European Journal of Medicinal Plants

20(2): 1-9, 2017; Article no.EJMP.35134
ISSN: 2231-0894, NLM ID: 101583475

The Effect of Hydroalcoholic Extract of *Ferulago angulata* on Liver Function Parameters and Antioxidant Status in Alloxan-Induced Diabetic Rats

Seyedeh-Fatemeh Mousavi-Ezmareh[1], Mohammad Mazani[1*], Esfandiar Heidarian[2], Reza Alipanah Moghadam[1], Mahmoud Rafieian-kopaei[3], Garshasp Mardanian[3], Lotfollah Rezagholizadeh[1] and Ali Shamsazar[4]

[1]Department of Biochemistry, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran.
[2]Clinical Biochemistry Research Center and Department of Clinical Biochemistry, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran.
[3]Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran.
[4]Department of Biology, Payame Noor University, Tehran, Iran.

Authors' contributions

This work was carried out in collaboration between all authors. Authors SFME, MM and EH designed the study, performed the statistical analysis and wrote the protocol. Authors RAM, MRK and GM managed the analyses of the study and literature searches. Author LR managed the literature searches, wrote the first draft of the manuscript, corrected the manuscript and approved the final submission. Author AS managed the literature searches and helped to write this article. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2017/35134
Editor(s):
(1) Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Maha Rizk, National Research Center, Egypt.
(2) Salwa Refat El-Zayat, National Research Centre, Egypt.
(3) Bhaskar Sharma, Suresh Gyan Vihar University, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/20530

Original Research Article

Received 29th June 2017
Accepted 10th August 2017
Published 17th August 2017

ABSTRACT

Aim: The aim of this study was to evaluate the hepatoprotective and antioxidant activities of *Ferulago angulata* Extract (FAE) in experimental diabetic rats.
Materials and Methods: 54 adult male Wistar rats divided into 6 groups (n=9). Diabetes was induced in all animals except those in group 1 by the daily intraperitoneal injection of 120 mg/kg

**Corresponding author: E-mail: mohammad.mazani@gmail.com;*
E-mail: reza34055@gmail.com;

alloxan monohydrate for 3 consecutive days. Experimental diabetic rats in groups 3-5 were orally administered with FAE (200,400, and 800 mg/kg/day, respectively). Group 6 was treated with 150 mg/kg of metformin. At the end of week 4, the rats were anesthetized and then sacrificed by cardiac puncture. Then, the levels of liver markers, malondialdehyde (MDA), and antioxidant enzymes capacity were evaluated in each group.

Results: Treatment with FAE resulted in a significant reduction in aspartate transaminase (AST) and alanine transaminase (ALT) activities as well as in the serum and liver tissue contents of MDA in comparison to the diabetic control group ($P<0.001$). The FAE-treated diabetic rats showed a significant increase in catalase, glutathione peroxidase (GPx), and super oxide dismutase (SOD) activities of the liver (P-values were dose-dependent). Furthermore, the extract has an ameliorative effect on the histopathological changes of the liver in alloxan induced diabetes.

Conclusions: These findings suggest that FAE can reduce the complications of diabetes, prevent oxidative stress, and improve antioxidant status in diabetic rats.

Keywords: Ferulago angulata; antioxidant; oxidative stress; diabetes mellitus.

1. INTRODUCTION

Diabetes Mellitus (DM), a major worldwide health problem, is a chronic metabolic disease that is characterized by increased concentration of glucose in the blood, resulting from the defects in insulin secretion, insulin action, or both [1,2]. It has been found that diabetes-induced hyperglycemia can cause various structural and metabolic problems in many body organs such as kidney, muscle, nerve, etc. [3].

One of the major complications of diabetes is its effect on the liver and hepatocytes [4,5]. In liver, diabetes can lead to glycogen deposition, steatosis and nonalcoholic steatohepatitis (NASH), fibrosis and cirrhosis, biliary disease, cholelithiasis, cholecystitis, and other related complications [6]. There has been extensive research regarding the liver disease occurring as a consequence of diabetes mellitus. For example, Wild et al. showed that type 2 DM is related to the increased risk associated with different types of liver diseases [7] and Hazlehurst et al. demonstrated that non-alcoholic fatty liver disease (NAFLD) is prevalent among up to 70% of the patients with diabetes [8]. Several experimental studies suggest that increased production of reactive oxygen species (ROS) in diabetes plays an important role in the onset of hyperglycemia-related liver damage [9]. However, antidiabetic medications are now used for the treatment of diabetic patients many of which are incapable of treating the complications caused by diabetes such as liver disease. Furthermore, due to the serious adverse effects of synthetic drugs, using these medications for long periods of time has now been limited [10]. Therefore, medicinal plants which have traditionally been used for herbal remedies are now used for the treatment of many diseases and illnesses.

