by Masod Azadbakht Prof. of Sana University of Mazandaran, Sari, Iran and under record number: E1-39-2191 preserved in the School of Pharmaceutics, Mazandaran University of Medical Sciences, Sari, Iran.

Toxicity test of AA extracts. The median lethal doses (LD_{50}) of extracts were obtained from a new method at 3 stages. At the first stage, 4 mice were selected for each extract and injected i.p. with different doses (10, 100, 300, 600 mg/kg) of AA extracts. Mortality as well as behavioral changes were monitored for 24h. Because there was no mortality in mice, the next stage started with injection of the higher doses of 100, 1500, 2000 mg/kg. Since there was no mortality, third stage started at doses of 300, 4000, 5000 mg/kg. Toxic behavioral was monitored for 14 days. The median lethal dose was calculated according to the formula: $LD_{50}=[M_0+M_1]/2$; where M_0=highest dose of tested extract without mortality and M_1=lowest dose of extract tested that induced mortality.

Preparation of AA extracts. The aerial parts of AA were cleaned, washed, dried, powdered and 450 ml of distilled water was added to 500 g of the powder. Then, for providing a cold-water extract, process of extraction was done for consecutive 3 days at room temperature (away from the sunlight). For providing a hot-water extract, the mixture of water and plant was boiled for 6 h and finally filtered. This stage was repeated 2 times. For preparing methanol extract, 450 ml of 95% methanol was used. The extraction process was done after 48 h. Finally, obtained water and alcoholic extracts were concentrated, frozen, dried, and stored at –20 °C.

Determination of total phenolic and total flavonoid content. The total phenolic content of hot water, cold water, and alcoholic AA extracts was detected by the Folin-Ciocalteu method (Vinatoru et al. 1997; Dilshad et al. 2016). The total flavonoid content of hot water, cold water, and alcoholic AA was determined by the aluminum chloride ($AlCl_3$) colorimetric method (Vinatoru et al. 1997; Chang et al. 2002; Dilshad et al. 2016).

Antioxidant activity assays. The antioxidant activities of hot water, cold water, and alcoholic AA extracts were determined using DPPH (2',2'-diphenyl-1-picrylhydrazyl) and FRAP (ferric ion reducing antioxidant power) assays. The scavenging capacity of DPPH free radical (IC_{50}) was measured by using a previously published method (Ydjedd et al. 2017; Xiao et al. 2019). The FRAP value of extracts of AA assay was detected according to the published method (Xiao et al. 2019).

Assessment of oxidative stress markers. In the deep anesthesia, all the animals were sacrificed by decapitation. The liver tissue of mice was removed and frozen at –80 °C until specific analysis. The liver tissue was homogenized and then centrifuged at 4 °C for 20 min at 2000xg. The obtained liver supernatants were used for MDA, ROS, and GSH measurements.

ROS content was quantified by 2',7'dichlorofluorescin diacetate (DCFH-DA). Briefly, 20 µl of DCFH-DA was added to 2000 µl of liver tissue homogenate. The obtained mixture was kept at low temperature (4 °C) for 15 min. Then, the levels of ROS were assessed by reading the absorbance of the color at 312 nm and 420 nm (Lund et al. 1993).

Content of GSH was determined by 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) as the indicator of spectrophotometry method. Briefly, 0.1 ml of the liver tissue homogenate was added into 0.1 mol/l of phosphate buffer and 0.04% DTNB in a total volume of 0.3 ml (pH 7.4). Then, yellow color of samples gets red at 412 nm on a spectrophotometer (UV-1601 PC, Shimadzu, Japan). GSH content was expressed as nM and calculated using the draw standard curve (Jamshidi et al. 2018; Shaki et al. 2021).

Content of MDA, an end product of lipid peroxidation, was measured using Shaki et al. (2021) method. Briefly, 0.1 ml of thiobarbituric acid (TBA) was added to 0.2 ml of liver tissue homogenate. All the supernatants were placed in a boiling water bath for 30 min. Finally, the micro tubes were shifted to an ice-bath to decrease temperature for 3 min and 0.2 ml n-butanol was added to each micro tube. Then, all tubes were centrifuged at 3500 rpm for 10 min. The content of MDA formed in each micro tube was assessed by reading the absorbance of the color at 532 nm. Tetramethoxypropane ($TBAR_S$) was used as standard, and amount of MDA was expressed as nmol/mg and calculated using the draw standard curve (Jamshidi et al. 2018; Shaki et al. 2021).

Statistical analysis. The results were reported as mean±standard error of the mean (mean±SEM). The data were analyzed by GraphPad Prism 8 statistical software using one-way ANOVA analysis followed by the post hoc Tukey test. The significance level was set at $p<0.05$. The Kolmogorov-Simonov normality test was used for variables in this study.

