Antioxidant and anti-inflammatory effects of *Acrocarpus fraxinifolius* on hyperglycemia, hyperlipidemia and liver/kidney dysfunctions against alloxan induced Type 1 diabetes in rats

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Many factors such as oxidative stress and pro-inflammatory cytokines cause β-cell dysfunction and death. The aim of this study was to investigate antioxidant and anti-inflammatory effects of *Acrocarpus fraxinifolius* (*A. fraxinifolius*) on hyperglycemia, hyperlipidemia and liver/kidney dysfunctions against alloxan induced Type 1 diabetes in rats. Thirty six male white albino rats were divided into 6 groups: Control group, diabetic group and two prophylactic group with two different doses and two therapeutic group with two different doses (250 or 500 mg/kg b.w; for 28 consecutive days). Diabetes was induced by i.p. injection of 75 mg/kg b.w.alloxan monohydrate for 5 consecutive days. In this study showed that *A. fraxinifolius* extract [especially prophylactic treatment] significantly suppressed diabetic complications in alloxan-induced diabetes in rat model by alleviating body weight loss, hyperglycemia, hypo-insulinemia and dyslipidemia through activating the antioxidant defense system, decreasing of oxidative/nitrosative stress as well as lipid peroxidation, and increasing/decreasing the production of anti-inflammatory/pro-inflammatory cytokines, respectively. The percentage of disease recovery was 15.00 ± 5.472 % in high dose *A. fraxinifolius* of prophylactic treatment compared with alloxan only group 73.70 ± 22.09 % (p < 0.05).The data confirmed property of *A. fraxinifolius* as an antioxidant and anti-inflammatory that ameliorates oxidative/nitrosative stress and revealed that it is efficiently improves diabetic complication in alloxan-induced diabetic rats.

**Keywords:** *Acrocarpus fraxinifolius*, Alloxan, Antioxidant, Cytokines, Diabetes.

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Diabetes mellitus is a global problem and the number of people suffering from it worldwide. Type 1 diabetes mellitus is a chronic autoimmune disease associated with selective destruction of insulin-producing pancreatic β-cells. Deficiency in insulin leads to uncontrolled lipolysis and elevated levels of free fatty acids in the plasma, which suppresses glucose metabolism in peripheral tissues such as skeletal muscle. Hyperglycemia in type 1 diabetes probably results from a long-term negative balance between immune mediated β-cell damage and β cell repair/regeneration. Once macrophages and T-cells have been attracted to the islets and activated, they secrete soluble mediators such as pro-inflammatory cytokines, oxygen free radicals, and nitric oxide (NOx), which probably contribute to β-cell dysfunction and death.

Alloxan is a commonly used chemical to generate diabetic animals in the laboratory for its ability to destroy insulin producing cells. It is generally accepted that free radicals, especially superoxide radicals, induced by alloxan cause cellular damage, that is key to its role as a diabetogenic action. Also, alloxan induced diabetes is due to a cellular-mediated autoimmune destruction of the β-cells of the pancreas. Hypoglycemic drugs have certain adverse effects like causing hypoglycemia at higher doses, liver problems, lactic acidosis and diarrhea. Thus searching for a new class of compounds is essential to overcome diabetic problems. The medicinal plants may provide the useful source of new oral hypoglycemic compounds for the development of pharmaceutical entities or as dietary adjunct to existing therapies. These antioxidant compounds include polysaccharides, terpenoids, flavonoids, sterols and alkaloids. Furthermore, after the
recommendation made by WHO on diabetes mellitus, investigation on hypoglycemic agents from medicinal plants have become more important. The ethnobotanical information reports state that more than 1200 traditional plants may have been used for real or perceived benefit of medicinal purposes for the treatment of diabetes. Natural antioxidants extracts are safe, potentially nutritional and have therapeutic effects.

Acerocarpus fraxinifolius Arn. belongs to the Fabaceae family. This species is distributed naturally in countries such as India, Burma and China and is widely cultivated in Egypt. Our previous study showed that GC-MS analysis of n-hexane extract (nHE) of A. fraxinifolius leaves revealed the presence of 37 compounds from which 29 compounds (95.9 %) were identified. These compounds may be responsible for its radical scavenging activity. The extracts of the plant were reported to have anti-proliferative, anti-inflammatory, antioxidant and antidiabetic as well as hepato-protective activities in vivo. Until now, there is not enough relevant data on the antidiabetic effect of nHE A. fraxinifolius leaves. The aim of the present study was designed to investigate the hypoglycemic effect of nHE of A. fraxinifolius leaves in either protective or therapeutic studies against alloxan-induced diabetes mellitus in rats.

