Antidiabetic Efficacy of *Solanum torvum* Extract and Glycoalkaloids against Diabetes Induced Mutation in Experimental Animals

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Abstract

**Aim**: Estimation of the antihyperglycemic activity of pure glycoalkaloids and *Solanum torvum* leaf extract obtained from Giza district, Egypt. Besides, to study the antihyperlipidemic and antigenotoxicity activities associated with diabetic syndrome.

**Method**: Mice were turned diabetic by 200 mg/kg i.p. alloxan administration twice. Animals with fasting blood glucose levels (FBG) ≥ 400 mg/dl were included. Plant materials (100 mg/kg) were orally administered to diabetic mice. Daonil was used as standard drug at dose 30 mg/kg. Phytochemical constituents were analyzed using HPLC & LC/MS/MS of the methanolic extract and pure glycoalkaloids.

**Results**: The analysis revealed presence of glycoalkaloids, saponins, flavonoids and coumarins. Biochemical investigation showed significant increase in FBG, glycosylated hemoglobin and lipid profile in diabetic mice. Likewise, increase in somatic and germ cells mutation. Results showed significant reduction in FBG in treated mice, besides improvement in the HB and HB A1C. Treated mice recorded significant decrease in total lipids, triglycerides, T. cholesterol, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol. The amelioration effect of glycoalkaloids as hypoglycemic agents was higher than total leaf extract. Plant treatments also, illustrated a significant inhibition in elevated DNA damage induced by DM regarding chromosomal aberrations in somatic and germ cells at the tested dose and duration. Meanwhile, Daonil showed no significant inhibition in DM induced mutation.

**Conclusion**: Both *S. torvum* leaves extract and pure glycoalkaloids possessed potent antidiabetic effectiveness in addition to remarkable anti mutagenic and antihyperlipidemic efficacy. Current outcomes could be instructors for natural antidiabetic drugs production to dominate health riskiness conducted from diabetes syndrome.

**Key words**: *S. torvum*, Glycoalkaloids, Diabetes, Lipid panel; Mutation.

INTRODUCTION

Diabetes mellitus (DM) is a widespread chronic disease affecting humankind of all ages and obliges a large economic load on healthcare system. DM is dangerous metabolic disorder that associated with serious health disruption as cardiovascular and renovascular problems [1]. Evidence link cancer incidence with diabetes and glycosylated hemoglobin could be considered as cancer risk marker [2]. It was reported that diabetes is a risk factor in colorectal, colon adenomas and hepatocellular carcinoma [3-5]. Diabetic persons have a higher recurrence of cardiovascular, neurological diseases, myocardial infarction, stroke, renal failure and severe vision complications [6]. Diabetes is also connected to gene mutation as mutations of mitochondrial DNA [7]. Hypercholesterolemia, hyperlipidemia, elevated triglycerides, oxidative stress and diabetic vessel troubles are reported to be related to hyperglycemia [8,9]. DM is characterized by chronic increase of blood glucose, which is a major element in the metabolism of reactive oxygen species (ROS) that, in turn, enhances cellular injury and participates in the development and progression of complications associated with diabetes [10]. Particularly, pancreatic β-cells are oversensitive to damage by oxidative stress when exposed to chronic hyperglycemia because they are low in free-radical repress enzymes [11]. ROS can suppress the response to insulin and share in the evolution of insulin resistance, a clef characteristic pathological sign of type 2 DM [12]. It is known that glucose intolerance results from either decrease of insulin secretion or deficiency in its action or both [8]. Thence, enough glycemic control is base for prohibiting complications related with type 2 diabetes. Dense glucose-lowering process is found to decrease the risk of microvascular endpoints in type 2 diabetic patients by up to 25% [13].

Unfortunately, oral hypoglycemic agents in current use have severe side effects, especially sulfonlureas and are connected with higher cancer rate [14]. On the other hand, medicinal plants could be an alternative safer and more effective antidiabetic remedy. Some plants of genus *Solanum* have been used in folk medicine as antidiabetic [9]. *S. torvum* is an edible plant known as wild or pear eggplant and has wide range of pharmaceutical effects as antiviral, analgesic, anti-inflammatory, antioxidant, anti-platelet aggregation and DNA-conservation power, moreover is used as antidiabetic [15]. In the current work, comprehensive study of the effect of *S. torvum* leaf methanolic extract and isolated glycoalkaloids are undertaken to correlate between the hypoglycemic efficacy and active metabolites. Different effects on the biochemical parameters of diabetic and treated mice besides antimutagenic influence against diabetes-induced genotoxicity are studied.

