Can ursolic acid be beneficial against diabetes in rats?
Ursolik asit sıçanlardaki diyabete karşı yararlı olabilir mi?

Abstract

Objective: Diabetes mellitus, a heterogeneous metabolic and chronic disease, is a growing health problem especially in developing countries. It is claimed that diabetes associated with increased formation of free radicals and decrease in antioxidant potential and also alterations in lipid profile and enzyme levels. Ursolic acid is commonly used in traditional Chinese medicine due to its beneficial effects. The aim of this study was to investigate the effects of ursolic acid on streptozotocin-induced diabetes in Wistar albino rats.

Methods: DNA damage was evaluated in the blood and liver cells of rats by alkaline comet assay. The activities of antioxidant enzymes, oxidative stress parameters, biochemical parameters, hepatic enzyme levels and lipid profile parameters were also evaluated.

Results: The results of this study demonstrate that diabetes caused genotoxic damage, changes in hepatic enzyme and lipid profile, biochemical and antioxidant enzyme activities and oxidative stress parameters in rats. Ursolic acid was found to be protective against diabetes induced effects in blood and liver samples of rats.

Conclusions: According to our results, it seems that ursolic acid may be beneficial against diabetes and its adverse effects in rats.

Keywords: Diabetes; DNA damage; Oxidative stress; Hepatic enzymes; Lipid profile; Ursolic acid.

ÖZET


Metod: DNA hasarı sıçanların kan ve karaciğer hücrelerinde alkali comet yöntemiyle değerlendirildirilmiştir. Antioksidan enzimlerin aktiviteleri, oksidatif stres göstergeleri, biyokimyasal parametreler, hepatik enzim düzeyleri, lipit profil göstergeleri de değerlendirilmiştir.

Bulgular: Bu çalışmanın sonuçları sıçanlarda diyabetin genotoksik hasara, hepatik enzimlerde ve lipit profilinde, biyokimyasal ve antioksidan enzim aktivitelerinde ve oksidatif stres parametrelerinde değişikliğe neden olduğunu göstermiştir. Ursolik asidin sıçanların kan ve karaciğer örneklerinde diyabetin neden olduğu etkileri karşı koruyucu olduğu bulunmuştur.

Sonuç: Sonuçlarımızda göre, ursolik asidin sıçanlardaki diyabet ve diyabetin istenmeyen etkilerine karşı yararlı olabileceği görülmektedir.
Introduction

Diabetes mellitus, a heterogenous metabolic and chronic disease, is caused by an absolute or relative lack of insulin. It is a growing health problem in most countries, especially in developing countries [1]. Despite advances in understanding the management of the disorder, the mortality and morbidity due to this disease is increasing. Approximately 150 million people have diabetes mellitus worldwide, and this number may be doubled by the year 2025 [2].

The previous studies have shown that alloxan and streptozotocin (STZ) induced diabetes leads to changes in body hemostasis [3]. It is claimed that diabetes is associated with increased formation of free radicals and decrease in antioxidant potential. In general terms, common diabetic complications may be due to increased or uncontrolled oxidative activity. Also, it is known that diabetes causes some alterations in lipid profile and hepatic enzymes [4].

The studies on diabetes therapy has unwanted effects in human life e.g. lifestyle changes lead to reduced physical activity, and increased obesity [5]. Most of antidiabetic drugs have high costs, limited efficacy and tolerability and/or significant side effects [6]. Patients often use alternative forms of therapy such as herbal medicines especially in West Africa, Central America and Asia [7].

Plants often contain various amounts of phenolics, flavonoids and tannins. Most of the studies are focused on the antidiabetic effects of these phytochemicals due to their antioxidant properties [8]. For example, epidemiological studies have associated a diet rich in isoflavones with a lower risk of diabetes and diabetes related complications [9]. However, the studies about the antidiabetic activity of plants and plant derived compounds are limited.