Ferulago angulata (Schlecht) Boiss., belongs to the Apiaceae, is a medicinal plant of West Asia, which contains essential oils and has many therapeutic uses in traditional medicine [11]. The phenolic and flavonoids compounds of *Ferulago angulata* extract (FAE) exhibit antioxidant, antimicrobial and anti-cancer properties [12,13]. The lipid-lowering effects and lipid peroxidation-decreasing properties of this plant have been shown [10].

In the previous study, we showed the beneficial effects of FAE on lipid profile and blood sugar [14]. However, we could not find any studies on the hepatoprotective and antioxidants effects of FAE in experimental diabetes mellitus. Therefore, in the present study, we aimed to evaluate the liver enzymes and antioxidant effects of FAE in alloxan-induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Animals

Healthy adult male Wistar albino rats with an average weight of 200-250 g were purchased from Pasture Institute, Tehran, Iran and housed in standard and clean cages (2 per cage) under controlled environmental conditions at room temperature of 22 ± 3℃ and in a 12:12 light–dark cycle with free access to a standard rat chow and clean water [5]. The animal experiments ethics committee approved the study (Approval number IR.ARUMS.REC.1394.129).

2.2 Diabetes Induction

Diabetes was induced in rats by the intraperitoneal (i.p.) injection of fresh solution of alloxan monohydrate (Sigma Ltd., USA) (120 mg/kg) for 3 days [15]. Elevated glucose level (>300 mg/dl) in the blood from the tail vein (OKmeter Match, Taiwan) on days 3 and 7 after the injection of alloxan was taken as the confirmation of diabetes [16]. Normal rats were injected with an equal volume of vehicle (sodium citrate buffer).

2.3 Preparation of Plant Extract

The plant samples were collected at the humidity level of 45-60% in May and June from Shahr-e Kord, the capital of Chaharmahal and Bakhtiari Province, Iran. The whole plant of FAE was shade-dried at room temperature and powdered in a Wiley mill. Fifty grams of powdered herb was solved in 200 ml of equal volume (1:1) of water and methanol for 72 hours at 15℃ to 20℃. The extract was filtered and placed in a vacuumed rotary evaporator solvent [17]. Then, it was incubated at the temperature of 40℃ for 2 days. The lyophilized powder was stored in a dry and cold place. Then, appropriate amounts of the lyophilized extract were weighed and orally gavaged to each rat according to their weekly body weights for 4 weeks on a daily basis.

2.4 Experimental Groups and Treatment

Control and diabetic animals were divided into 6 groups of at least 9 animals each and treated by an appropriate regimen for 4 weeks as follows:

Group I : Normal control rats.
Group II : Diabetic control.
Group III : Diabetic rats treated with hydroalcoholic extract of the whole plant of FAE (200 mg/kg).
Group IV : Diabetic rats treated with hydroalcoholic extract of the whole plant of FAE (400 mg/kg).
Group V : Diabetic rats treated with hydroalcoholic extract of the whole plant of FAE (800 mg/kg).
Group VI : Diabetic rats treated with metformin (150 mg/kg).

Rats in group I and II received normal rat regimen. Groups III-V treated with 200, 400, and 800 mg/kg hydroalcoholic extract of FAE respectively. In group VI, the standard anti-hyperglycemic drug (metformin) was administered once daily through oral gavage for 28 consecutive days.

After the last treatment, rats were fasted for 12 h and then anesthetized with chloroform. Blood samples were taken from all the experimental animals by heart puncture and the collected samples were allowed to clot for 30 min and centrifuged at 3000 RPM for 10 min to obtain serum in order to estimate the biochemical parameters. Slices of livers obtained from each animal were washed with isotonic saline solution and homogenized. Then, aliquots were immediately frozen in liquid nitrogen and stored at −80℃ until the measurement of parameters such as catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) activities, and malondialdehyde (MDA) concentration.