Results

The effect of hot-water, cold-water, and alcoholic extracts of AA on body weight in T2DM mice. During the experiment period, the body weight of all animals was measured at the end of each week. Comparison of results showed that during the first 8 weeks, the body weight of mice in all diabetic groups under high-fat diet was remarkable higher than in mice of the control group fed with normal diet ($p<0.05–0.001$) (Figure 1). After that, all animals of untreated or treated diabetic groups were fed with a normal diet and their body weight showed a declined trend. At the end of 9, 10, 11 weeks, the body weight of mice in the untreated diabetic group was significantly higher than in mice of the control group ($p<0.05–0.01$). The body weight of mice in all diabetic groups treated with AA decreased to a normal range (control group level) (Figure 1).

The effect of hot-water, cold-water, and alcoholic extracts of AA on serum levels of glucose in T2DM mice. Serum glucose levels significantly increased in diabetic mice in comparison with the control group ($p<0.0001$). Four-week treatment of diabetic mice with metformin and/or 100, 200 or 400 mg/kg doses of hot-water, cold-water, and alcoholic AA extracts significantly decreased the serum glucose in comparison with the untreated diabetic group ($p<0.0001$) (Table 1).

Total phenolic and flavonoids content. The total phenolic content of hot-water, cold-water and alcoholic extract of AA was 142.742±0.413, 108.492±5.984, and 68.908±3.263 mg gallic acid equivalents (GAE) per gram of plant dry extract, respectively. Total flavonoids content of hot-water, cold-water, and alcoholic extract of AA was 24.937±0.413, 7.228±0.325, and 33.583±0.548 mg quercetin equivalents per gram of dry extract, respectively.

Ferric reducing antioxidant power (FRAP) and DPPH free radical scavenging activity. FRAP value for hot-water, cold-water, and alcoholic extract was 7.917±0.149 µg/ml, 4.427±0.016 µg/ml, and 6.088±0.014 µg/ml, respectively. DPPH free radical scavenging activity (IC_{50} value) for the hot-water extract group showed no significant difference compared to the control group (vit C). While IC_{50} value of the cold-water and alcoholic AA extracts showed a significant increase compared to the control group (both $p<0.001$) (Figure 2).

The effect of hot-water, cold-water, and alcoholic AA extracts on ROS of T2DM mice liver. The induction of T2DM in mice significantly increased ($p<0.0001$) the ROS production in liver tissues and metformin treatment significantly decreased ($p<0.0001$) the ROS levels in liver tissues in diabetic mice (Figure 3). Treatment of diabetic mice with all doses of AA extracts significantly reduced the ROS levels ($p<0.0001$) compared to the T2D group, except the dose of 100 mg/kg of cold-water AA extract. Doses of 100 and 200 mg/kg of hot-water, doses of 200 and 400 mg/kg of cold-water, and alcoholic AA extracts showed similar effectiveness to metformin. However,

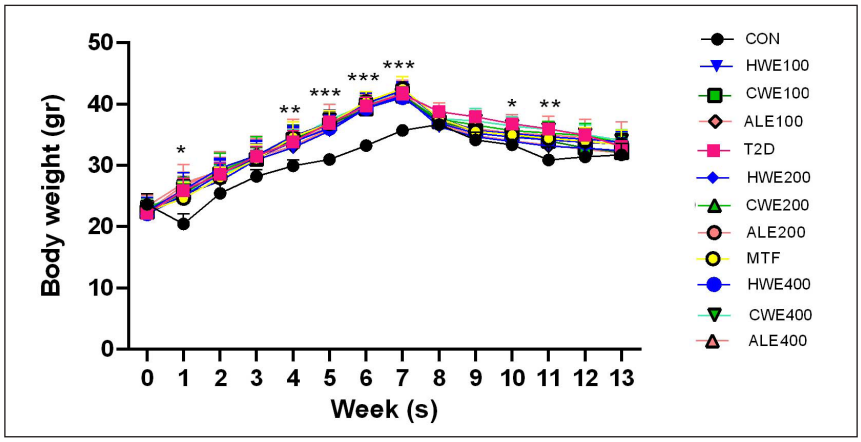

Figure 1. The effects of metformin and different doses (100, 200 or 400 mg/kg) of hot-water, cold-water, and alcoholic extracts of *Artemisia annua* L. (AA) on body weights in HFD/STZ-induced diabetic mice. Data are expressed as the mean±SEM (n=5). *p<0.05, **p<0.01, ***p<0.001 vs. CON group. Abbreviations: HFD – high-fat diet; STZ – streptozotocin; CON – control group; T2D – type 2 diabetes group; T2D+MTF – T2D+metformin group; T2D+HWE – T2D+hot-water AA extract group; T2D+CWE – T2D+cold-water AA extract group; T2D+ALE – T2D+alcoholic AA extract group.