Ursolic acid (3β-hydroxy-12-urs-12-en-28-oic acid) is a well-known pentacyclic triterpene which is commonly used in traditional Chinese medicine (Figure 1). Malus pumila, Ocimum basilicum, Vaccinium spp., Vaccinium macrocarpon, Olea europaea, Origanum vulgare, Rosmarinus officinalis, Salvia and Thymus plants are the main sources of ursolic acid [10]. In recent years, interest in ursolic acid has increased due to its various beneficial effects and low toxicity. Ursolic acid has been used against different diseases including osteoarthritis, rheumatoid arthritis, ulcer, cancer and diabetes [11]. Ursolic acid has been suggested to increase insulin levels with the preservation of pancreatic β-cells and modulate blood glucose levels in diabetic mice [12]. In our previous in vitro study, we showed the antioxidant capacity of ursolic acid by trolox equivalent antioxidant capacity (TEAC) assay [13].

In this paper, protective role of ursolic acid on diabetes induced oxidative damage in the blood and liver cells of rats was evaluated. To determine the DNA damage, Comet assay was used. The activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GSH-Px) and oxidative stress parameters including 8-hydroxy-2-deoxyguanosine (8-OHdG), total glutathione (GSH) and malondialdehyde (MDA) levels in the plasma and liver tissues; the hepatic enzyme parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) levels in the serum; insulin levels in the plasma and blood lipid profiles such as triglyceride, total cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) levels were also measured to investigate the effects of ursolic acid on diabetes induced damage STZ, a drug well known to cause diabetes in vivo with changes in blood lipid and hepatic enzyme profiles [3].

Materials and methods

Chemicals

The chemicals used in the study were purchased from the following suppliers: normal melting agarose (NMA) and low melting point agarose (LMA) from Boehringer Manheim (Mannheim, Germany); sodium chloride (NaCl),
sodium hydroxide (NaOH), and potassium chloride (KCl) from Merck Chemicals (Darmstadt, Germany); dimethyl sulfoxide (DMSO), ethidium bromide (EtBr), Triton-X-100, phosphate-buffered saline (PBS) tablets, ALT assay kit, AST assay kit, GGT assay kit, insulin assay kit, bicinchoninic acid (BCA) protein kit, HDL-LDL assay kit, total cholesterol assay kit, triglyceride assay kit, streptozotocin (STZ) and ursolic acid from Sigma-Aldrich Chemicals (St Louis, MO, USA); ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA-Na2), sodium lauroyl sarcosinate, and Tris from ICN Biomedicals Inc. (Aurora, OH, USA), 8-OHdG assay kit, SOD assay kit, CAT assay kit, GR assay kit, GSH-Px assay kit and GSH assay kit from Cayman Chemicals Co. (Ann Arbor, MI, USA), ketamin hydrochloride from Eczacıbaşı (İstanbul, Türkiye).

**Animals**

Male Wistar albino rats (180–250 g, totally 24 rats) were used in all experiments. They were housed in plastic cages with stainless steel grid tops. Rats were maintained on a 12 h light-dark cycle, at controlled temperature (23 ± 2 °C) and humidity (%50). Animals were fed with standard laboratory chow and allowed to access feed and drinking water ad libitum. The blood glucose levels of animals were measured via glucometer (Plusmed) before the experiments. The animals were treated humanely and with regard for alleviation of suffering and the study was approved by Ankara University Animal Ethical Committee (2015-12-138).

**Streptozotocin induced diabetes mellitus model**

Wistar rats were subjected to type 1 diabetes by STZ injection (60 mg/kg in freshly prepared PBS, i.p. in 0.01 M citrate buffer pH of 4.3) as previously described [14]. After 1 week, blood samples were obtained by tail prick, and diabetes was confirmed by fasting (8 h) blood glucose value of 250 mg/dL higher using glucometer (Plusmed). Insulin and other parameters were also measured in fasting state.

Blood glucose levels and body weights in experimental groups were assessed weekly (Table 1).

**Experimental design**

The rats were divided into four groups:

1. Group 1: Sham group. Control rats treated with PBS (per oral) (n = 6).
2. Group 2: Diabetic group. Diabetic rats treated with PBS (per oral) (n = 6).
3. Group 3: Ursolic acid treated group. Control rats treated with ursolic acid (50 mg/kg b.w.) for 28 days (per oral) (n = 6).
4. Group 4: Ursolic acid treated diabetic group. Diabetic rats treated with ursolic acid (50 mg/kg b.w.) for 28 days following the induction of diabetes with STZ (per oral) (n = 6). Ursolic acid treated diabetic group (n = 6).