2.5 Phytochemical Screening

The hydroalcoholic extract of FAE was analyzed for the presence of phenolic compounds and total flavonoids. Phenolic compounds was quantified using Folin-Ciocalteau's assay [18] with some differences. The assay mixture was made with 100 µl of sample, 0.5 ml of 10% Folin-Ciocalteau's reagent, and 0.4 ml of sodium bicarbonate 7.5% solution. Gallic acid was used as the standard in the concentrations of 0.25, 50, 75, 100, and 125 PPM in methanol 60% solution. The absorbance was measured spectrophotometrically against a blank at 725 nm after the incubation of assay mixture for 30 min.

Total flavonoids measurement of flavonoid concentration in hydroalcoholic extract of FAE was done based on the method described by Dowd using the aluminum chloride colorimetric protocol [19]. A 0.5 ml extract was mixed with methanol (1.5 ml), to which 2% aluminum chloride (1 ml), 5% potassium acetate (6 ml), and deionized water (2.8 ml) were added. The solution was vortexed and allowed to sit for 40 minutes at room temperature. After that, the absorbance was measured at 415 nm using a spectrophotometer. The total flavonoid content was quantified according to the standard curve prepared with gallic acid and the concentrations of flavonoids were reported as mg per gram of extract.

2.6 Determination of Biochemical Parameters

The liver biomarkers (AST and ALT) were analyzed using an auto-analyzer (BT 3000, Italy)

by commercially available kits from Pars Azmoon (Pars Azmoon Inc., Tehran, Iran).

2.7 Determination of Lipid Peroxides in Plasma and Liver

MDA (as a lipid peroxidation marker) level in serum and liver homogenate was measured as described previously by Agarwal et al. [20] using a reverse phase high-pressure liquid chromatography (HPLC) (Agilent 1100 Series HPLC system) after derivatization with 2, 4-dinitrophenylhydrazine were purchased from Merck, Germany.

2.8 Assay of Catalase

Catalase was assayed according to the method described previously by Hugo Aebi [21]. The assessment was done spectrophotometrically following the decrease in absorbance at 240 nm. 1 g of the liver tissue was homogenized in 50 mM phosphate buffer (pH 7.0) at the temperature of 4℃ and then centrifuged at 4500 RPM. The reaction mixture contained 0.01 M phosphate buffer (pH 7.0), 2 mM Hydrogen peroxide (Merck, Germany), and the enzyme extract. The specific activity of catalase was expressed in terms of unit/mg protein.

2.9 Assay of Superoxide Dismutase (SOD)

The specific activity of superoxide dismutase in liver homogenate was determined by the method proposed by Sun et al. [22]. The principle of the method is based on the inhibition of nitroblue tetrazolium (NBT) (Sigma) reduction by the xanthine/XO system as a superoxide generator. In so doing, 250 mg of tissue was homogenized in 4 ml Tris buffer (50 mM Tris, 225 mM sucrose, 1 mM EDTA, PH=74) 0.8 ml of assay reagent (containing 0.3 mM xanthine, 0.6 mM Na_2EDTA (All chemical were purchased from Merck), 0.15 mM nitroblue tetrazolium (NBT), and 0.4 M (Na_2CO_3) (Merck,) was mixed with 100 µl of liver homogenate and 100 µl Xanthine oxidase. Spectrophotometric absorbance was measured at 560 nm. Activity was expressed as unit/mg protein.

2.10 Assay of Glutathione Peroxidase (GPx)

To assay the GPx, 250 mg of tissue was homogenized in 4 ml Tris buffer (50 mM Tris, 225 mM sucrose, 1 mM EDTA, PH=74). 100 µl of tissue homogenate was mixed with 750 µl assay buffer (171 mM potassium phosphate buffer, 4.28 mM sodium azide, 2.14 mM EDTA, 6 mM reduced glutathione, and 0.9 mM NADPH) and 5 µl of glutathione reductase 2 U/mL. The reaction started with the addition of 150 µl of 0.72 mM H2O2. The absorbance was measured by spectrophotometer at 340 nm [23].