Materials and methods

Chemicals
Alloxan monohydrate (C₄H₂N₂O₄·H₂O; molecular weight 160.08 Da) was purchased from Sigma-Aldrich (St Louis, MO, USA).

Plant material and preliminary phytochemical screening
A. fraxinifolius leaves (2 kg) were collected from Giza Zoo Botanical Garden (Giza, Egypt). These leaves were authenticated by Mrs. Tereize Labib, the taxonomy specialist in El-Orman Botanical Garden (Giza, Egypt). Preparation of nHE was designed according to a previous study. nHE of A. fraxinifolius was subjected to preliminary phytochemical screening for the detection of various plant constituents.

Animals and experimental design
Thirty six male albino rats (Rattus norvegicus), weighing about 140 ± 5 g, were obtained from the National Research Centre in Giza, Egypt. Animals were housed in suitable cages and acclimatized to laboratory conditions for a period of one week before the commencement of the experiments. They were fed standard diet and maintained at 37 °C, 12-12 h dark/light periods and water ad libitum.

The rats were divided randomly into six groups (6 rat each): Animals of the first group served as negative control group. In diabetic groups, injection of alloxan monohydrate (75 mg/kg b.w/i.p) in 0.9 % sterile saline sodium chloride, pH 7 was given to rats for five consecutive days. Rats of the second group served as diabetic control group. The animals of the third and fourth group served as prophylactic groups and received orally A. fraxinifolius at a dosage of either 250 or 500 mg/kg b.w for 28 days; then followed by i.p. injection of alloxan for 5 consecutive days. Rats of 5th and 6th groups served as therapeutic groups and received orally A. fraxinifolius at a dosage of either 250 or 500 mg/kg b.w for 28 days after i.p. injection of alloxan for 5 consecutive days.

At the end of the experiment, the animals were weighted and sacrificed under anesthesia with diethyl ether. A blood samples were collected into clean test-tubes without anti-coagulant. The serum was separated and divided into aliquots and preserved at -80 °C for further analysis.

Measurements
Body-weight gain or loss was calculated. All biochemical analyses were manually done using commercial kits. Serum glucose, total cholesterol, triglycerides, HDL-cholesterol, reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), malondialdehyde (MDA), nitric oxide (NOx), and hydrogen peroxides (H₂O₂) concentrations were determined using Bio-diagnostic kits (Bio-diagnostic, Giza, Egypt). LDL-cholesterol concentration was calculated according to the equation: [LDL-chol.= total chol. – (TAG/5) – HDL-chol.]. Atherogenic indexes were calculated as follows: atherogenic index (total chol.: HDL-chol. ratio); atherogenic index (LDL- chol.: HDL-chol. ratio). Serum insulin concentration was determined by enzyme-linked immunosorbent assay by using a diagnostic kit (Crystalchem, USA) according to the method of Judzewitsch et al. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as the product of the fasting serum glucose (mg/dL) and fasting insulin levels (μIU/mL) divided by a constant. Quantitative insulin sensitivity check index (QISCI) was derived by the following equation:
Qisci = \left[ \frac{1}{\log \text{(fasting insulin (μIU/mL))} + \log \text{(fasting glucose mg/dL)}} \right].

Serum tumor necrosis factor alpha (TNF-α), interleukine-IL-4, IL-6, and IL-10 were determined using commercially available diagnostic kit (R&D system, Minneapolis, USA).

Statistics

Results are expressed as mean ± their standard error of mean (SEM). All statistical comparisons between the groups are made by means of One Way ANOVA with post hoc Tukey’s multiple comparison test using Graph Pad Prism version 4.03 for Windows (Graph Pad Software Inc., San Diego, CA, USA). The p value < 0.05 was regarded as significant.