Ursolic acid dose (50 mg/kg b.w. per oral) was selected according to our unpublished studies. At the end of the

| Table 1: Weekly levels of blood glucose and body weights in experimental groups. |
|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| **Group 1** | **Group 2** | **Group 3** | **Group 4** |
| 0th day | | | |
| Body weight (g) | 220.9 ± 7.3 | 248.5 ± 20.9 | 269.6 ± 8.3 | 254.8 ± 20.3 |
| Blood glucose (mg/dL) | 118.9 ± 13.9b | 526 ± 96.1a | 169.2 ± 5.6b | 458.3 ± 27.8ab |
| 7th day | | | |
| Body weight (g) | 233.4 ± 11.7 | 266 ± 29.5 | 272.4 ± 3.8 | 262.6 ± 27.3 |
| Blood glucose (mg/dL) | 124.7 ± 14.0b | 425 ± 92.4a | 130 ± 12.8b | 414.8 ± 65.2a |
| 14th day | | | |
| Body weight (g) | 245.1 ± 12.6 | 225 ± 10.0 | 285 ± 3.9 | 265 ± 26.45 |
| Blood glucose (mg/dL) | 133.4 ± 13.2b | 465 ± 10.0a | 120.2 ± 11.2b | 412.8 ± 41.3ab |
| 21st day | | | |
| Body weight (g) | 249.1 ± 12.8 | 253 ± 33.1 | 288.8 ± 3.8 | 263 ± 30.3 |
| Blood glucose (mg/dL) | 120.4 ± 14.3b | 419.7 ± 141.1a | 115.8 ± 11.2b | 399.3 ± 76.5ab |
| 28th day | | | |
| Body weight (g) | 259.9 ± 13.6b | 255 ± 33.9 | 290.1 ± 4.3 | 284 ± 31.6 |
| Blood glucose (mg/dL) | 156.1 ± 29.4b | 408.5 ± 92.1a | 112.5 ± 18.6b | 382.4 ± 24.6ab |

The values are expressed as mean ± SD; a statistically different from sham group (p < 0.05); b statistically different from diabetes group (p < 0.05). Group 1: sham; Group 2: diabetic rats; Group 3: ursolic acid (50 mg/kg) treated rats; Group 4: ursolic acid (50 mg/kg) treated diabetic rats.
experimental period, all animals were decapitated under
the anesthesia (90 mg/kg ketamine hydrochloride, i.p.).
Cardiac blood was collected into preservative-free heparin
tubes. Livers were removed. The organs were examined for
changes in size, color and texture. The samples were kept in
the dark at 4°C and processed within 4 h for COMET assay.

Whole blood samples were obtained via the intracardiac
method. The serum was immediately separated by
centrifugation at 448 g for 15 min and the plasma was imme-
diately separated by centrifugation at 1792 g for 5 min at 4°C.

The serum and plasma samples and liver homo-
genates were kept in −80°C for the determination of
oxidative stress parameters and hepatic enzyme and
lipid profiles.

**Determination of biochemical parameters**

The determination of insulin, total bilirubin and BCA
protein levels in the plasma samples was performed
spectrophotometrically with assay kits (Sigma-Aldrich,
St Louis, MO, USA) at 450, 530 and 562 nm, and
results were expressed as μIU/mL, mg/dL and μg/mL,
respectively.

**Determination of liver enzyme levels**

The determination of ALT, AST and GGT levels in the
serum samples was performed spectrophotometrically (Spectramax, Molecular Devices, USA) with assay kits
(Sigma-Aldrich, St Louis, MO, USA) at 570, 450 and 418 nm,
respectively. Results were expressed as mU/mL.

**Determination of lipid levels**

The determination of HDL, LDL, total cholesterol and
triglyceride levels in the serum samples was performed
spectrophotometrically (Spectramax, Molecular Devices,
USA) with assay kits (Sigma-Aldrich, St Louis, MO, USA)
at 570 nm. Results were expressed as μg/mL for HDL, LDL
and total cholesterol and mg/dL for triglyceride levels.

**Determination of antioxidant enzymes
and oxidative stress parameters**

Oxidative stress parameters were assayed in the plasma
samples and in the liver homogenates. The liver tissues
were weighted and extracted following the homogeniza-
tion and sonication procedure [15].