2.11 Histomorphological Studies

Some pieces of liver tissue were fixed in 40 g/L buffered formaldehyde, dehydrated with alcohol, embedded in paraffin, cut into 4-5 µm thick sections, and stained with hematoxylin and eosin (H&E) for general histopathological examinations according to a described procedure [24].

2.12 Statistical Analysis

Data were analyzed statistically using SPSS Software, Version 20 and the results were expressed as mean ± SEM. One-way analysis of variance (ANOVA) was used to compare different groups and determine overall effect of each treatment with the confidence level of 95% ($p<0.05$) considered to be significant.

3. RESULTS

3.1 Body Weight

No significant differences were observed in the weight of the healthy and diabetic control animals after 28 days. Furthermore, no significant difference was observed after treatment with FAE or metformin as compared to the diabetic control group.

3.2 Blood Glucose

As showed in the previous study [14], there was a significant increase in blood glucose ($P< 0.001$) in diabetic rats as compared with those in normal control group. Administration of FAE extract at 200 mg, 400 mg, 800 mg, and metformin decreased blood glucose in diabetic rats by 26% ($P<0.01$), 59.3% ($P<0.001$), 69.4% ($P<0.001$), and 28.8% ($P<0.01$), respectively.

3.3 Toxicity Study

A toxicity study revealed the non-toxic characteristics of the extract. The rats treated with different doses of FAE did not show any

drug-induced physical symptoms of toxicity during the whole experimental period and no deaths were reported.

3.4 Liver Enzymes

Induction of diabetes caused a significant increase ($p< 0.001$) in the activities of AST and ALT as compared to the corresponding control animals. Serum activities of AST of diabetic rats significantly decreased ($P<0.01$ for FAE (200 mg) and $P<0.001$ for both FAE (400 mg) and FAE (800 mg) after 28 days of treatment. Administration of FAE with the doses of 400 mg and 800 mg significantly decreased ALT activity ($P<0.05$ and $P<0.01$, respectively) as compared to the un-treated diabetic group (Table 1). Treatment with metformin (150 mg/kg) did not show significant decrease in the activity of AST and ALT compared to the diabetic control group.

Table 1. Comparison of measured liver markers between the experimental groups

Parameters / Group	AST (IU/ml)	ALT (IU/ml)
Normal	11.38±5.6	57±2.5
Diabetic control	69.8±4.7$^{a♣}$	112.2±7.9$^{a♣}$
Diabetic + FAE (200 mg)	33±7.5$^{b♠}$	91.6±3.5
Diabetic + FAE (400 mg)	5.4±3.1$^{b♣}$	80.8±3.2^{b*}
Diabetic + FAE (800 mg)	7.8±5.4$^{b♣}$	63.6±3.6$^{b♠}$
Diabetic + metformin	56.6±5.02	97.8±9.6

*AST: Aspartate transaminase, ALT: Alanine transaminase, a; Compared to normal group, b; Compared to diabetic control group, *; P<0.05, ♠; P<0.01, ♣; P<0.001. Values are given as mean ± S.E.M for 9 rats in each group. A P-value of less than 0.05 was considered as statistically significant.*

3.5 Antioxidant Enzymes

As presented in Table 2, there is a significant decrease in the level of catalase ($P<0.01$), SOD, and GPx ($P<0.001$ for both) in the diabetic group compared with the healthy group. Administration of FAE (400 mg) increased catalase, SOD, and GPx significantly ($P<0.01$ for catalase and CAT and $P<0.001$ for GPx). Treating with FAE (800 mg) also increased these three enzymes significantly by $P<0.001$. Treatment with metformin did not change catalase, however it increased SOD and GPx activity ($P<0.01$).

3.6 Oxidative Stress Marker

Concentrations of serum MDA (s-MDA) and tissue MDA (t-MDA) increased in alloxan-induced diabetic rats compared with the healthy control group. Administration of FAE extract for 28 days decreased s-MDA ($P<0.001$) and t-MDA ($P<0.01$) significantly as compared with the diabetic control group. Also, in the group treated with metformin, s-MDA and t-MDA decreased significantly ($P<0.05$ and $P<0.01$, respectively (Table 2).

3.7 Antioxidant Compounds in Extract

The amounts of total phenol, flavonoid, and flavonol were 13, 42, and 4 mg in one gram of hydroalcoholic extract of FAE, respectively.