Results

Modulatory effects of A. fraxinifolius extract on change in body weight, serum glucose concentration and liver/kidney functions in diabetic rats

Table 1 revealed that the significant decrease (p < 0.001) in the body weight gain shown in alloxan only treated group that received vehicle, compared with the normal control group. The animals that received A. fraxinifolius with alloxan (both prophylactic and therapeutic treatments) showed significantly less loss of body weight (p < 0.01-0.001) when compared with rats receiving alloxan only, but the utmost modulation on the changes in body weight was shown in diabetic groups that received prophylactic doses of A. fraxinifolius. Serum glucose concentration significantly increased (p < 0.001) in alloxan only treated group compared with the normal control group. Oral administration of A. fraxinifolius extract in both prophylactic and therapeutic groups with A. fraxinifolius extract showed decrease (p < 0.001) in serum glucose concentration compared with alloxan only group. Alloxan only treated group showed significant decrease (p < 0.001) in the serum ALAT, ASAT, urea and creatinine levels compared to the normal control group. A significant decrease (p < 0.001) in the serum ALT, ASAT, urea and creatinine levels were observed in both prophylactic and therapeutic groups with A. fraxinifolius extract compared with compared with rats receiving alloxan only. The utmost modulation on the serum liver/kidney enzymes markers were shown in diabetic groups that received high dose in prophylactic treatment with A. fraxinifolius (p < 0.05-0.01, compared to the normal control group).

Modulatory effects of A. fraxinifolius extract on serum lipid profile and atherogenic indexes in diabetic rats

Fig. 1 illustrated that alloxan only treated group induced a significant increase (p < 0.01) in serum total cholesterol, triglycerides and LDL-C concentrations as well as atherogenic indexes compared with the normal control animals. A significant decrease (p < 0.01-0.001) in the serum

Table 1—Body weight and serum markers of liver/kidney function in control and diabetic rat model groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal Control</th>
<th>Alloxan only</th>
<th>A. fraxinifolius 250 + Alloxan</th>
<th>A. fraxinifolius 500 + Alloxan</th>
<th>Alloxan + A. fraxinifolius 250</th>
<th>Alloxan + A. fraxinifolius 500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>180.50±1.48</td>
<td>179.90±0.68</td>
<td>181.00±0.93</td>
<td>179.90±1.06</td>
<td>180.50±1.48</td>
<td>179.90±0.68</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>211.90±2.59</td>
<td>181.60±0.86</td>
<td>193.80±1.36</td>
<td>194.50±1.36</td>
<td>189.20±0.68</td>
<td>190.10±1.11</td>
</tr>
<tr>
<td>Body weight change (g)</td>
<td>31.38 ± 2.95</td>
<td>1.67 ± 0.35</td>
<td>12.77± 0.62</td>
<td>14.68 ± 1.26</td>
<td>8.72 ± 1.37</td>
<td>10.20± 0.94</td>
</tr>
<tr>
<td>Serum glucose (mg/dL)</td>
<td>84.89 ± 1.69</td>
<td>295.40±2.39</td>
<td>215.80±2.27</td>
<td>216.20±1.94</td>
<td>270.40±2.69</td>
<td>215.80±2.27</td>
</tr>
<tr>
<td>Serum ALAT (IU/L)</td>
<td>47.37±1.88</td>
<td>95.24±4.27</td>
<td>70.32±1.06</td>
<td>62.54±1.74</td>
<td>80.81±2.30</td>
<td>76.45±2.96</td>
</tr>
<tr>
<td>Serum ASAT (IU/L)</td>
<td>38.70±1.58</td>
<td>91.63±3.45</td>
<td>62.40±2.22</td>
<td>52.22±2.54</td>
<td>76.55±1.88</td>
<td>69.74±1.21</td>
</tr>
<tr>
<td>Serum urea (mg/dL)</td>
<td>27.24±1.72</td>
<td>67.50±1.39</td>
<td>42.24±1.02</td>
<td>35.69±1.82</td>
<td>51.69±1.54</td>
<td>49.83±1.55</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>0.53±0.03</td>
<td>1.89±0.04</td>
<td>0.69±0.01</td>
<td>0.63±0.02</td>
<td>0.80±0.01</td>
<td>0.71±0.02</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SE., n = 6 rats per group. A. fraxinifolius: Acrocarpus fraxinifolius. * p<0.05, ** p<0.01, *** p<0.001: compared with the normal control group; † p<0.05, †† p<0.01, ††† p<0.001: compared with the alloxan only group that received vehicle; (One-Way ANOVA with Tukey's multiple comparison test).
lipid profile and atherogenic indexes were observed in both prophylactic and therapeutic groups with *A. fraxinifolius* extract compared with compared with rats receiving alloxan only. On the other hand, diabetic rats that received low dose of *A. fraxinifolius* extract in therapeutic groups did not show any modulation on serum HDL-chol. (p > 0.05) compared with alloxan only group. The utmost modulation on the changes in lipid profile and atherogenic indexes were shown in diabetic groups that received prophylactic doses of *A. fraxinifolius*.