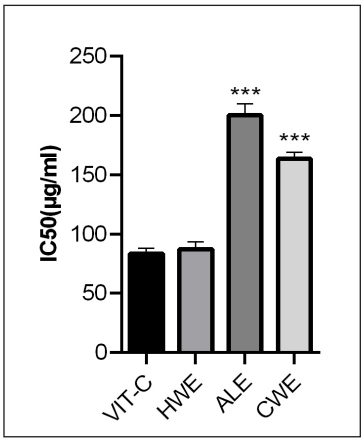

Figure 2. Comparison of DPPH (IC50) value in groups of hot-water, cold-water, and alcoholic extracts of *Artemisia annua* L. with control group (vit C). Data are expressed as the mean±SEM. ***p<0.001 vs. vit C group.

400 mg/kg dose of hot-water AA extract showed higher significancy in comparison to metformin ($p<0.0001$) (Figure 3).

A comparison of the doses used among 3 different extracts showed that the efficacy of hot-water extract in low production of ROS was significantly higher than that of two other extracts at dose of 100 mg/kg ($p<0.01$), more than that of cold-water at dose of 200 mg/kg ($p<0.05$), and more than that of two other extracts at dose of 400 mg/kg ($p<0.01$) (Figure 3).

The effect of hot-water, cold-water, and alcoholic AA extracts on GSH production in T2DM mice liver. GSH production in liver tissues after induction of diabetes in mice was significantly reduced in comparison with the diabetic group ($p<0.0001$); while after treatment with metformin and AA extracts (100, 200 or 400 mg/kg), GSH levels significantly increased compared to the diabetic mice ($p<0.0001$) (Figure 4). The effects of 100 and 200 mg/kg doses of hot-water, 200 and 400 mg/kg doses of cold-water, and 100 mg/kg dose of alcoholic extracts in GSH production of liver tissues were similar to that of metformin; while, the effect of 400 mg/kg dose of hot-water ($p<0.05$) and 200 and 400 mg/kg of alcoholic AA extracts were higher than in the metformin group ($p<0.05$, $p<0.0001$, respectively). The effect of 100 mg/kg dose

Table 1
The effects of metformin and different doses (100, 200 or 400 mg/kg) of hot-water, cold-water, and alcoholic extracts of *Artemisia annua* L. (AA) on the glucose serum levels in HFD/STZ-induced diabetic mice

Group	Glucose (mg/dl)
CON	98.00±5.42
T2D	272.6±5.9[a]
T2D+MTF	115.6±5.9[b]
T2D+HWE100	156.80±7.24[b]
T2D+HWE200	109.2±7.7[b]
T2D+HWE400	108.2±5.6[b]
T2D+CWE100	152.40±10.73[bc]
T2D+CWE200	196.0±9.7[b]
T2D+CWE400	160.6±16.6[b]
T2D+ALE100	142.2±15.5[b]
T2D+ALE200	106.20±7.93[b]
T2D+ALE400	101.2±8.3[b]

Data are expressed as the mean±SEM (n=5). [a]$p<0.0001$ vs. CON group; [b]$p<0.0001$ vs. T2D group; [c]$p<0.0001$ vs. T2D+MTF group. Abbreviations: HFD – high-fat diet; STZ – streptozotocin; CON – control group; T2D – type 2 diabetes group; T2D+MTF – T2D+metformin group; T2D+HWE – T2D+hot-water AA extract group; T2D+CWE – T2D+cold-water AA extract group; T2D+ALE – T2D+alcoholic AA extract group.