3.8 Liver Histology

The central vein, hepatocyte, and portal space were observed to be normal in the normal control group (Fig. 1-A). The damage to the liver cells in the form of damaged central vein, hepatocytes, and portal trial could be seen clearly in the alloxan-induced diabetic rats (Fig. 1-B). These damages were reversed in the FAE treated groups (Fig. 1-C, D, and E). It seems that the amelioration effect of FAE on liver cells is dose-dependent. The therapeutic effect of metformin is shown in (Fig. 1-F).

4. DISCUSSION

There are numerous studies and clinical evidences showing that the production of high levels of oxygen free radicals increases in Type 1 diabetes and that the onset of diabetes is closely related to the level of oxidative stress [25,26]. The diabetogenic substance of alloxan monohydrate has three different pathological effects that intervene in the physiological function of pancreatic beta cells. First, alloxan selectively inhibits glucose-induced insulin secretion through its capability to specifically inhibit glucokinase, the glucose sensor of the beta cell. Second, alloxan is a urea derivative which causes a state of insulin-dependent diabetes mellitus through its ability to induce a selective necrosis of the beta cells. Third, alloxan-induced beta-cell toxicity is mediated by the formation of oxygen free radicals such as superoxide. These three effects of alloxan can be assigned to its specific chemical properties that cause islet beta cell destruction and increase plasma glucose [27-29].

Table 2. Comparison of measured antioxidant enzyme activity and lipid peroxidation marker between the experimental groups

Group	CAT (U/mg protein)	SOD (U/mg protein)	GPx (U/mg protein)	s-MDA (μM)	t-MDA (μM)
Normal	327.5±14	27.5±1	88.9±5.8	1.4±0.1	1.3±0.1
Diabetic control	215±20$^{a♠}$	14.3±0.8$^{a♣}$	43.2±3.8$^{a♣}$	7.9±0.4$^{a♣}$	7.9±0.4$^{a♣}$
Diabetic + FAE (200 mg)	232.8±20	20.4±2.4	46.2±5.3	2.5±0.2$^{b♣}$	5.0±0.1$^{b♠}$
Diabetic + FAE (400 mg)	341.6±16$^{b♠}$	23.8±1.9$^{b♠}$	87±4.2$^{b♣}$	2.8±0.2$^{b♣}$	5.1±0.3$^{b♠}$
Diabetic + FAE (800 mg)	369.1±27$^{b♣}$	27.4±0.8$^{b♣}$	86±5.0$^{b♣}$	2.4±0.1$^{b♣}$	4.6±0.2$^{b♠}$
Diabetic + metformin	288.4±15	23±1.6$^{b♠}$	74±6.8$^{b♠}$	6.8±0.2^{b*}	5.0±0.3$^{b♠}$

*CAT: Catalase, SOD: Superoxide dismutase, GPx: Glutathione peroxidase, sMDA: Serum Malondialdehyde, tMDA: Tissue Malondialdehyde. a; Compared to normal group, b; Compared to diabetic control group, *; P<0.05, ♠; P<0.01, ♣; P<0.001. Values are given as mean ± S.E.M for 9 rats in each group. A P-value of less than 0.05 was considered as statistically significant.*