**Modulatory effects of *A. fraxinifolius* extract on serum insulin hormone, HOMA-IR and QISCI in diabetic rats**

As shown in Fig. 2, alloxan only treated group showed highly significant decrease (p < 0.05), however, it showed non-significant changes in HOMA-IR and QISCI compared to the normal control group. Only prophylactic groups with *A. fraxinifolius* extract significantly increased (p < 0.001) the insulin level shown in diabetic rats. While, in therapeutic groups with *A. fraxinifolius* extract showed non-significant changes in the insulin level, as compared with alloxan only treated group. HOMA-IR and QISCI showed non-significant changes in all studied groups compared with the normal control/alloxan only groups.

**Modulatory effects of *A. fraxinifolius* extract on serum antioxidant/oxidant status in diabetic rats**

The observed data shown in Fig. 3 indicated that alloxan only treated group induced a significant
decrease (p < 0.001) in serum GSH and antioxidant enzymes (GSH-Px and CAT); and a significant increase in serum SOD, MDA, NOx and H2O2 compared with the normal control animals. Oral administration of A. fraxinifolius extract in both prophylactic and therapeutic groups with A. fraxinifolius extract showed an elevation (p < 0.05-0.001) in GSH, GSH-Px and CAT; and reduction in serum SOD, MDA, NOx and H2O2 (p < 0.05-0.001) compared with alloxan only treated group. The utmost modulation of A. fraxinifolius extract on the changes in antioxidant/oxidant status of diabetic rats was shown in those receiving the prophylactic doses of A. fraxinifolius extract (p < 0.001), especially high dose.

**Modulatory effects of A. fraxinifolius extract on serum pro- and anti-inflammatory cytokines in diabetic rats**

Levels of serum pro-inflammatory cytokines (TNF-α and IL-6) were significantly increased (p < 0.001) in alloxan only treated group compared with the normal control rats (Fig. 4). In contrast, prophylactic and therapeutic groups with A. fraxinifolius extract significantly decreased (p < 0.05–0.001) the elevation in levels of serum pro-inflammatory cytokines compared with alloxan only treated group. The utmost modulation on the elevation in levels of serum pro-inflammatory cytokines of diabetic rats was shown in those receiving the prophylactic doses of A. fraxinifolius extract. On the other hand, levels of serum anti-inflammatory cytokines (IL-4 and IL-10) were significantly decreased (p < 0.001) in alloxan only treated group compared with the normal control rats. In contrast, prophylactic and therapeutic groups with A. fraxinifolius extract significantly increased (p < 0.05–0.001) the reduction in levels of serum anti-inflammatory cytokines compared with alloxan only treated group. The utmost modulation on the decrease in levels of serum anti-inflammatory cytokines of diabetic rats was shown in those receiving the prophylactic doses of A. fraxinifolius extract.
The percentages of changes of all parameters measured, compared with the normal control group, in diabetic groups that received vehicle, *A. fraxinifolius* 250, *A. fraxinifolius* 500 in prophylactic study or *A. fraxinifolius* 250, *A. fraxinifolius* 500 in therapeutic study 73.70 ± 22.09, 20.85 ± 8.766, 15.00 ± 5.472, 51.22 ± 15.95, and 37.13 ± 12.07, respectively, indicating that the anti-diabetic activity of *A. fraxinifolius*, especially high dose in prophylactic treatment exceeded (p < 0.05) that of low dose in prophylactic treatment and both doses in therapeutic treatment in alloxan-induced diabetes in rat model.