MATERIAL AND METHODS

**Plant Materials**

*Solanum torvum* Swartz (Solanaceae) leaves were collected at the flowering period (April- May) from medicinal plant farm, Faculty of Pharmacy, Cairo University at Giza, Egypt. The plant was identified by the director of the farm and Prof. Zakaria Fouad at the Agricultural and Biological Division, National Research Centre, Egypt.

**Chemicals and Equipments**

Standard solasonine, solamargine and solasodine (Sigma Co.); Alpha-solanie (Roth Co.); DPPH• (Sigma Co.); Vitamin C (Fluka Co.); Daonil "glibenclamide of chemical name 5-chloro-N-[2-[4-((cylohexy carbamoylsulfanyloxy)phenyl)ethyl]-2-methoxybenzamide" (Sanofi-aventis Co.).

HPLC: Hewlett Packard series 1050, with UV detector. C18 column (5 μm, 0.4×25 cm), FR. 0.9 ml/min.

ESR: Bruker, Elexys, X-band modulation frequency, 500 MHz, the sample inserted via quartz liquid flat cell, average scans 1, average sampling time (s) 0.04096, state of aggregation C, field Mod. Amplitude 0.0002, field Mod. Frequency (Hz) 100,000 microwave frequency (Hz) 9.77568e + 09, microwave power (w) 0.00202637, receiver gain 65, receiver harmonic 1.

Mass spectrometry: LC-MS/MS 4000 QTRAP, AB SCIEX, Foster City, USA.
Light microscope; Olympus, Saitama, Japan, Eyepiece: 25X, Oil, objective: 100X.
Accu-Check: Active, Roche, UK
Autoanalyzer: Dimension Clinical Chemistry System, Dimension Max. Germany.

LC/MS/MS and HPLC analyses of secondary phytochemicals
Leaves were shade dried, crushed and macerated in methanol three times (1x 3eacch 50 ml) until complete exhaustion. Macerated materials were filtered and the combined filtrate was evaporated under reduced pressure and temperature using rotator evaporator. LC/MS/MS analysis of the methanol extract was performed at, Agricultural Research Centre, Egypt; Regional Center for food and Feed (RCFF, ARC). Mass spectra were measured using both –ve and +ve modes by adapting hybrid triple quadrupole/linear ion trap mass spectrometer. Mobile phase: Methanol: water: formic acid (70: 30: 0.1 %), Spectral range: 100: 1100 (mz, Da).
For HPLC analysis, the methanol residue (1mg dissolved in 1 ml methanol HPLC grade) was filtered through 0.45 μ Millipore membrane filter then analyzed by HPLC in triplicates using isocratic mobile phase (40 % methanol: 60 % H2O). Flow rate; 0.9 ml/min. Column temperature; 32°C.Wavelength; 210 nm. Pressure; 6 bars. Glycoalkaloids were identified by comparison with standards at the same analytical conditions.
Pure glycoalkaloids were separated by acid base precipitation. The methanicl residue was acidified by 5% acetic acid then treated with 25% ammonia till pH 10; the mixture was allowed to cool to deposit glycoalkaloids.

Free radical holding potential
Free radical scavenging power of the methanolic extract and isolated glycoalkaloids was established using Electron spin resonance technique (ESR) [16]. Diphenylpicrylhydrazyl (DPPH•) was the target free radical. Solanine and Vit. C were utilized as standard antioxidants.
MDPΦH10⁻³ (1 ml) was added to 1 mg of solanine or 1 mg of the methanolic extract or 1 mg of the glycoalkaloids. Decrease of DPPH• area designated the antioxidant activity. Measurements were taken after 5 minutes.
% of antioxidant activity = A0-A1 / A0 x100
A0 = Area of DPPH•
A1 = Area of tested sample + DPPH•

Biological Study
Experimental design
Swiss albino male mice of 10-12 weeks (20-25 g) obtained from the National Research Center, Cairo, Egypt. Animals were kept under oversight for one week before experiment and fed standard pellet diet and water ad libitum. Handling and experimental procedures were carried out according to NRC ethical committee instructions for animal care and use (registration No: 16 445).
Plant material was tested for safety before start by oral administration of the chosen tested dose to mice for 30 days and found to be within normal range biochemical indices regarding liver function (alanine aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, total bilirubin, total protein ), kidney function (uric acid, blood urea, creatinine) and tumor markers ( alpha feto-protein).
The duration of the experiment for antidiabetic evaluation was one month from starting time. Mice were divided into six groups (five mice /each) as follow:
Group 1: Control (untreated).
Group 2: Alloxan induced diabetic mice (a double i.p injection 3 days interval at 200 mg/kg, in distilled water). After 72 h from last injection, animals with fasting serum glucose levels ≥ 400 mg/dl were considered diabetic and included in the study. Animals were then kept for the next 24 hours on 5% glucose to prevent hypoglycemia.
Group 3 (a and b): Mice received oral S. torvum methanol leaf extract and pure glycoalkaloids at dose 100 mg/kg which equal 1/10 safe dose on solanine base [17].
Group 4: Mice received oral Daonil as standard treatment at dose 30 mg/ kg which equivalents to 10 mg daily human dose of pure glibenclamide [18].
Group 5 (a and b): Diabetic mice received oral total S. torvum extract and glycoalkaloids (100 mg/kg).
Group 6: Diabetic mice received oral Daonil (30 mg/kg).