The determination of CAT, SOD, GR and GSH-Px
enzyme activities, GSH and MDA levels in the plasma
samples and liver tissues and 8-OHdG levels in the plasma samples were performed spectrophotometrically
(Spectramax, Molecular Devices, USA) with a CAT, SOD,
GR, GSH-Px, GSH, 8-OHdG and MDA assay kits (Cayman
Chemicals Co., Ann Arbor, MI, USA) at 540, 440, 340, 340,
420, 420 and 535 nm, respectively. Results were expressed
as mmol/min/mg for enzyme activities, μM for GSH levels,
pg/mL for 8-OHdG levels and nmol/g for MDA levels.

**Comet assay**

The DNA damage was determined by COMET assay. For
COMET assay, blood samples and liver homogenates were
used. The liver tissues were carefully dissected from their
attachments and totally excised. Preparation of single-
cell suspension from the livers was performed accord-
ing to standard procedures [16]. Briefly, approximately
0.2 g of each organ was placed in 1 mL chilled mincing
solution (HBSS with 20 mM EDTA and 10% DMSO) in a
petri dish and chopped into pieces with a pair of scis-
sors. The pieces were allowed to settle and the superna-
tagant containing the single-cell suspension was taken.
The concentrations of renal and hepatic tissue cells in
the supernatant were adjusted to approximately 2 × 10^6
cells/mL in HBSS containing 20 mM EDTA/10% DMSO.

Alkaline Comet assay technique of Singh et al. [17], as
further described by Aydin et al. [18] and Bacanli et al. [19]
was followed.

The dried microscopic slides were stained with EtBr
(20 μg/mL in distilled water, 60 μL/slide), covered with
a cover-glass prior to analysis with a Leica® fluorescence
microscope under green light. The microscope was con-
ected to a charge-coupled device camera and a personal
computer-based analysis system (Comet Analysis Soft-
ware, version 3.0, Kinetic Imaging Ltd., Liverpool, UK) to
determine the extent of DNA damage after electrophoretic
migration of the DNA fragments in the agarose gel. In
order to visualize DNA damage, 100 nuclei per slide were
examined at 40× magnification. Results were expressed
as percent of DNA in tail (“tail intensity”).

**Statistical analysis**

Statistical analysis was performed by SPSS for Windows
20.0 computer program. Differences between the means
of data were compared by the one way variance analysis
(ANOVA) test and post hoc analysis of group differences
was performed by least significant difference (LSD) test.
Kruskal-Wallis K test followed by Mann–Whitney U-test was used in comparing the parameters displaying abnormal distribution between groups. The results were given as the mean ± SD. p Values of less than 0.05 were considered as statistically significant.

Results

Blood glucose levels

The blood glucose levels in the groups were shown in Table 1. The blood glucose levels were significantly increased in the diabetic group compared to sham group (p < 0.05). The blood glucose levels in the ursolic acid treated diabetic group were found to be significantly lower than in the diabetic group at the 1st, 14th, 21st and 28th days of treatment (p < 0.05).

Biochemical parameters

The insulin, total bilirubin and BCA protein levels in the plasma samples were shown in Table 2.

The insulin levels were significantly decreased in the diabetic group compared to sham group (p < 0.05). However, the insulin levels in the plasma increased significantly in the ursolic acid treated diabetic group compared to diabetic group (p < 0.05).

Liver enzyme levels

The serum ALT, AST and GGT levels in the groups were shown in Table 3.

The ALT, AST and GGT levels were significantly increased in the diabetic group compared to sham group (p < 0.05). The serum AST and GGT levels in the ursolic acid treated diabetic group were found to be significantly lower than in the diabetic group (p < 0.05). However, there was no significant decrease between diabetic and ursolic acid treated diabetic group in the serum ALT levels.

Lipid levels

The HDL, LDL, total cholesterol and triglyceride levels in the serum samples were shown in Table 4.

The LDL, total cholesterol and triglyceride levels were significantly higher in the diabetic group compared to sham group (p < 0.05). The LDL, total cholesterol and triglyceride levels in the serum samples in the ursolic acid treated diabetic group were found to be significantly lower than in the diabetic group (p < 0.05). The HDL levels were

Table 2: Biochemical findings of plasma samples of experimental groups.