**Discussion**

Alloxan which selectively destroy β-cells of the islet Langerhans of pancreas was used to induce type 1 diabetes mellitus. Depletion of β-cells will, therefore, result in a massive reduction in insulin release and hyperglycemia which will lead to a disorder in protein and fat metabolism, similar disorders were found in the present study. Additionally, hyperglycemia may induce macrophage production of IL-12, which can stimulate CD4+ cell production of IFN-γ which initiate and induce further pro-inflammation and oxidative stress as well as decrease anti-inflammatory cytokines as seen in this study. Suress & Das reported that animals which developed alloxan-induced diabetes mellitus showed a significant decrease in body weight due to uncontrolled diabetes mellitus. The reduction of body weight might be due to the low utilization of uptake blood sugar in cell as well as lipolysis/hyperlipidemia and gluconeogenesis during diabetes. Also, diabetes is characterized by impairment of liver/kidney functions as well as elevation oxidative stress as seen in alloxan-treated groups. Elevation of serum hepatic/renal markers indicate cellular infiltration and disturbance in the functioning of the hepatic/renal cell membranes.

In this study showed that *A. fraxinifolius* extract (especially prophylactic treatment) significantly suppressed (p < 0.05–0.001) diabetic complications in alloxan-induced diabetes in rat model by alleviating body weight loss, hyperglycemia, hypo-insulinemia and dyslipidemia. The enhancement in body weight in *A. fraxinifolius* groups may be attributed to anabolic action of phytochemicals compounds that can ameliorate physiological response to stress. Also, the modulation in body weight resulted mainly from decrease/increase in the lipid peroxidation/antioxidant system and by decreasing pro-inflammatory/pyrogenic cytokines such as TNF-α, as seen the present study. El-Rafie et al. reported that the anti-diabetic activity exhibited by 100 mg of *A. fraxinifolius* extract bark extract was 74.38 % relative to metformin (100 % potency) in alloxan rats model. In addition, total ethanol and aqueous ethanol extracts of *A. fraxinifolius* leaves exhibited high potencies as compared with metformin in decreasing the glucose level after four weeks. Polyphenols show natural...
antioxidant properties, therefore, impart a notifying action\textsuperscript{11}. El-Kashak \textit{et al.}\textsuperscript{15} reported that aqueous methanolic extract of \textit{A. fraxinifolius} leaves contains 8 flavonoids (quercetin, quercetin 3-0-β-D-glucopyranoside, quercetin3-O-α-L-rhamnopyranoside, myricetin, myricetin 3-O-β-D-galactopranoside, myricetin 3-O-α-L-rhamnopyranoside, desmanthin-1, and naringenin), in addition to 4 phenolic acids (brevifolin carboxylic acid, ellagic acid, gallic acid, and methyl gallate). It is well-known that flavonoids and phenolic acids are potent antioxidant agents and exhibit therapeutic potential, including hepato-protection and the inhibition of liver fibrosis, against many diseases\textsuperscript{15}. Also, \textit{A. fraxinifolius} extracts showed an in \textit{vivo} antioxidant activity when compared to vitamin E\textsuperscript{22}. \textit{A. fraxinifolius} extract treatment modulated the serum hepatic/renal markers in all animals treated with alloxan compared to untreated diabetic group. El-Rafie \textit{et al.}\textsuperscript{16} reported that the hepato-protective activity of \textit{A. fraxinifolius} extract (100 mg) was evidenced by significant decrease in liver function enzymes compared to the CCl\textsubscript{4} untreated group.

The hypo-lipidemic lowering effect of \textit{A. fraxinifolius} extract may be related to several active constituents extracted. Abd El-Ghffar \textit{et al.}\textsuperscript{12} reported that the mechanism of antioxidant ability of nHE of \textit{A. fraxinifolius} may be attributed to numerous bioactive compounds such as steroids (9.82 %); triterpenes (12.47 %); α-tocopherol (18.23 %); labda 8 (20)-13-dien-15-oic acid (13.15 %), lupeol (11.93 %), phytol (10.95 %), and squalene (7.19 %). Most of them (especially flavonoids, and triterpenoids) showed a mechanism to improve the function of many organs such as liver and pancreas cells\textsuperscript{23,24}. Flavonoids, like antioxidants may prevent the progressive impairment of pancreatic β-cell function due to oxidative stress and may thus reduce the occurrence of type 2 diabetes\textsuperscript{25}.