Separation of Blood Serum and analysis of biochemical parameters
At the end of experiment, animals were killed by decapitation after an overnight fasting. Portion of collected blood was centrifuged at 3000 rpm for 20 min to obtain the serum, which was kept in deep-freezer for analysis. Another portion of blood was kept for hematological studies.
Serum fasting blood glucose (FBG) was measured using Accu-Check diabetic test strips. For confirmation, FBG was also assayed on analyzer using Flex reagent cartridges, supplied by Dade Behring, Germany.
The onset of action and duration of antidiabetic activities of both plant materials and Daonil were determined.
Hemoglobin Hb % was determined according to the adopted method [19].
Glycosylated Hemoglobin (HbA1c) was assayed on autoanalyzer “Hb Gold Analyzer”, using Gold Reagent Kit- HbA1c, provided by Drew Scientific Ltd., Germany.
Bioassay of lipid panel was carried out according to the reported methods [19]. The analyzed parameters included total lipids, triglycerides, total cholesterol, HDL-Cholesterol and Low-density lipoproteins- cholesterol (LDL & VLDL- cholesterol). Statistical analysis was done by Duncon's Method. The data of diabetic group was compared to control group. The data of treated groups were compared to both control and diabetic groups.

Mutagenicity Study
Chromosome preparations from bone marrow (somatic cells) and spermatocyte (germ cells) were carried out according to the adapted protocol [20]. Hundred well spread metaphases were analyzed per mouse. Metaphases with abnormalities were recorded using light microscope (100X).
Evaluation of the effect of S. torvum extract, glycoalkaloids and Daonil to inhibit DNA damage induced in diabetic group was carried out applying the following equation:
Inhibitory index (II) = [1 - (Plant extract plus diabetic group – control) / (diabetic group - control)] X 100.
Significance of the differences from the untreated control data and between plant materials plus diabetic group compared to diabetic group was calculated using t-test.

RESULTS AND DISCUSSION
HPLC and LC/MS/MS analyses
HPLC analysis revealed the presence of solasoline, solanine and solamargine glycoalkaloids in addition to solasodine aglycone in S. torvum leaf extract (Fig.1). The results were confirmed through LC/MS/MS analyses of the methanolic extract by comparing fragmentation data including (M+H)⁺ and (M-H)⁻ with that of previously recorded compounds and average molecular weights (Mw expressed as g/mol) are listed. LC/MS/MS analyses illustrated the presence of glycoalkaloids solasoline, solamargine and or solanine together with solasodine (Table 1 & Fig. 2). The results disclosed the presence of flavonoids and coumarins phenolics (Table 1 & Fig. 3).The existed compounds were;
neochlorogenin 6-O-β-rhamnopyranosyl-
illustrated presence of spirostanol saponins; saponin 1: VLDL (T. cholesterol, Total lipid, Cholesterol), were illustrated in the methanol extract as scopoletine, methyl caffeate, and kaempferol. Table 2: LC/MS/MS data of S. torvum leaf methanol extract and main detected compounds.

**Table 1:** LC/MS/MS data of S. torvum leaf methanol extract and main detected compounds

<table>
<thead>
<tr>
<th>Glycoalkaloids</th>
<th>MW [M-H]+</th>
<th>[M+H]+</th>
<th>Fragments m/z</th>
<th>Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>884</td>
<td>883</td>
<td>855</td>
<td>solasamine</td>
</tr>
<tr>
<td>2</td>
<td>868</td>
<td>867</td>
<td>869</td>
<td>solamargine and/or solanine</td>
</tr>
<tr>
<td>3</td>
<td>413</td>
<td>-</td>
<td>414</td>
<td>solasodine</td>
</tr>
</tbody>
</table>

**Flavonoids**

| 4              | 302       | 301    | 303           | quercetin |
| 5              | 286       | 285    | 287           | kaempferol |
| 6              | 610       | 609    | 611           | rutin |
| 7              | 194       | 193    | 195           | methyl caffeate |

**Coumarins**

| 8              | 192       | 191    | 193           | scopoletine |
| 9              | 206       | 205    | 207           | scopolamine |
| 10             | 216       | 215    | -             | bergapten and/or xanthotoxin |