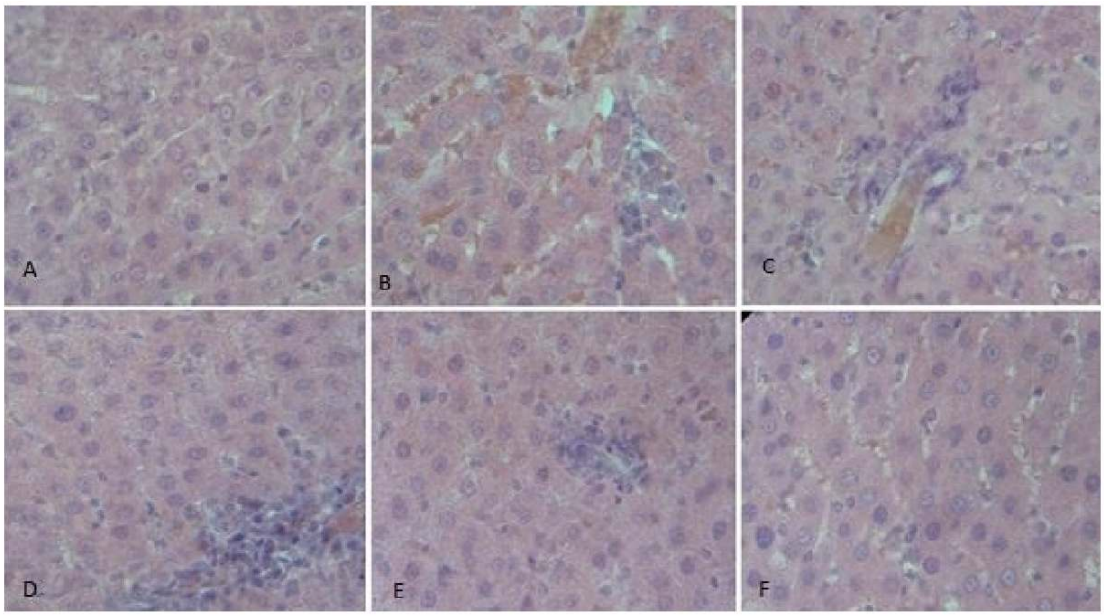

Fig. 1. Effects of *Ferulago angulate* extract (FAE) on hepatic morphological analysis (paraffin-embedded sections were stained with hematoxylin and eosin (H&E)). (A) Control group with no noticeable histological changes, (B) Alloxan-induced diabetic control group showing severe histopathological changes and necrosis, (C) Diabetic + FAE(200 mg/kg), (D) Diabetic + FAE(400 mg/kg), (C) Diabetic + FAE(800 mg/kg), and metformin (150 mg/kg). Rats treated with 800 mg/kg dose showed more improvement than those treated with 400 mg/kg and 200 mg/kg doses so its therapeutic effects are dose dependent (Original magnification, ×400)

Injury to the liver in diabetes, which can be induced by alloxan, releases and also increases the level of liver enzymes in the bloodstream [30]. The significant dose-dependent reduction in the serum levels of these enzymes after the administration of FAE indicates its anti-hepatotoxic effect in diabetes which may have been brought about by the stability of plasma membrane as well as the repair of tissue damage.

The results of our study indicated that the plant extract administered to the diabetic animals during 28 days reduced liver and serum MDA concentrations (P<0.001). It has been revealed that in hyperglycemia, antioxidant defenses change due to the presence of diabetic conditions leading to the increase in free radical production [31,32]. FAE significantly inhibited the increase of serum and

liver MDA levels and indicated a protective effect against oxidative stress.

Antioxidant scavenger systems deficiency under diabetic conditions can increase lipid peroxidation due to oxidative stress [33,34]. The results of our study showed that catalase, SOD, and GPx activities were significantly lower in the diabetic animals compared to the healthy ones ($P<0.01$ for catalase and $P<0.001$ for two others). After 28 days, treatment with FAE significantly increased the activity of these enzymes (P values were dose dependent and was not significant in FAE 200 mg/kg). These effects might be related to the antioxidant activity of the extract. The increase in the activity of enzymatic antioxidants may be helpful in reversing the action of the alloxan monohydrate through the antioxidant mechanism [35].

In this study, we observed some lesions in the livers of diabetic animals including structural changes in hepatocytes, both sinusoids and portal areas, and enlargement of sinusoids. The same results were reported by Mahmoud and Al-Salahy who demonstrated in their study that alloxan-induced hyperglycemia plays a crucial role in the genesis of liver injury [36]. The structural abnormalities and degenerative changes induced by alloxan monohydrate in the livers of diabetic rats ameliorated by the administration of FAE and metformin. This effect was more prominent at the dose of 800 mg/kg FAE. The presence of phenolic compounds and flavonoids in our extract might be the reason for its antioxidant and thus liver treating activity [37].