<table>
<thead>
<tr>
<th></th>
<th>Insulin levels (μIU/mL)</th>
<th>Total bilirubin levels (mg/dL)</th>
<th>BCA protein levels (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>192.349 ± 50.998b</td>
<td>4.374 ± 1.654</td>
<td>3668.371 ± 68.988</td>
</tr>
<tr>
<td>Group 2</td>
<td>68.058 ± 35.779a</td>
<td>7.717 ± 1.821</td>
<td>3726.975 ± 25.091</td>
</tr>
<tr>
<td>Group 3</td>
<td>278.298 ± 21.942b</td>
<td>1.677 ± 0.400</td>
<td>3710.8 ± 78.864</td>
</tr>
<tr>
<td>Group 4</td>
<td>206.132 ± 31.415b</td>
<td>4.141 ± 1.141</td>
<td>3707.82 ± 25.997</td>
</tr>
</tbody>
</table>

The values are expressed as mean ± SD; a statistically different from sham group (p < 0.05); b statistically different from diabetes group (p < 0.05). Group 1: sham; Group 2: diabetic rats; Group 3: ursolic acid (50 mg/kg) treated rats; Group 4: ursolic acid (50 mg/kg) treated diabetic rats.

Table 3: Liver enzyme levels in the plasma samples of experimental groups.

<table>
<thead>
<tr>
<th></th>
<th>ALT levels (mU/mL)</th>
<th>AST levels (mU/mL)</th>
<th>GGT levels (mU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>1984.5 ± 348.3a</td>
<td>79.198 ± 24.9b</td>
<td>1926.5 ± 696.5b</td>
</tr>
<tr>
<td>Group 2</td>
<td>8457.1 ± 684.4a</td>
<td>1535.849 ± 924.8a</td>
<td>4213.3 ± 157.8a</td>
</tr>
<tr>
<td>Group 3</td>
<td>1580.6 ± 31.3a</td>
<td>99.723 ± 15.2b</td>
<td>1271.2 ± 165.9b</td>
</tr>
<tr>
<td>Group 4</td>
<td>3718.9 ± 552.1</td>
<td>716.377 ± 38.1b</td>
<td>3733.5 ± 361.1</td>
</tr>
</tbody>
</table>

The values are expressed as mean ± SD; a statistically different from sham group (p < 0.05); b statistically different from diabetes group (p < 0.05). Group 1: sham; Group 2: diabetic rats; Group 3: ursolic acid (50 mg/kg) treated rats; Group 4: ursolic acid (50 mg/kg) treated diabetic rats. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutammyl transferase.
significantly decreased in the diabetic group compared to sham group (p < 0.05). The HDL levels in the serum samples of the ursolic acid treated diabetic group were found to be significantly higher than in the diabetic group (p < 0.05).

**Antioxidant enzymes and oxidative stress parameters**

The CAT, SOD, GSH-Px enzyme activities and GSH levels were found to be significantly lower in the diabetic group compared to the sham group (p < 0.05). CAT, SOD, GSH-Px enzyme activities and GSH levels in both plasma and liver increased significantly in the ursolic acid treated diabetic group compared to diabetic group (p < 0.05). There was no significant increase between sham and ursolic acid treated diabetic group.

Plasma 8-OHdG levels and plasma and hepatic GR enzyme activities and MDA levels were found to significantly increased in diabetic group compared to the sham group (p < 0.05). However, the levels were found to significantly decrease in the ursolic acid treated diabetic group.

**Table 4: Lipid levels in the serum samples of experimental groups.**

<table>
<thead>
<tr>
<th></th>
<th>HDL levels (µg/mL)</th>
<th>LDL levels (µg/mL)</th>
<th>Cholesterol levels (µg/mL)</th>
<th>Triglyceride levels (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>0.702 ± 0.157a</td>
<td>0.325 ± 0.051a</td>
<td>0.283 ± 0.014a</td>
<td>1590 ± 260.7a</td>
</tr>
<tr>
<td>Group 2</td>
<td>0.345 ± 0.294a</td>
<td>0.522 ± 0.046a</td>
<td>0.700 ± 0.053a</td>
<td>5144.9 ± 2890.0a</td>
</tr>
<tr>
<td>Group 3</td>
<td>0.691 ± 0.183a</td>
<td>0.390 ± 0.062a</td>
<td>0.302 ± 0.040a</td>
<td>1564.8 ± 247.6a</td>
</tr>
<tr>
<td>Group 4</td>
<td>0.568 ± 0.011a</td>
<td>0.432 ± 0.121a</td>
<td>0.264 ± 0.026a</td>
<td>4425.9 ± 3211.6a</td>
</tr>
</tbody>
</table>

The values are expressed as mean ± SD; a statistically different from sham group (p < 0.05); b statistically different from diabetes group (p < 0.05). Group 1: sham; Group 2: diabetic rats; Group 3: ursolic acid (50 mg/kg) treated rats; Group 4: ursolic acid (50 mg/kg) treated diabetic rats. HDL, high density lipoprotein; LDL, low density lipoprotein.