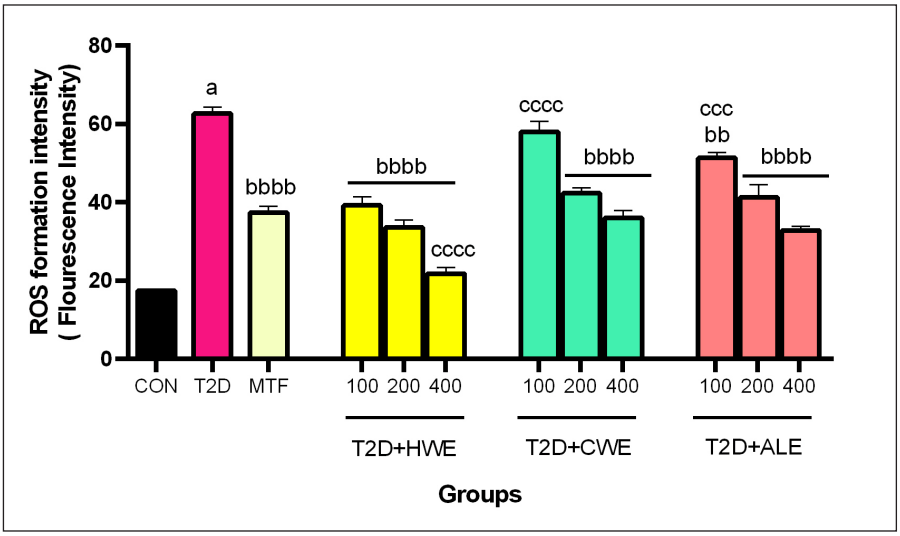

Figure 3. The effects of metformin and different doses (100, 200 or 400 mg/kg) of hot-water, cold-water, and alcoholic extracts of *Artemisia annua* L. (AA) on ROS levels in HFD/STZ-induced diabetic mice. Data are expressed as the mean±SEM (n=5). [a]$p<0.0001$ vs. CON group; [bb]$p<0.01$ and [bbbb]$p<0.0001$ vs. T2D group; [ccc]$p<0.001$ and [cccc]$p<0.0001$ vs. MTF group. Abbreviations: HFD – high-fat diet; ROS – reactive oxygen species; STZ – streptozotocin; CON – control group; T2D – type 2 diabetes group; MTF group – metformin group; T2D+MTF – T2D+metformin group; T2D+HWE – T2D+hot-water AA extract group; T2D+CWE – T2D+cold-water AA extract group; T2D+ALE – T2D+alcoholic AA extract group.

of cold-water AA extract on GSH production was significantly lower compared to the metformin group ($p<0.0001$) (Figure 4).

A comparison of the doses used among 3 different extracts showed that the efficacy of alcoholic AA extract in the production of GSH reached a higher significance than that of hot-water ($p<0.01$) and cold-water ($p<0.001$) AA extracts; at the dose of 100 mg/kg, more than that of hot-water extract at the dose of 200 mg/kg ($p<0.01$), and hot-water ($p<0.05$) and cold-water ($p<0.01$) extracts at the dose of 400 mg/kg ($p<0.01$). The effectiveness of hot water extract was also significantly higher than that of cold-water extract at doses of 100 and 400 mg/kg ($p<0.01$) (Figure 4).

The effect of hot-water, cold-water, and alcoholic AA extracts on lipid peroxidation of T2DM mice liver. The MDA levels, the end product of lipid peroxidation, were significantly increased in diabetic mice compared to the control group ($p<0.0001$), while metformin treatment and all doses of AA extracts decreased MDA levels in T2DM mice ($p<0.0001$) (Figure 5). The effects of the 100 mg/kg dose of hot-water extract, 100, 200 and 400 mg/kg doses of cold-water extract and 100 mg/kg dose of alcoholic AA extract were similar to that of metformin, while the effects of 200 and 400 mg/kg of hot-water extract ($p<0.01$, $p<0.0001$, respectively) and 200 and 400 mg/kg ($p<0.0001$) of alcoholic AA extract were significantly higher than that of metformin (Figure 5).

A comparison of the all doses used among 3 different AA extracts showed that at dose of 100 mg/kg, the efficacy of alcoholic and hot-water extracts in low production of MDA were significantly higher than that of cold-water extract ($p<0.01$). At the dose of 200 mg/kg, the effectiveness of alcoholic extract was higher than that of hot-water ($p<0.05$) and cold-water ($p<0.01$) extracts. At the dose of 400 mg/kg, the effectiveness of hot-water extract was higher than that of cold-water extract ($p<0.05$), and the effectiveness of the alcoholic AA extract was higher than that of cold-water extract ($p<0.05$) (Figure 5).

Discussion

In the present study, administration of AA extracts and metformin for 4 weeks significantly improved the oxidative stress-induced liver damage created by T2DM in mice. In the current study, in agreement with the previous studies, we showed an increase in blood glucose in T2DM mice induced by a high-fat diet and a single dose of STZ injection, which confirmed the accuracy of the study (Deayu Putri et al. 2020).