5. CONCLUSION

The present investigation suggested that hydroalcoholic extract of FAE exerts an anti-hepatotoxic effect via inhibiting oxidative stress due to the presence of phenolic and flavonoid compounds and their anti-oxidant nature. However, further investigations are needed to elucidate the precise molecular mechanism of specific bio-active agents of FAE for the protection against, and treatment of, the diabetes induced by alloxan or other toxic agents and validate these findings.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Ardabil University of Medical Sciences for funding of this project, and Shahrekord University of Medical Sciences for cooperating in this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ozougwu JC, Obimba KC, Belonwu CD, Unakalamba CB. The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. Journal of Physiology and Pathophysiology. 2013;4(4):46-57.
2. Shrivastava SR, Shrivastava PS, Ramasamy J. Role of self-care in management of diabetes mellitus. Journal of Diabetes & Metabolic Disorders. 2013; 12(14).
3. Kazemi S, Asgary S, Moshtaghian J, Rafieian M, Adelnia A, Shamsi F. Liver-protective effects of hydroalcoholic extract of *Allium hirtifolium* Boiss. In rats with alloxan-induced diabetes mellitus. ARYA Atherosclerosis Journal. 2010;6(1):11-15.
4. Paschos P, Paletas K. Non alcoholic fatty liver disease and metabolic syndrome. Hippokratia. 2009;13(1):9-19.
5. Rezagholizadeh L, Pourfarjam Y, Nowrouzi A, et al. Effect of *Cichorium intybus* L. on the expression of hepatic NF-κB and IKKβ and serum TNF-α in STZ− and STZ+ niacinamide-induced diabetes in rats. Diabetology & Metabolic Syndrome. 2016; 8:11.
6. Levinthal GN, Tavill AS. Liver disease and Diabetes mellitus. Clinical Diabetes. 1999; 17(2).
7. Wild SH, Morling JR, McAllister DA, et al. Type 2 diabetes, chronic liver disease and hepatocellular cancer: A national retrospective cohort study using linked routine data. Journal of Hepatology; 2016.

8. Hazlehurst JM, Woods C, Marjot T, Cobbold JF, Tomlinson JW. Non-alcoholic fatty liver disease and diabetes. Metabolism; 2016.
9. Lucchesi AN, Tavares de Freitas N, Cassettari LL, Marques SFG, Spadella CT. Diabetes mellitus triggers oxidative stress in the liver of alloxan-treated rats: A mechanism for diabetic chronic liver disease. Acta Cir. Bras. 2013;28(7):502-508.
10. Rafieian-kopaei M, Shahinfard N, Rouhi-Boroujeni H, Gharipour M, Darvishzadeh-Boroujeni P. Effects of *Ferulago angulata* extract on serum lipids and lipid peroxidation. Evidence-Based Complementary and Alternative Medicine. 2014;2014:1-4.
11. Khalighi-Sigaroodi F, Hadjiakhoondi A, Shahverdi A, Mozaffarian V, Shafiee A. Chemical composition and antimicrobial activity of the essential oil of *Ferulago bernardii* Tomk. and M. Pimen. DARU Journal of Pharmaceutical Sciences. 2005; 5.
12. Khanahmadi M, Janfeshan K. Study on antioxidation property of *Ferulago angulata* plant. Asian Journal of Plant Sciences. 2006;5:521-526.
13. Tabatabaei Yazdi F, Alizade Behbahani B, Heidari Sureshjani M. The comparison of antimicrobial effects of chevil (*Ferulago angulata*) extract with a variety of common therapeutic antibiotics *in vitro*. Arak Medical University Journal. 2014;17(3):35-46.
14. Musavi Ezmareh F, Mazani M, Heidarian E, et al. Effect of hydroalcoholic extract of chevil (*Ferulago angulata*) on glucose and lipid in diabetic male rats. Iranian Journal of Endocrinology and Metabolism. 2015; 17(3):230-237.
15. Verma L, Singour PK, Chaurasiya PK, Rajak H, Pawar RS, Patil UK. Effect of ethanolic extract of *Cassia occidentalis* Linn. for the management of alloxan-induced diabetic rats. Pharmacognosy Research. 2010;2(3):132-137.
16. Sarfraz M, Khaliq T, Khan JA, Aslam B. Effect of aqueous extract of black pepper and ajwa seed on liver enzymes in alloxan-induced diabetic Wister albino rats. Saudi Pharmaceutical Journal. 2017/05/01/. 2017;25(4):449-452.
17. Parsaei P, Karimi M, Asadi SY, Rafieian-Kopaei M. Bioactive components and preventive effect of green tea (*Camellia sinensis*) extract on post-laparotomy intra-abdominal adhesion in rats. International Journal of Surgery (London, England). 2013;11(9):811-815.
18. Pitcheshwar P. Phytochemical screening, anti-inflammatory activity and anti-oxidant activity assessment of black tea, green tea and rooibos tea. Journal of Biological and Information Sciences. 2014;3(4):23-26.
19. DOWD LE. Spectrophotometric determination of quercetin. Analytical Chemistry. 1959;31(7):1184-1187.
20. Agarwal R, Chase SD. Rapid, fluorimetric–liquid chromatographic determination of malondialdehyde in biological samples. Journal of Chromatography B. 2002;775: 121-126.
21. Aebi H. Catalase *in vitro.* Methods in Enzymology. 1984;105:121-126.
22. Sun Y, Obertly LW, Li Y. A Simple method for clinical assay of superoxide dismutase. Clinical Chemistry. 1988;34(3):497-500.
23. Cichoski AJ, Rotta RB, Scheuermann G, Cunha Junior A, Barin JS. Investigation of glutathione peroxidase activity in chicken meat under different experimental conditions. Ciênc. Tecnol. Aliment., Campinas. 2012;32(4):661-667.
24. Sun X, Han F, Yi J, Han L, Wang B. Effect of aspirin on the expression of hepatocyte NF-κB and serum TNF-α in Streptozotocin-induced Type 2 diabetic rats. J Korean Med Sci. 2011;26:765-770.
25. Bisht S, Sisodia SS. Diabetes, dyslipidemia, antioxidant and status of oxidative stress. IJRAP. 2010;1(1):33-34.
26. Matough FA, Budin SB, Hamid ZA, Alwahaibi N, Mohamed J. The role of oxidative stress and antioxidants in diabetic complications. Sultan Qaboos Univ Med J. 2012;12(1):5-18.
27. Ezeja MI, Anaga AO, Asuzu IU. Antidiabetic, antilipidemic, and antioxidant activities of Gouania longipetala methanol leaf extract in alloxan-induced diabetic rats. Pharm Biol. 2015;53(4):605-614.
28. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia. 2008;51:216-226.
29. Tripathi V, Verma J. Different models used to induce diabetes: A comprehensive review. Int J Pharm Pharm Sci. 2014; 6(6):29-32.
30. Edet EE, Atangwho IJ, Akpanabiatu MI, Edet TE, Uboh FE, David-Oku E. Effect of *Gongronema latifolium* leaf extract on some liver enzymes and protein levels in