An oxygen reduction cycle would then take place in which superoxide radicals (O\textsubscript{2}−) would be produced during the oxidation of the dialuric acid (the metabolites of alloxan). O\textsubscript{2}− generated during the reoxidation of alloxan resulted in the formation of H\textsubscript{2}O\textsubscript{2} which forms hydroxyl radicals (OH) toxic to the pancreatic β-cells\textsuperscript{3}. In addition, hyperglycemia increases oxidative stress through the overproduction of ROS which results in an imbalance between free radicals/ non-radical and the antioxidant defense systems of the cell. The H\textsubscript{2}O\textsubscript{2} formed by SOD and other processes is scavenged by CAT that catalyzes the dismutation of H\textsubscript{2}O\textsubscript{2} into H\textsubscript{2}O and O\textsubscript{2}. ROS could cause inactivation of SOD and CAT activities lead to elevation of free radicals (O\textsubscript{2}−) and non-radical form (H\textsubscript{2}O\textsubscript{2}), as seen in the present study.

GSH-Px is an enzyme that detoxifies peroxides with GSH acting as an electron donor in the reduction reaction, producing GSSG as an end product. ROS could cause inactivation of GSH-Px activity and decreased availability of its substrate, GSH, which has been shown to be depleted during diabetes, as seen in the present study. Another view, lowered levels of GSH may also due to utilization of GSH by the GSH-Px and GST as their substrate. Also, a reduction in GSH-Px activity results in increased H\textsubscript{2}O\textsubscript{2} levels and hence severe cellular damage is observed, as seen in the present study. This data agree with other results of some authors\textsuperscript{19,26,27}.

Polyunsaturated fatty acids, when exposed to ROS, can also be oxidized to hydro-peroxides that decompose to hydrocarbons and aldehydes such as MDA. Also, the increased lipid peroxidation such as MDA in diabetes may be due to the insufficient antioxidant system\textsuperscript{7}, as seen in the present study. In diabetes, the loss of endothelium-derived NOx permits increased activity of the pro-inflammatory transcription factor nuclear factor kappa β (NF-κ β), resulting in expression of leukocyte adhesion molecules and production of chemokines and cytokines. Of great importance for pro-inflammatory cytokine toxicity in β-cells of pancreas are in particular the generation of NOx via induction of the inducible nitric oxide synthase (iNOS) and production of ROS. Cytokine-induced nitrosative and oxidative stresses trigger eventually β-cell death. In addition, β-cells of pancreas produce a large amount of NOx into circulation when exposed to pro-inflammatory cytokines, as seen in the present study.

In this research, the diabetic treated groups with \textit{A. fraxinifolius} extract (especially prophylactic treatment) showed a significant amelioration in the reduction serum CAT, GSH-Px activities, and GSH concentration through alleviating in the elevation SOD, MDA, NOx and H\textsubscript{2}O\textsubscript{2} as well as pro-inflammatory cytokines (will discuss below). The data presented revealed that \textit{A. fraxinifolius} extract offers enhanced antioxidant potential and protection against tissue lipid peroxidation. Aparadh \textit{et al.}\textsuperscript{11} showed that antioxidants are considered important nutraceuticals on account of their many health benefits and are widely used in the food industry as inhibitors of lipid
peroxidation. In addition, some flavonoids act as powerful electron scavengers of free radicals and electron donors to the $\text{H}_2\text{O}_2$ scavenging peroxidases. Abd El-Ghffar et al. reported that the nHE of *A. fraxinifolius* contains $\alpha$-tocopherols. Tocopherols are the most important lipophilic antioxidants and are believed to play a preventive role in diseases associated with oxidative stress like cancer, cardiovascular diseases and diabetes mellitus. $\alpha$-Tocopherol has a powerful antioxidant activity in detoxifying free radicals, stabilization of the cell membrane and structure restoration. Also, $\alpha$-tocopherol was reported to reduce the elevated lipid peroxidation and improve the oxidative damages. $\alpha$-Tocopherol appears to be the first line of defense against the peroxidation of polyunsaturated fatty acids that are contained in cellular and sub-cellular membrane phospholipids, because it binds to peroxyl free radicals and forms stable molecules. It also binds to a variety of active oxidant species such as singlet oxygen and superoxide free radicals. Phytol, another compound in nHE of *A. fraxinifolius* which is an acyclic diterpene alcohol, acts as a precursor for vitamin E and K1 and it has antioxidant and anti-cancer activities. The antioxidant activities of flavonoids involve ROS scavenging, transition metal ion chelation, increase of enzymatic/non-enzymatic antioxidants, and reduction of lipid peroxidation, and hence results in restoration the oxidant/antioxidant balance. A possible explanation for this effect is the antioxidant activity of *A. fraxinifolius* extract polyphenols and their redox properties that allow them to act directly as reducing agents by donating hydrogen, quenching singlet oxygen or acting as metal chelators. Also, the protective effect of *A. fraxinifolius* extract can be brought indirectly by elevating GSH. GSH protects the cell against oxidative stress by reacting with peroxides and hydro-peroxides.