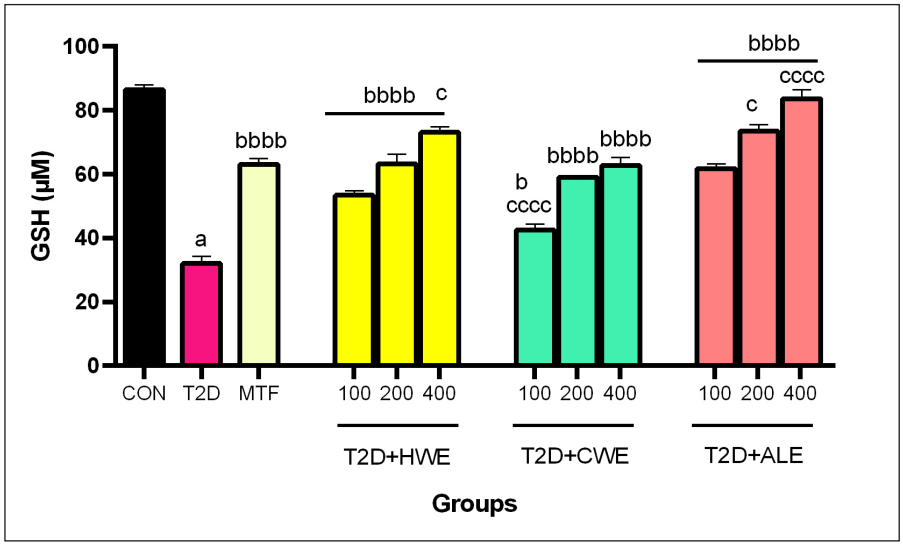

Figure 4. The effects of metformin and different doses (100, 200 or 400 mg/kg) of hot-water, cold-water, and alcoholic extracts of *Artemisia annua* L. (AA) on GSH levels in HFD/STZ-induced diabetic mice. Data are expressed as the mean±SEM (n=5). [a]$p<0.0001$ vs. CON group; [b]$p<0.05$ and [bbbb]$p<0.0001$ vs. T2D group; [c]$p<0.05$ and [cccc]$p<0.0001$ vs. MTF group. Abbreviations: GSH – glutathione; HFD – high-fat diet; STZ – streptozotocin; CON – control group; T2D – type 2 diabetes group; MTF group – metformin group; T2D+MTF – T2D+metformin group; T2D+HWE – T2D+hot-water AA extract group; T2D+CWE – T2D+cold-water AA extract group; T2D+ALE – T2D+alcoholic AA extract group.

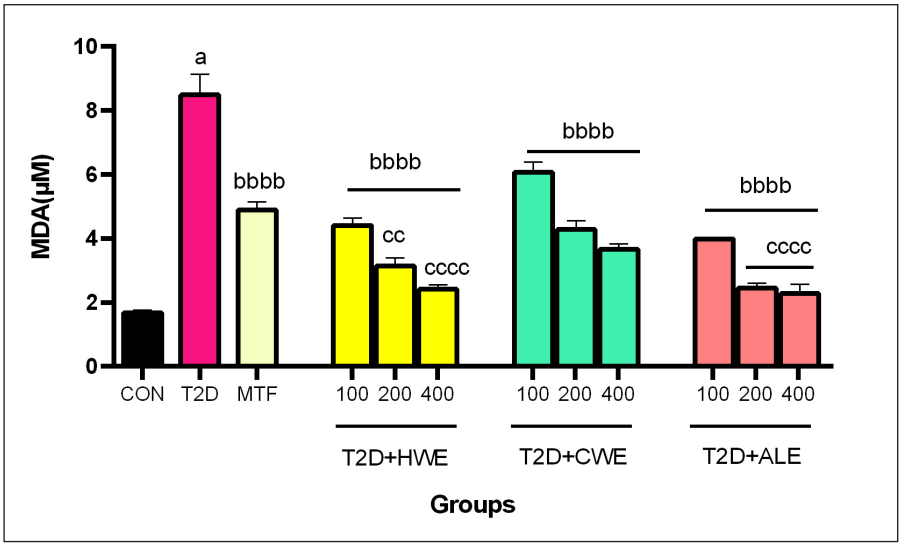

Figure 5. The effects of metformin and different doses (100, 200 or 400 mg/kg) of hot-water, cold-water, and alcoholic extracts of *Artemisia annua* L. (AA) on MDA levels in HFD/STZ-induced diabetic mice. Data are expressed as the mean±SEM (n=5). $^{a}p<0.0001$ vs. CON group; $^{bbbb}p<0.0001$ vs. T2D group; $^{cc}p<0.01$ and $^{cccc}p<0.0001$ vs. MTF group. Abbreviations: MDA – malondialdehyde; HFD – high-fat diet; STZ – streptozotocin; CON – control group; T2D – type 2 diabetes group; MTF group – metformin group; T2D+MTF – T2D+metformin group; T2D+HWE – T2D+hot-water AA extract group; T2D+CWE – T2D+cold-water AA extract group; T2D+ALE – T2D+alcoholic AA extract group.