**Saponins**

| 11             | 578       | 577    | -             | saponin 1 |
| 12             | 710       | 709    | 711           | saponin 2 |
| 13             | 724       | 723    | 725           | saponin 3 |
| 14             | 576       | 575    | 577           | saponin 4 |
| 15             | 722       | 721    | 723           | saponin 5 |
| 16             | 726       | 725    | 727           | saponin 6 |
| 17             | 740       | 739    | 741           | saponin 7 |

**Free sugars**

| 18             | 180       | 179    | 181           | glucose |
| 19             | 164       | 163    | 165           | galactosyl |
| 20             | 342       | 341    | 343           | galactosyl |

**Table 2:** Effect of S. torvum extract, glycoalkaloids and Daonil on biochemical parameters in non treated mice and after treatments of diabetic mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Leaf ext.</th>
<th>Glycoalkaloids</th>
<th>Daonil</th>
<th>diabetic mice</th>
<th>Leaf ext. treated diabetic mice</th>
<th>Glycoalkaloids treated diabetic mice</th>
<th>Daonil treated diabetic mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total lipid (mg/dl)</td>
<td>330.45 ± 20.6</td>
<td>327.10 ± 11.35</td>
<td>325.75 ± 4.82</td>
<td>322.63 ± 3.47</td>
<td>650.26 ± 22.06 *</td>
<td>408.66 ± 19.3b</td>
<td>388.87 ± 17.55 a</td>
<td>411.63 ± 7.89 ab</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>72.42 ± 14.98</td>
<td>70.3 ± 2.78</td>
<td>73.35 ± 2.64</td>
<td>69.6 ± 5.6</td>
<td>296.7 ± 5.95 *</td>
<td>135 ± 4.87 ab</td>
<td>129.82 ± 8.64 a</td>
<td>130 ± 4.61 ab</td>
</tr>
<tr>
<td>T. cholesterol (mg/dl)</td>
<td>94.89 ± 13.24</td>
<td>93.3 ± 4.68</td>
<td>87.95 ± 3.31</td>
<td>92.5 ± 4.7</td>
<td>306.55 ± 11.01 a</td>
<td>145.3 ± 6.55 a</td>
<td>138.42 ± 6.84 ab</td>
<td>161.85 ± 3.71 a</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>44.64 ± 8.35</td>
<td>39.46 ± 4.71</td>
<td>40.62 ± 4.57</td>
<td>42.48 ± 2.52</td>
<td>81.57 ± 0.35 a</td>
<td>65.5 ± 3.41 ab</td>
<td>55.92 ± 0.46 ab</td>
<td>67.34 ± 4.27 ab</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>36.51 ± 7.21</td>
<td>35.78 ± 4.63</td>
<td>37.07 ± 0.32</td>
<td>37.62 ± 3.8</td>
<td>167.65 ± 6.18 a</td>
<td>53.25 ± 0.21 ab</td>
<td>60.27 ± 0.86 ab</td>
<td>69.83 ± 6.79 a</td>
</tr>
<tr>
<td>VLDL-cholesterol (mg/dl)</td>
<td>14.37 ± 4.57</td>
<td>13.9 ± 2.50</td>
<td>14.20 ± 1.34</td>
<td>14.12 ± 2.32</td>
<td>59.65 ± 2.11 a</td>
<td>27.9 ± 0.50 ab</td>
<td>24.67 ± 0.38 ab</td>
<td>31.88 ± 1.42 ab</td>
</tr>
<tr>
<td>B. Glucose (mg/dl)</td>
<td>79.33 ± 11.12</td>
<td>77.54 ± 5.34</td>
<td>75.49 ± 3.86</td>
<td>76.9 ± 3.1</td>
<td>460.33 ± 10.12 a</td>
<td>190.55 ± 5.71 a</td>
<td>124.92 ± 8.21 a</td>
<td>109.63 ± 5.81 a</td>
</tr>
<tr>
<td>Hb Alc %</td>
<td>5 ± 0.4</td>
<td>4.9 ± 0.1</td>
<td>4.79 ± 0.2</td>
<td>4.8 ± 0.6</td>
<td>13.5 ± 0.47 a</td>
<td>7.3 ± 0.05 ab</td>
<td>6.9 ± 0.24 ab</td>
<td>7.83 ± 0.34 ab</td>
</tr>
<tr>
<td>Hb %</td>
<td>13.8 ± 0.6</td>
<td>13.9 ± 0.4</td>
<td>13.7 ± 0.8</td>
<td>13.6 ± 0.6</td>
<td>10.6 ± 0.8</td>
<td>13.1 ± 0.28 ab</td>
<td>12 ± 0.31 ab</td>
<td>11.8 ± 0.4 a</td>
</tr>
</tbody>
</table>