**Table 5: Antioxidant enzyme activities and oxidative stress parameters in the plasma samples of experimental groups.**

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT activity (nmol/min/mL)</td>
<td>153.659 ± 13.276a</td>
<td>89.282 ± 34.180a</td>
<td>146.137 ± 40.449a</td>
<td>142.448 ± 10.403a</td>
</tr>
<tr>
<td>SOD activity (U/mL)</td>
<td>0.960 ± 0.661a</td>
<td>0.403 ± 0.314a</td>
<td>1.333 ± 0.115a</td>
<td>1.075 ± 0.700a</td>
</tr>
<tr>
<td>GSH-Px activity (nmol/min/mL)</td>
<td>114.456 ± 2.462a</td>
<td>42.451 ± 9.452a</td>
<td>106.222 ± 1.159a</td>
<td>115.452 ± 6.489a</td>
</tr>
<tr>
<td>GR activity (nmol/min/mL)</td>
<td>2.456 ± 0.452a</td>
<td>9.135 ± 3.458a</td>
<td>2.964 ± 1.023a</td>
<td>4.915 ± 4.372a</td>
</tr>
<tr>
<td>GSH levels (µM)</td>
<td>4.195 ± 1.259a</td>
<td>2.103 ± 0.146a</td>
<td>4.326 ± 1.056a</td>
<td>9.153 ± 2.449a</td>
</tr>
<tr>
<td>8-OHdG levels (ng/mL)</td>
<td>11.136 ± 5.961a</td>
<td>20.193 ± 7.058a</td>
<td>5.407 ± 6.130a</td>
<td>10.604 ± 8.895a</td>
</tr>
<tr>
<td>MDA levels (nmol/g)</td>
<td>11.452 ± 4.125a</td>
<td>26.478 ± 4.479a</td>
<td>11.956 ± 3.896a</td>
<td>14.230 ± 5.067a</td>
</tr>
</tbody>
</table>

The values are expressed as mean ± SD; a statistically different from sham group (p < 0.05); b statistically different from diabetes group (p < 0.05). Group 1: sham; Group 2: diabetic rats; Group 3: ursolic acid (50 mg/kg) treated rats; Group 4: ursolic acid (50 mg/kg) treated diabetic rats. CAT, catalase; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; MDA, malondialdehyde.

**Table 6: Antioxidant enzyme activities and oxidative stress parameters in the livers of experimental groups.**

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT activity (nmol/min/mL)</td>
<td>15.130 ± 2.189a</td>
<td>10.463 ± 4.711a</td>
<td>12.347 ± 4.244a</td>
<td>15.888 ± 2.055a</td>
</tr>
<tr>
<td>SOD Activity (U/mL)</td>
<td>0.451 ± 0.304a</td>
<td>0.241 ± 0.147a</td>
<td>0.367 ± 0.373a</td>
<td>0.364 ± 0.223a</td>
</tr>
<tr>
<td>GSH-Px activity (nmol/min/mL)</td>
<td>124.126 ± 2.563a</td>
<td>56.514 ± 5.466a</td>
<td>89.479 ± 6.487a</td>
<td>107.452 ± 1.125a</td>
</tr>
<tr>
<td>GR activity (nmol/min/mL)</td>
<td>3.737 ± 1.281a</td>
<td>11.382 ± 6.265a</td>
<td>4.003 ± 0.956a</td>
<td>8.099 ± 2.820a</td>
</tr>
<tr>
<td>GSH levels (µM)</td>
<td>9.938 ± 3.628a</td>
<td>5.419 ± 0.584a</td>
<td>3.198 ± 1.045a</td>
<td>8.520 ± 0.603a</td>
</tr>
<tr>
<td>MDA levels (nmol/g)</td>
<td>13.452 ± 5.128a</td>
<td>24.367 ± 4.785a</td>
<td>13.027 ± 5.487a</td>
<td>15.853 ± 6.752a</td>
</tr>
</tbody>
</table>