The pro-inflammatory cytokines, such as IL-1b, TNF-$\alpha$ and IFN-$\gamma$, are putative mediators of the progressive loss of pancreatic $\beta$-cells in type 1 diabetes mellitus by the formation of oxygen free radicals, lipid peroxides and aldehydes. These mediators produced by infiltrating macrophages, Th1, and monocytes, in infiltrated islets $\beta$-cells and cause impaired function and ultimately cell death by apoptosis or necrosis. On the other hand, the release of anti-inflammatory cytokines, in particular IL-4, IL-13 and IL-10, is related to the protection of pancreatic $\beta$-cells and the prevention of destructive insulitis. In addition, hyperglycemia induced pathways get together to elevate the level of NF-k$\beta$, a pro-inflammatory master key, which activates pro-inflammatory cytokines gene expressions and apoptosis cascade leading to programmed cell death of islet $\beta$-cells and increase oxidative stress. IL-4 is a pleiotropic cytokine produced by Th2 cells, mast cells and NK cells as well as other specialized subsets of T cells, basophils and eosinophils. It suppresses the production of Th1 cells and enhances the synthesis/the production of tissue inhibitors of MMPs; and hence suppresses MMPs activation; and reduced NOx production has been considered an important element for this beneficial effect. IL-10 is an anti-inflammatory cytokine that identified as an inhibitor of IFN-$\gamma$ synthesis in Th1 cells, IL-10 is an important immune-regulatory cytokine. It inhibits the synthesis of a number of cytokines involved in the inflammatory process including IL-2, IL-3, GM-CSF, TNF-$\alpha$ and IFN-$\gamma$. Based on its cytokine-suppressing profile, it also functions as an inhibitor of Th1 cells. Anti-inflammatory cytokines have been thought to be upstream regulators that control the progression of diabetes negatively. Souza et al. reported that the anti-inflammatory cytokines counteracted the cytotoxic effects of pro-inflammatory cytokines in insulin-producing cells. This was achieved through the reduction of nitrosative stress. Thus, a balance between the anti-inflammatory/pro-inflammatory cytokines is of crucial importance for the prevention of $\beta$-cell destruction. Moreover, Dudhgaonkar et al. demonstrated the suppression of the oxidative stress and inflammatory response related to diabetes through the inhibition of pro-inflammatory cytokines. Other studies have revealed that treatment of mice model of type 1 diabetes with IL-4 delays the onset of spontaneous diabetes and reduces its incidence.

In the present study, anti-inflammatory and pro-inflammatory cytokines were increased and decreased, respectively, in the alloxan plus *A. fraxinifolius*. Abd El-Ghffar et al. reported that the nHE of *A. fraxinifolius* contains triterpenes. The anti-inflammatory properties of triterpenes may be attributed to inhibit the transcription of pro-inflammatory genes by blocking the transactivation of NF-k$\beta$, so these compounds acts as anti-inflammatory. The oxidative stability that is induced by *A. fraxinifolius* extract may mediate a down regulation of NF-k$\beta$ activation, which results in the suppression of the inflammatory cascade and the low concentrations of TNF-$\alpha$ and IL-6 that were observed.
Also, the anti-inflammatory effect of *A. fraxinifolius* extract may be related to steroid compounds which is considered as anti-inflammatory agents.\(^\text{12}\)

**Conclusion**

From the above mentioned results we can conclude that oral administration of *A. fraxinifolius* extract might reduce the risk of type 1 diabetes, perhaps through its antioxidant/anti-inflammatory action. Prophylactic-treatment with *A. fraxinifolius* extract showed a better protective action against alloxan-induced diabetes mellitus than therapeutic treatment group did. The modulatory effect of *A. fraxinifolius* was partial but significant, and dose dependent.

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