Dysfunction of liver by oxidative stress induced by hyperglycemia affects many endogenous organs (Yazdi et al. 2019). It has been reported that the most common outcome of liver damage following oxidative stress is the disturbance in carbohydrates, proteins, and lipid metabolism in diabetic patients (Mohamed et al. 2016; Yazdi et al. 2019; Albasher et al. 2020). The results of the present study showed that treatment of diabetic mice with doses of 100, 200 and 400 mg/kg of AA extracts improved the oxidative stress markers such as ROS, MDA (decreasing production), and GSH (increasing production) in the liver tissue and improved hyperglycemia. These improving effects were hierarchical and dose-depend. Higher doses of the extracts showed an efficacy similar to or even higher than that of metformin in improving the liver oxidative markers. Beneficial action of hot-water, cold-water, and alcoholic AA extracts might be due to presence of antioxidant effects of AA, especially by terpenoids, phenols, and flavonoids as shown in the results of another studies (Yazdi et al. 2019; Kim et al. 2020).

The effectiveness of hot-water AA extract in reducing ROS production was higher than that of other AA extracts. The lower amount of IC_{50} and also the higher value of FRAP in hot-water AA extract confirmed the higher antioxidant activity of hot-water AA extract in comparison with the other AA extracts. In addition, the presence of more phenols in the hot-water AA extract also confirms the more antioxidant content in this extract (Song et al. 2016). Phenol is one of the major groups of phytochemicals that can be found in the medicinal plants. Phenolic compounds are powerful antioxidants and free radical scavengers that can act as hydrogen donors, reducing agents, metal chelators, and singlet oxygen quenchers (Chew et al. 2011; Song et al. 2016).

The effectiveness of alcoholic AA extract in increasing the GSH production was higher than that of other AA extracts. This effect might be due to higher amount of total flavonoids content in the alcoholic AA extract. Flavonoids are phenolic compounds, which are present in a wide variety of medicinal plants. Phenolic compounds are predominant antioxidants that reduce free radical production and increase scavenging free radicals (Afolayan and Sunmonu 2013; Wan et al. 2016).

In agreement with the results of the present study, it has been reported that water extract of AA showed a hepatoprotective effect in an *in vitro* experimental system as amelioration of GSH levels suppressed by organic peroxidase and normalizing ROS production

induced by oxidative stress (Iqbal et al. 2012; Choi et al. 2020).

The results of the present study showed that the effectiveness of hot-water and alcoholic AA extracts in reducing MDA production in liver tissue in diabetic mice was approximately the same. This effect may be due to high level of the total phenol and flavonoids content in these extracts (Gouveia and Castilho 2013; Wan et al. 2016). It has been reported that hot and cold extract of *Artemisia* fruit include several secondary metabolites such as phenols, flavonoids, terpenes, and alkaloids, which have hepatoprotective and antidiabetic potentials leading to restoring and normalizing of insulin and glucose in diabetes (Ghaffari et al. 2012).

Conclusion

Artemisia annua L. showed an antioxidant potential in forms of its extracts prepared in hot water, cold water, and methanol solvents. This effect can be attributed to the liver antioxidant enzymes activation and free radical scavenging activity of AA extracts. It seems that higher doses of AA extracts (between 200 and 400 mg/kg body weight) have a more antioxidant activity in treatment of T2DM in mice when compared with the efficacy of a standard drug such as metformin. In addition, hot-water and alcoholic extracts indicated a higher antioxidant capacity and were more effective in improving oxidative stress markers and accordingly hyperglycemia in HFD/STZ T2DM mice. Hot water may open the cell walls of plants and can release more effective substances and hence it showed higher effectiveness than the cold-water extract.

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References

Afolayan AJ, Sunmonu TO. Protective role of Artemisia afra aqueous extract on tissue antioxidant defense systems in streptozotocin-induced diabetic rats. Afr J Tradit Complement Altern Med 10, 15–20, 2013.

Albasher G, Alwahaibi M, Abdel-Daim MM, Alkahtani S, Almeer R. Protective effects of Artemisia judaica extract compared to metformin against hepatorenal injury in high-fat diet/streptozotocine-induced diabetic rats. Environ Sci Pollut Res Int 27, 40525–40536, 2020.

Aouacheri O, Saka S, Krim M, Messaadia A, Maidi I. The investigation of the oxidative stress-related parameters in type 2 diabetes mellitus. Can J Diabetes 39, 44–49, 2015.

Bellou V, Belbasis L, Tzoulaki I, Evangelou E. Risk factors for type 2 diabetes mellitus: An exposure-wide umbrella review of meta-analyses. PLoS One 13, e0194127, 2018.

Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 10, 178–182, 2002.