The values are expressed as mean ± SD; a statistically different from sham group (p < 0.05); b statistically different from diabetes group (p < 0.05). Group 1: sham; Group 2: diabetic rats; Group 3: ursolic acid (50 mg/kg) treated rats; Group 4: ursolic acid (50 mg/kg) treated diabetic rats. CAT, catalase; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; MDA, malondialdehyde.
group compared to the diabetic group \( (p < 0.05) \). There was no significant difference between sham and ursolic acid treated diabetic group in MDA levels but the 8-OHdG levels of ursolic acid treated diabetic group were significantly higher than sham group \( (p < 0.05) \).

Ursolic acid alone did not cause significant changes in all studied oxidative stress parameters, compared to sham group.

**Comet assay**

The DNA damage expressed as tail intensity in the blood and liver cells of rats was shown for Comet assay in Figure 2.

In all biological samples studied, there were no statistically significant differences in tail intensity between the sham group and the ursolic acid-treated groups \( (p > 0.05) \). The DNA damage was found significantly higher in the diabetic group compared to the sham group \( (p < 0.05) \). Ursolic acid treatment in the diabetic group was found to decrease the DNA damage significantly \( (p < 0.05) \).

**Discussion**

In the present study, we evaluated the beneficial effects of ursolic acid against STZ-induced diabetes mellitus and its effects in Wistar albino rats. We found that genotoxic damage, hepatic enzyme and lipid profile, biochemical, antioxidant enzyme and oxidative stress parameter changes are significantly altered in STZ-induced diabetic rats. Ursolic acid was found to be protective against diabetes induced effects in blood and liver samples of rats. As far as we know, our study is the first study that demonstrated the beneficial effects of ursolic acid against genotoxicity and liver enzyme alterations in diabetes.

STZ-induced hyperglycemia has been described as a notable experimental model to diabetes mellitus [20]. STZ causes a massive reduction in insulin release, by the destruction of the \( \beta \)-cells of the islets of Langerhans, thereby inducing hyperglycemia [21].

Different studies have shown that multiple factors can cause oxidative stress in diabetes. The most important factor is glucose autoxidation leading to the production of free radicals. Other factors include cellular oxidation/reduction imbalances and reduction in antioxidant defenses (including decreased cellular antioxidant levels and a reduction in the activity of enzymes that dispose of free radicals) [22]. Numerous reports have documented elevations in peroxide levels in plasma, red blood cells and tissues of animals with chemically-induced diabetes [23]. Both increases and decreases in the activities of antioxidant enzymes including CAT, SOD, GSH-Px and GR have been reported [24]. Seven et al. [25] demonstrated that SOD and GSH-Px enzyme activities and GSH levels were decreased in the livers of STZ-induced diabetic rats compared to their controls. In a study with 26 microangiopatic, 28 non-microangiopatic diabetic and 40 healthy subjects, SOD enzyme activity was found to be lower in the diabetic group [26].

MDA, a direct product of lipid peroxidation, has been identified to bind and damage DNA [27]. In a study with STZ-induced diabetic rats, it is demonstrated that thiobarbituric acid-reactive substances (TBARS) levels increased and total antioxidant activity decreased 4 weeks after from diabetes induction [28]. Martín-Gallán et al. [26] showed the increased MDA levels in diabetes patients. Also, Asayama et al. [29] demonstrated the induction in MDA levels in diabetic rats. Kushwaha et al. [30] showed that 8-OHdG levels was found significantly higher in diabetic group compared to non-diabetic group of rats. Our findings are consistent with the data of some recent studies showing the changes in oxidative stress parameters in

![Figure 2: DNA damage of the experimental groups of the rats expressed as (A) blood cells; (B) liver cells. The values are expressed as mean ± SD; *statistically different from sham group \( (p < 0.05) \); †statistically different from diabetic group \( (p < 0.05) \). Group 1: sham; Group 2: diabetic rats; Group 3: ursolic acid (50 mg/kg) treated rats; Group 4: ursolic acid (50 mg/kg) treated diabetic rats.](image-url)
diabetes. In this study we also observed that GR enzyme activities and 8-OHdG and MDA levels were significantly increased and GSH levels and CAT, SOD and GSH-Px enzyme activities were significantly decreased in diabetic rats.