a: significant compared to untreated group (P < 0.01); b: significant compare to diabetic group (P < 0.01). Each value represents the mean of 5 mice's ± S.E.
Table 3: Percentage of aberrations in bone marrow cells of diabetic and treated groups with S. torvum extract, glycoalkaloids (100 mg/kg) and Daonil (30 mg/kg).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total Abnormal Metaphases</th>
<th>No. of Different Types of Abnormal Metaphases</th>
<th>Inhibitory Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (%) ± SE</td>
<td>G. Frag. and/or Br. Del. Polyp. Excluding Gaps</td>
<td>(II)</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------------------------</td>
<td>-----------------------------------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>I. Control</td>
<td>19</td>
<td>3.80 ± 0.55</td>
<td>9</td>
</tr>
<tr>
<td>II. DM</td>
<td>56</td>
<td>15.20 ± 0.65</td>
<td>12.20 ± 0.48</td>
</tr>
<tr>
<td>III. Solanum a)</td>
<td>25</td>
<td>5.0 ± 0.55</td>
<td>10</td>
</tr>
<tr>
<td>IV. Daonil</td>
<td>27</td>
<td>5.40 ± 0.40</td>
<td>3.60 ± 0.72</td>
</tr>
<tr>
<td>V. Solanum + DM</td>
<td>33</td>
<td>6.60 ± 0.70</td>
<td>4.20 ± 0.75</td>
</tr>
<tr>
<td>VI. Daonil + DM</td>
<td>65</td>
<td>13.00 ± 0.55</td>
<td>10.60 ± 0.50</td>
</tr>
</tbody>
</table>

**Number of examined metaphases** = 500 (100 metaphase/animal, 5 animals/group); **G.**: Gap; **Frag.**: Fragments; **Br.**: Breaks; **Del.**: Deletions; **Polyp.**: Polyploidy.

Table 4: Percentage of spermatocyte abnormalities of diabetic and treated groups with S. torvum extract, glycoalkaloids (100 mg/kg) and Daonil (30 mg/kg).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total Abnormal Metaphases</th>
<th>No. of different types of abnormal metaphases</th>
<th>Inhibitory index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (%) ± SE</td>
<td>XY-uni. Auto.uni. XY-uni.+ Auto.uni. Chain (IV)</td>
<td>(II)</td>
</tr>
<tr>
<td>I. Control</td>
<td>14</td>
<td>2.80 ± 0.40</td>
<td>9</td>
</tr>
<tr>
<td>II. DM</td>
<td>65</td>
<td>13.00 ± 0.45</td>
<td>41</td>
</tr>
<tr>
<td>III. Solanum a)</td>
<td>19</td>
<td>3.60 ± 0.52</td>
<td>13</td>
</tr>
<tr>
<td>IV. Daonil</td>
<td>26</td>
<td>5.20 ± 0.48</td>
<td>15</td>
</tr>
<tr>
<td>V. Solanum + DM</td>
<td>43</td>
<td>8.60 ± 0.50</td>
<td>26</td>
</tr>
<tr>
<td>VI. Daonil + DM</td>
<td>50</td>
<td>10.80 ± 0.42</td>
<td>32</td>
</tr>
</tbody>
</table>

**Number of examined metaphases** = 500 (100 metaphase/animal, 5 animals/group); **XY-uni.**: XY-univalent; **Auto.uni.**: Autosomal univalent; **XY-uni.+ Auto.uni.**: XY-univalent + Autosomal univalent.

Free radical capturing activity
Current data demonstrated that both leaf methanolic extract of S. torvum and glycoalkaloids possessed promising free radical scavenging activity. The present results illustrated higher antioxidant capacity of total methanolic extract (81.77 %) compared with pure glycoalkaloids (60.69 %). Standard solanine has had 44 % activity at concentration 1 mg/ml each against DPPH. 

Biochemical investigation
Total lipids, triglycerides and total cholesterol were measured in the serum to evaluate the role of S. torvum extract and glycoalkaloids on changes in these glycemic dominance parameters at the treatment period on normal mice and diabetic treated ones. The results illustrated elevation of all biochemical parameters in diabetic group compared to normal one. The results also, recorded elevation of fasting blood glucose (FBG), hemoglobin (Hb) and glycosylated hemoglobin (HbA1c) levels in hyperglycemic mice compared to normal mice. The results are shown in Table 2. 

Table 2 also, illustrates that treated normal mice with total extract, glycoalkaloids and Daonil had no significant differences compared to control untreated mice regarding all biochemical parameters. While, diabetic group were significantly different compared to control group with respect to all tested parameters. Whereas, treatment of diabetic mice with leaf extract, glycoalkaloids and Daonil induced significant decrease in elevated fasting blood glucose, glycosylated hemoglobin and lipid profile. The onset of action for both leaf extract and glycoalkaloids was 3± 0.17 h and lasting for 18± 0.29 h, whereas, their hypoglycemic activities declined after 20± 0.43 h. On the other hand, Daonil onset of action was 1± 0.14 h and lasting for 24± 0.23 h after which, the action declined.