Chew YL, Chan EW L, Tan PL, Lim YY, Stanslas J, Goh JK. Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of Leguminosae medicinal plants in Peninsular Malaysia. BMC Complement Altern Med 11, 12, 2011.

Choi EY, Choi JO, Park CY, Kim SH, Kim D. Water extract of Artemisia annua L. exhibits hepatoprotective effects through improvement of lipid accumulation and oxidative stress-induced cytotoxicity. J Med Food 23, 1312–1322, 2020.

Das S. Artemisia annua (Qinghao): a pharmacological review. Int J Pharm Sci Res 3, 4573–4577, 2012.

Deayu Putri M, Wiboworini B, Dirgahayu P. The effect of strawberry on type 2 diabetes mellitus: A review. Int J Nutr Sci 5, 1–6, 2020.

Dilshad E, Zafar S, Ismail H, Waheed MT, Cusido RM, Palazon J, Mirza B. Effect of rol genes on polyphenols biosynthesis in Artemisia annua and their effect on antioxidant and cytotoxic potential of the plant. Appl Biochem Biotechnol 179, 1456–1468, 2016.

Eltayef EM, Jouda J, Saeed MK, Khadem AAJ. Artemisia L. fruit extract normalized diabetic and liver function parameters in streptozotocin-induced diabetic mice. Ann Tropical Med Public Health 23, 134–139, 2020.

Gao J, Cheng Y, Hao H, Yin Y, Xue J, Zhang Q, Li L, Liu J, Xie Z, Yu S. Decitabine assists umbilical cord-derived mesenchymal stem cells in improving glucose homeostasis by modulating macrophage polarization in type 2 diabetic mice. Stem Cell Res Ther 10, 259, 2019.

Ghaffari H, Ghassam BJ, Prakash H. Hepatoprotective and cytoprotective properties of Hyptis suaveolens against oxidative stress-induced damage by CCl4 and H2O2. Asian Pac J Trop Med 5, 868–874, 2012.

Ghazanfar K, Ganai BA, Akbar S, Mubashir K, Dar SA, Dar MY, Tantry MA. Antidiabetic activity of Artemisia amygdalina Decne in streptozotocin induced diabetic rats. Biomed Res Int 2014, 185676, 2014.

Gouveia SC, Castilho PC. Artemisia annua L.: essential oil and acetone extract composition and antioxidant capacity. Indust Crops Products 45, 170–181, 2013.

Guven A, Yavuz O, Cam M, Ercan F, Bukan N, Comunoglu C, Gokce F. Effects of melatonin on streptozotocin-induced diabetic liver injury in rats. Acta Histochem 108, 85–93, 2006.

Iqbal S, Younas U, Chan KW, Zia-Ul-Haq M, Ismail M. Chemical composition of Artemisia annua L. leaves and antioxidant potential of extracts as a function of extraction solvents. Molecules 17, 6020–6032, 2012.

Jabir FA, Shaker AS. Roles of superoxide dismutase (SOD), malondialdehyde (MDA), 8-iso-prostaglandin F2α (8-iso-PGF2α) as oxidative stress in development and progression of breast cancer in Iraqi females patients. Al-Qadisiyah J Pure Sci 25, 1–4, 2020.

Jamshidi HR, Zeinabady Z, Zamani E, Shokrzadeh M. Attenuation of diabetic nephropathy by carvacrol through anti-oxidative effects in alloxan-induced diabetic rats. Res J Pharmacognosy 5, 57–64, 2018.

Jung UJ, Baek NI, Chung HG, Bang MH, Yoo JS, Jeong TS, Lee KT, Kang YJ, Lee MK, Kim HJ, Yeo JY, Choi MS. The anti-diabetic effects of ethanol extract from two variants of Artemisia princeps Pampanini in C57BL/KsJ-db/db mice. Food Chem Toxicol 45, 2022–2029, 2007.

Kang YJ, Jung UJ, Lee MK, Kim HJ, Jeon SM, Park YB, Chung HG, Baek NI, Lee KT, Jeong TS, Choi MS. Eupatilin, isolated from Artemisia princeps Pampanini, enhances hepatic glucose metabolism and pancreatic beta-cell function in type 2 diabetic mice. Diabetes Res Clin Pract 82, 25–32, 2008.

Kennedy L, Sandhu JK, Harper ME, Cuperlovic-Culf M. Role of glutathione in cancer: From mechanisms to therapies. Biomolecules 10, 1429, 2020.

Kim HH, Vetrivel P, Ha S, Bhosale P B, Lee HJ, Kim JA, Park KI, Kim SM, Kim GS. Functions of flavonoids in three Korean native varieties of Artemisia species. J Biomed Transl Res (JBTR) 21, 39–49, 2020.