The liver plays a pivotal role in glycolysis and gluconeogenesis [31]. Elevation of liver enzymes has been reported to be associated with increased risk for diabetes. Nakanishi et al. [32] reported higher GGT enzyme levels in diabetic patients. ALT and AST levels is also associated with incident diabetes [33]. In a study in Italy, it is found that GGT enzyme levels of 3124 male and females with diabetes and cardiovascular disorders were related with diabetes [34].

In our study, we found that STZ-induced diabetes caused an increase in hepatic enzyme (ALT, AST and GGT) levels and a decrease in insulin levels compared to sham group. There was no significant increase between sham and diabetic group in the total bilirubin levels in the plasma samples of rats. As far as we know, this is the first study about the correlation between diabetes and BCA protein levels and we found no significant difference between sham and diabetic group in the BCA protein levels.

Yin and Chan [35] have found that oleanolic acid and ursolic acid could inhibit in vitro formation of pentosidine and Nε-(carboxymethyl)lysine (CML) which have been implicated in the pathogenesis of diabetic nephropathy and other diabetic complications. It is concluded that, ursolic acid significantly inhibited sorbitol dehydrogenase as well as aldose reductase activity, and increased glucokinase activity. While decreasing glucose-6-phosphatase activity, it elevated the hepatic glycogen content and lowered the plasma total cholesterol, free fatty acid, and triglyceride concentrations compared with the diabetic control group. It also normalized hepatic triglyceride concentration in the livers of STZ-induced diabetic mice [36]. Similarly, it is found that ursolic acid (0.05% w/w) improved blood glucose levels, glucose intolerance, and insulin sensitivity in diabetic rats [12] and at the doses of 0.01% w/w and 0.05% w/w, it improved blood glucose, glycosylated hemoglobin, glucose tolerance, insulin tolerance and plasma leptin levels as well as aminotransferase activity in diabetic mice [37].

In our study, ursolic acid treatment was found to significantly decrease GR enzyme activities and 8-OHdG and MDA levels and significantly increase GSH levels and CAT, SOD and GSH-Px enzyme activities in diabetic rats. The insulin levels in the plasma samples of the ursolic acid treated diabetic group were found to be significantly lower than the sham group. The AST, GGT, LDL, total cholesterol and triglyceride levels in the serum samples of the ursolic acid treated diabetic group were found to be significantly lower than the diabetic group and the HDL levels in the serum samples in the ursolic acid treated diabetic group were found to be significantly higher than the diabetic group.

There are limited studies about genotoxic damage caused by diabetes mellitus. In these studies, it is claimed that reactive oxygen species (ROS) production could cause DNA damage. Kushwaha et al. [30] determined the DNA damage in lung, liver, aorta, heart, kidney, pancreas and blood samples of the experimentally induced diabetic rats by comet and endonuclease III and formamidopyrimidine (fpg) modified comet assays. They showed that DNA damage was found significantly higher in diabetic group compared to non-diabetic group.

In our previous study, we reported the antigenotoxic effect of ursolic acid against hydrogen peroxide (H$_2$O$_2$) induced oxidative DNA damage in the human lymphocytes and Chinese hamster fibroblast cells (V79) [13]. In this study, increases in the tail intensity in the blood and livers of diabetes-induced rats treated with ursolic acid determined by the standard comet assay. The DNA damage in the blood and liver cells of the diabetic rats have been found to be higher compared to the sham group. Ursolic acid (50 mg/kg/day, per oral) treatment was found to significantly decrease the DNA damage in the blood and liver cells of the diabetic rats.

Our results are in accordance with previous reports about diabetes, its complications and the effects of natural antioxidants against diabetes.

### Conclusion

The results of this study demonstrate that diabetes caused genotoxic damage, changes in hepatic enzyme and lipid profile, biochemical and antioxidant enzyme activities and oxidative stress parameters in rats. Ursolic acid was found to be protective against diabetes induced effects in blood and liver samples of rats. However, further investigations are needed to prove protective role and clinical availability of ursolic acid for diabetes mellitus therapy.

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### Conflict of interest

The authors declare that there are no conflicts of interest.
References