diabetic and non diabetic rats. Journal of Pharmaceutical and Biomedical Sciences. 2011;1(5):104-107.
31. Aragno M, Mastrocola R, Catalano MG, Brignardello E, Danni O, Boccuzzi G. Oxidative stress impairs skeletal muscle repair in diabetic rats. Diabetes. 2004; 53(4):1082-1088.
32. Kamalakkannan N, Prince PS. Antihyperglycaemic and antioxidant effect of rutin, a polyphenolic flavonoid, in streptozotocin-induced diabetic wistar rats. Basic Clin Pharmacol Toxicol. 2006; 98(1):97-103.
33. Anuradha CV, Selvam R. Effect of oral methionine on tissue lipid peroxidation and antioxidants in alloxan-induced diabetic rats. The Journal of Nutritional Biochemistry. 1993;4(4):212-217.
34. Rezaei-Moghadam A, Mohajeri D, Rafiei B, et al. Effect of turmeric and carrot seed extracts on serum liver biomarkers and hepatic lipid peroxidation, antioxidant enzymes and total antioxidant status in rats. Bioimpacts. 2012;2(3):151-157.
35. Shajeela PS, Kalpanadevi V, Mohan VR. Potential antidiabetic, hypolipidaemic and antioxidant effects of Nymphaea pubescens extract in alloxan induced diabetic rats. Journal of Applied Pharmaceutical Science. 2012;2(2):83-88.
36. Mahmoud AB, Al-Salahy MB. Physiological and Histological studies on the effect of lupine seeds on the fish, *Clarias lazera* treated with glucose and alloxan. Fish Physiology and Biochemistry. 2004;30(3): 213-229.
37. Eidi A, Mortazavi P, Bazargan M, Zaringhalam J. Hepatoprotective activity of cinnamon ethanolic extract against ccl4-induced liver injury in rats. EXCLI Journal. 2012;11:495-507.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/20530