Komeili G, Ghasemi F, Rezvani AR, Ghasemi K, Khadem Sameni F, Hashemi M. The effects of a new antidiabetic glycinium [(pyridine-2, 6-dicarboxylato) oxovanadate (V)] complex in high-fat diet of streptozotocin-induced diabetic rats. Arch Physiol Biochem 128, 80–86, 2019.

Lund BO, Miller DM, Woods JS. Studies on Hg (II)-induced H2O2 formation and oxidative stress in vivo and in vitro in rat kidney mitochondria. Biochem Pharmacol 45, 2017–2024, 1993.

Messaili S, Colas C, Fougere L, Destandau E. Combination of molecular network and centrifugal partition chromatography fractionation for targeting and identifying Artemisia annua L. antioxidant compounds. J Chromatogr A 1615, 460785, 2020.

Mohamed J, Nafizah AN, Zariyantey A, Budin S. Mechanisms of diabetes-induced liver damage: the role of oxidative stress and inflammation. Sultan Qaboos Univ Med J 16, e132–141, 2016.

Nofal SM, Mahmoud SS, Ramadan A, Soliman G, Fawzy R. Anti-diabetic effect of Artemisia judaica extracts. Res J Med Sci 4, 42–48, 2009.

Ogbonna C, Ogbonna A, Itelima J, Umar A, Onyezili N, Istifanus M. Combined Anti-diabetic effects of extracts of Artemisia annua var. chiknensis (CBGE/CHNA/09/LTNGS/G) and each of three other plants (Momordica charantia Linn. Vernonia amygdalina Del. and Aegle marmelos Correa) traditionally used in Nigeria for the treatment of diabetes. J Sci Res Rep 16, 1–12, 2017.

Shaki F, Teymoori M, Motafeghi FS, Hemmati N, Arab-Nozari M. L-arginine ameliorated mitochondrial oxidative damage induced by sub-chronic exposure to cadmium in mice kidney. Pharmaceut Biomed Res 7, 79–86, 2021.

Shokrzadeh M, Zamani E, Mehrzad M, Norian Y, Shaki F. Protective effects of propofol against methamphetamine-induced neurotoxicity. Toxicol Int 22, 92–99, 2015.

Song Y, Desta KT, Kim GS, Lee SJ, Lee WS, Kim YH, Jin JS, Abd El-Aty A, Shin HC, Shim JH. Polyphenolic profile and antioxidant effects of various parts of Artemisia annua L. Biomed Chromatogr 30, 588–595, 2016.

Taile J, Arcambal A, Clerc P, Gauvin-Bialecki A, Gonthier MP. Medicinal plant polyphenols attenuate oxidative stress and improve inflammatory and vasoactive markers in cerebral endothelial cells during hyperglycemic condition. Antioxidants 9, 573, 2020.

Teow CC, Truong VD, McFeeters RF, Thompson RL, Pecota KV, Yencho GC. Antioxidant activities, phenolic and β-carotene contents of sweet potato genotypes with varying flesh colours. Food Chemistry 103, 829–838, 2007.

Vinatoru M, Toma M, Radu O, Filip P, Lazurca D, Mason T. The use of ultrasound for the extraction of bioactive principles from plant materials. Ultrason Sonochem 4, 135–139, 1997.

Wan X, Niu Y, Zheng X, Huang Q, Su W, Zhang J, Zhang L, Wang T. Antioxidant capacities of Artemisia annua L. leaves and enzymatically treated Artemisia annua L. in vitro and in broilers. Anim Feed Sci Technol 221, 27–34, 2016.

Xiao JQ, Liu WY, Sun HP, Li W, Koike K, Kikuchi T, Yamada T, Li D, Feng F, Zhang J. Bioactivity-based analysis and chemical characterization of hypoglycemic and antioxidant components from Artemisia argyi. Bioorg Chem 92, 103268, 2019.

Yazdi HB, Hojati V, Shiravi A, Hosseinian S, Vaezi G, Hadjzadeh MAR. Liver dysfunction and oxidative stress in streptozotocin-induced diabetic rats: protective role of Artemisia turanica. Journal Pharmacopuncture 22, 109–114, 2019.

Ydjedd S, Bouriche S, Lopez-Nicolas RN, Sanchez-Moya T, Frontela-Saseta C, Ros-Berruezo G, Rezgui F, Louaileche H, Kati DE. Effect of in vitro gastrointestinal digestion on encapsulated and nonencapsulated phenolic compounds of carob (Ceratonia siliqua L.) pulp extracts and their antioxidant capacity. J Agric Food Chem 65, 827–835, 2017.