Genotoxicity analyses
Percentage of inhibition of aberrations in bone marrow cells
Table 3 and Figure (5) show different types and percentage of abnormalities in all groups. Diabetic-group induced a high percentage of aberrations. S. torvum extract and glycoalkaloids treated groups at 100 mg/kg b.wt at the treatment period were statistically non-significant comparing to control. On the other side, Daonil at 30 mg/kg b.wt induced statistically significant aberrations in comparison with control. Diabetic mice received oral total S. torvum extract and glycoalkaloids (100 mg/kg b.wt) had the ability to reduce in statistically significant the number of aberrations. Leaf extract was more effective than glycoalkaloids in reduction of abnormalities, it reached to 45 % comparing to 33 % for glycoalkaloids. Meanwhile, Daonil did not induce statistically significant inhibition in the percentage of aberrations comparing to the diabetic group at the same period.

Percentage of inhibition of abnormalities in spermatocyte cells
Table 4 and Figure (6) show that diabetic group induced a high percentage of aberrations. While, percentage of aberrations in animal groups treated with S. torvum extract and glycoalkaloids (100 mg/kg b.wt) at the treatment period were close to control. Meanwhile, Daonil group at 30 mg/kg b.wt induced statistically significant aberrations comparing to control. Administration of leaf extract and glycoalkaloids to diabetic mice reduced in statistically significant number of abnormalities. Also, Daonil at 30 mg/kg b.wt induced statistically significant inhibition in aberrations comparing to diabetic group.
**Figure 1.** HPLC chromatogram of *S. torvum* where 1: solasonine, 2: solanine, 3: solamargine and 4: solasodine.

**Figure 2.** Glycoalkaloids detected by LC/MS/MS of *S. torvum* leaf methanol extract where: solasonine (A), solanine(B), solamargine (C) and solasodine (D).

**Figure 3.** Flavonoids and coumarins phenolics detected by LC/MS/MS of *S. torvum* leaf methanol extract where: rutin (A), methyl caffeate (B), kaempferol (C), quercetin (D), scoparone (E), scopoletin (F), bergapten (G) and xanthotoxin (H).
**Figure 4.** Saponins detected by LC/MS/MS of *S. torvum* leaf methanol extract where: Saponin 1 (A), Saponin 2 (B), Saponin 3(C) Saponin 4(D), Saponin 5 (E), Saponin 6 (F) and Saponin 7 (G).

**Figure 5.** Chromosomal aberrations in bone marrow cells of alloxan induced diabetic mice showing (a) normal, (b) fragment and (c) break.

**Figure 6.** Chromosomal abnormalities in diakinase - metaphase 1 cells in alloxan induced diabetic mice showing (a) normal, (b) autosomal univalent and (c) XY univalent
The current study has made the antihyperglycemic activity of *S. torvum* leaf extract and glycoalkaloids a focus of attention. The significant antidiabetic effects of *S. torvum* leaf extract and that of pure glycoalkaloids fraction in addition to their antimutagenic activities against diabetes-induced genotoxicity from the literature survey are the first. Besides, monitoring the glucose lowering capacity and enduring antidiabetic effect of *S. torvum* leaf extract and glycoalkaloids alone in the current search is newly studied. As well, the establishment of the antimutagenic activities of *S. torvum* leaf methanol extract and intact glycoalkaloids versus alloxan induced diabetes and associated mutation is a distinct novel study. As new findings also, is the pronounced lowering effects of *S. torvum* and glycoalkaloids extracts on glycated hemoglobin, besides the decrease in lipid panel achieved hereinafter which are likewise new flash points in controlling diabetes hazards especially cardiovascular risk. The data also, clarified no hazards effects of *S. torvum* and pure glycoalkaloids extracts regarding biochemical parameters and bone marrow & spermatocite chromosomes at the tested doses.

Concerning phytochemical investigation, results elucidated from LC/MS/MS data demonstrated the existence of spirotanol saponins, which are consistent with previously defined ones [22]. The present outcomes indicated the ability of *S. torvum* extract and glycoalkaloids to capture free radical reactive species DPPH. The predominant scavenging power of the methanolic extract in the present study might be due to additive influence of phytochemical groups that could be considered as premium provenance of antioxidants in the extract. Running results are in close agreements with oxidative hemolysis and lipid peroxidation inhibition of *S. torvum* fruit water extract [23]. Also, the presence of solasone, solanine and solamargine glycoalkaloids detected in the methanol extract could participate in the observed antioxidant activity. In the same line with current data, *S. tuberosum* callus methanol extract and *S. lacinum* glycoalkaloids were reported as antioxidants due to solandine and solasodine glycoalkaloids [16, 24] As well, *S. nigrum* showed strong antioxidant capacity [25]. Antioxidant activity and flavanol & glycoalkaloid contents of *S. lycopersicum* were also correlated [26]. Controversially to present results, hydroethanolic and aqueous extracts of *S. sessiliflorum* exhibited low antioxidant activity [27].

Regarding antihyperglycemic activity, current data demonstrated pronounced hypoglycemic activity of *S. torvum* leaf extract and glycoalkaloids that significantly (p < 0.01) decreased blood sugar in diabetic mice at treatment duration (Table 2). Percentages of blood glucose levels reduction were 58.61 and 72.86 %, respectively comparing with 76.18 % reduction upon Daonil treatment. Results of present study illustrated that *S. torvum* extract and glycoalkaloids enhanced the glycemic dominance, demonstrated by the significant lowering in both FBG and HbA1c in all measurements in the duration of study, compared to conformable hyperglycemia control group. This reflects fastness of *S. torvum* extract and glycoalkaloids short-term improvement in glycemic control. No reports about the significance antihyperglycemic activity of pure glycoalkaloids from *Solanum* species were notified. Another remarkable outcome is the determination of the onset of action (3h for plant materials and 1h for Daonil) as well as determination of duration of activities (18h for plant materials and 24h for Daonil). These results are of importance for determination of dose repetition. The decrease in HbA1c in present results which, reached 45.93 and 48.89% for the leaf and glycoalkaloids extracts (Table 2), is an additional novel influential outcome. Elevated glycosylated hemoglobin is considered as cancer risk marker [2]. It was reported that in clinical experiment, a 1 % decrease in HbA1c reduced cardiovascular complications by 14% [28].

Hypoglycemic activity of *S. torvum* leaf extract on diabetic treated mice, encountered herein, agrees with previous studies conducted on streptozotocin induced diabetic rats treated with fruit extract of the plant that appeared β-cells regeneration and reduction of glucose levels (17.04 - 42.10 %) at much higher doses; 200, 400 mg/kg, respectively [29]. However, in the present study lower dose of the leaf methanol *S. torvum* extract and pure glycoalkaloids(100mg/kg of diabetic mice) that compromise 1/2 and 1/4 the above mentioned reported doses, induced extremely higher glucose lowering effects (58.61 and 72.86 %, respectively) which considered unprecedented outcome. The obvious variation of hypoglycemic effect in our study and reported one could be virtue of higher concentrations of active ingredients in leaves utilized in the current study comparing to that of the fruits formerly applied due to intraspecific diversity that partitioned within and among plant individuals and because of ecological context. Plant maturation and environmental and genetic diversity are other influential agents. Also, genetic factor in experimental modeling performs critical role. While no report concerning the effect of pure glycoalkaloids was undertaken.

Antioxidant activity herein, could partially account for the observed antidiabetic activity. Oxidative stress is causative agent for emerge of diabetes and its complications besides induction of diabetes by alloxan might be associated with cytotoxic free radical release to β cells of pancreatic islets ; the most fastidious to reactive free radicals due to their lack of free-radical inhibiting enzymes [12, 30]. Also, free radicals may cause emerge of insulin resistance [12]. Present antidiabetic activity of glycoalkaloids is in good agreement with previous reports returned the antidiabetic and antihypertensive activity of *S. lycocarpum* to solasodine and solamargin glycoalkaloids [31]. Our results are in consistence with previous studies owed the antidiabetic activity of *S. nigrum* extract to antioxidant activity [32]. In spite that free radical scavenging capacity is an effective element influencing antidiabetic activity, current data demonstrated that hypoglycemia is not parallel to the antioxidant activity. Although, leaves methanol extract displayed higher antioxidant activity comparing to glycoalkaloids, it exhibited less antidiabetic efficacy. Thus, present study clarified that antioxidant capacity could partially account for the antihyperglycemic activity. However, there are other agents that could influence diabetic disorders as enhanced glucose tolerance, inflammation, insulin control activity and β-cell functional efficacious [33].

Antidiabetic effect of leaf extract in the present search could result from active metabolites present in addition to the glycoalkaloids. Presence of saponins, flavonoids and coumarins identified through LC/MS/MS in the current analyses could attribute to melatonin extract hypoglycemic effect. Saponins were reported to be perfect treatment of hyperglycemia [34]. Triterpenoid saponins may exert antidiabetic activity via increase insulin secretion and enhance β-cells [35]. Likewise, Glucose regulation, insulin stimulation, decreasing resistance to insulin and reducing oxidative stress could be enhanced by flavonoids uptake [36]. Methyl caffeate detected in the LC/MS/Ms spectra could attribute to the hypoglycemic effect via pancreatic β-cells regeneration [21]. Besides, flavonoids and saponins are considered as the antidiabetic agents in medicinal plants used in folk medicine [37]. The detected coumarins might account for the eventual hypoglycemic effect of leaf extract. This is in agreement with studies demonstrated that *Urtica dentate* coumarins have antidiabetic and anti-renal lesion activities in rats [38]. The increment of hypoglycemic activity of glycoalkaloids comparing to the methanol extract might be due to presence of other compounds having no antidiabetic activity in the extract. Besides
presence of glucose and sucrose free sugars in the methanol extract could restrain the effect of antidiabetic constituents. This is consistent with fact that glucose intake increases serum glucose concentration [39]. Also, sucrose could participate in insulin resistance and hyperglycemia development [40]. Sucrose is found in S. tovum as natural content [41]. While glucose could result from enzymatic hydrolysis by solanum β-glucosidase [42]. With respect to lipid panel, current results illustrated significant increase (p < 0.01) in triglycerides and cholesterol profiles in diabetic mice comparing to normal ones (Table 2). Elevation of lipid profile causes complicated health problems. It is reported that patients who had elevated LDL cholesterol levels are at high cardiovascular risk [43]. Elevated level of fasting triglyceride ≥ 150 mg/dl is one of accepted signs of cardiovascular diseases high risk [44]. Increased levels of lipoprotein perform independent risk factor for coronary heart disease alone or combined with increased cholesterol and LDL-cholesterol. Current data showed that treatment with plant materials induced significant decrease (p < 0.01) in total lipids, triglycerides, T. cholesterol, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol (Table 2). Thus, solanum plant materials could help to overcome the hazards of elevated lipid panel induced by diabetic syndrome especially cardiovascular risk. The results illustrated decrease in Hb level in diabetic mice at the tested period that might lead to anemia by time. Anemia is risk factor for cardio vascular disease and all-cause death rate in diabetes in persons who have chronic kidney disease [45]. The data, showed amelioration in HB levels upon treatment. The increment of the enhancement Hb level induced by methanol extract might be due to presence of iron detected in LC/MS/MS analysis (Mw: 56 Da) as S. tovum fruit accumulates high concentration of iron and is considered as hematinic supplier [46]. Regarding mutation study, the genotoxic activity of S. tovum and glycoalkaloids extracts were performed using chromosomal aberrations, a sensible protocol for DNA damage detection. Our data illustrated elevation in DNA damage induced by DM causing significant increase in chromosomal aberrations in somatic and germ cells in diabetic mice comparing to untreated ones (p < 0.01), which is compatible with previous findings [47]. Elevation in frequency chromosomal aberrations might be affected by oxidative stress and glutathione concentrations in DM subject [48]. Likewise, gene polymorphisms of antioxidant and DNA repairing genes were reported to influence DNA damage [49]. Significant novel findings obtained through this study demonstrated that S. tovum extract and glycoalkaloids had the capability to induce significant decrease (p < 0.01) of chromosomal aberrations frequency, which resulted from DM in mice bone marrow and spermatocytes. The plant materials displayed higher effectiveness comparing to glibenclamide that included XY- univalent, autosomal univalent; XY -univalent + breaks, deletions and polyploidy. Spermatocyte amelioration from diabetic mice bone marrow and spermatocytes.  The plant materials displayed higher effectiveness comparing to glibenclamide that was lasting for 18 h, which is important for determination of doses administration time is newly supervised. The significant repression of the glycosylated HB of diabetic mice by S. tovum and glycoalkaloids extracts (45.93 and 48.89 % reduction) is another remarkable novel outcome in addition to amendment of lipid profile. Another important new finding is that S. tovum extract and glycoalkaloids induced antimitogenic modulation effects at the tested dose and period against diabetes related mutation with inhibitory indices 45and 33 for bone marrow aberrations & 48 and 39 for spermatocytes mutation comparing with 16 and 36 for glibenclamide treatment, respectively. The data also, clarified no hazards effects of S. tovum and glycoalkaloids extracts at the tested doses. Built upon these outcomes, effective and secure antidiabetic medication from plant origin could be conducted from S. tovum leaf methanol and pure glycoalkaloids extracts. Besides, it is significantly important to seek for good blood glucose monitoring natural agents to prevent long-term complications of insulin-dependent and non insulin-dependent diabetes syndrome. Recently, searching for safe and efficacious medicinal plants, possessing antidiabetic, antiglycotoxic and antioxidant activities is very important for therapy of complications of chronic diabetes.

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1331


