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

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Abstract

Aim: Estimation of the antihyperglcemic 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 oblige a large economic load on health care system. DM is dangerous metabolic disorder that associated with serious health disruption as cardiac 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 recrudescence 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 2diabetes. 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 sulfonylureas 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-solanine (Roth Co.); DPPH• (Sigma Co.); Vitamin C (Fluka Co.); Daonil "glibenclamide of chemical name 5-chloro-N-[2-[4-(cyclohexylcarbamoylsulfamoyl) 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 3each 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 % H_2O). 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 methanolic 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 ($DPPH^{\bullet}$) was the target free radical. Solanine and Vit. C were utilized as standard antioxidants.
$MDPPH^{\bullet}10^{-3}$ (1 ml) was added to 1 mg of solanine or 1 mg of the methanolic extract or 1mg of the glycoalkaloids. Decrease of $DPPH^{\bullet}$ area designated the antioxidant activity. Measurements were taken after 5 minutes.
% of antioxidant activity = A_0-A_1 / A_0×100
A_0 = Area of $DPPH^{\bullet}$
A_1 = Area of tested sample + $DPPH^{\bullet}$

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.
Groups 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 autoanalyzer 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 solasonine, 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 solasonine, 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;

quercetin, kaempferol, rutin and methyl caffeate Coumarins as well, were illustrated in the methanol extract as scopoletine, scoparone and bergapten. Regarding saponins, comparing LC/MS/MS current data with previous reported information illustrated presence of spirostanol saponins; saponin1: neochlorogenin 6-O-β-D-quinovopyranoside, saponin2: neochlorogenin 6-O-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranoside, saponin3: neochlorogenin 6-O-α-L-rhamnopyranosyl-(1→3)-β-D-quinovopyranoside, saponin 4: solagenin 6-O-β-D-quinovopyranoside, saponin 5: solagenin 6-O-α-L-rhamnopyranosyl-(1→3)-β-D-quinovopyranoside, saponin 6: neosolaspigenin 6 - O- [β -D-xylopyranosyl-(1→3)- O - β -D-quinovopyranoside and saponin 7: neosolaspigenin 6- O -[α -L-rhamnopyranosyl- (1 →3)- O - β -D-quinovopyranoside (Table 1 & Fig. 4).

LC/MS/MS data also, disclosed the presence of free sugars in the methanol extract which were glucose, rhamnose and sucrose.

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

Groups	MW	$[M-H]^-$	$[M+H]^+$	Fragments m/z	Compounds
Glycoalkaloids					
1	884	883	885	807$[M+H-C_6H_6]^+$, 722 $[M+H-Rha]^+$,576 $[M+H- -Gal]^+$,415 $[M+H-Rha-Gal-Glu]^+$	solasonine solamargine and / or solanine solasodine
2	868	867	869	833$[M+H-2H_2O]$+, 791$[M+H-C_6H_6]^+$, 722 $[M+H-Rha]^+$, 576 $[M+H-2Rha]^+$	
3	413	-	414	415$[M+2H]^+$, 416	
Flavonoids					
4	302	301	303	283 $[M-H-H_2O]^-$, 255$[M-H- 46]^-$, 211$[M-H- 90]^-$,179, 151	quercetin kaempferol rutin methyl caffeate
5	286	285	287	269[M+ $H-H_2O]^+$, 209$[M+H- C_6H_6]^+$, 163$[M+H- C_6H_6$-46$]^+$, 257, 243	
6	610	609	611	533$[M+H-H_2O]^+$,449$[M+H- Glu]^+$, 302,274, 269, 137,118	
7	194	193	195	167[M+H-CO]+, 165$[M+H-CH_2O]$+,151$[M+H-CO_2]$+, 136$[M+H- CO_2CH_3]$+, 116$[M+H-C_6H_6]^+$	
Coumarins					
8	192	191	193	175$[M+H-H_2O]^+$, 165$[M+H-CO]^+$, 133 $[M+H-COCH_3OH]^+$, 119, 94, 81	scopoletine scoparone bergapten and/ or xanthotoxin
9	206	205	207	189$[M+H-H_2O]^+$, 163$[M+H-CO_2]$, 135$[M+H-72]^+$, 129$[M+H-C_6H_6]^+$,117$[M+H-CO-2OCH_3]^+$	
10	216	215	-	197$[M-H-H_2O]^-$, 171$[M-H-CO_2]^-$	
Saponins					
11	578	577	-	541$[M-H-2H_2O]^-$, 331$[M-H-Qui]^-$, 287$[M-H-Qui-144]^-$, 269$[M-H-Qui-144-H_2O]^-$	saponin 1
12	710	709	711	579$[M+H-Xyl]^+$, 433$[M+H-Xyl-Qui]^+$, 289$[M+H-Qui-144]^+$, 271$[M+H-Qui-144-H_2O]^+$	saponin 2
13	724	723	725	579$[M+H-Rha]^+$, 433$[M+H-Rha-Qui]^+$, 289$[M+H-Rha-Qui-144]^+$, 271$[M+H-Rha-Qui-144-H_2O]^+$	saponin 3
14	576	575	577	559$[M+H-H_2O]^+$, 499$[M+H-C_6H_6]^+$, 431$[M+H-Qui]^+$, 269$[M+H-Qui -144-H_2O]^{+,}$	saponin 4
15	722	721	723	577$[M+H-Rha]^+$, 431$[M+H-Rha-Qui]^{-+}$,269$[M+H- Qui-144-H_2O]^+$	saponin 5
16	726	725	727	709$[M+H-H_2O]^+$, 595$[M+H-Xyl]^+$, 449$[M+H-Xyl-Qui]^+$, 289$[M+H-Xyl-Qui-144]^+$	saponin 6
17	740	739	741	723$[M+H-H_2O]^+$,663$[M+H-C_6H_6]^+$, 449$[M+H-Xyl-Qui]^+$, 289$[M+H-Rha-Qui-144]^+$	saponin 7
Free sugars					
18	180	179	181	161 $[M-H-H_2O]^-$, 135 $[M-H-CO_2]$, 119, 101, 71	glucose
19	164	163	165	1 35$[M-H-CO]^-$,119$[M-H-CO_2]^-$	rhamnose
20	342	341	343	305 $[M-H-2H_2O]^-$, 179,161, 119, 101	sucrose

Qui: Quinovosyl, Xyl: Xylosyl, Rha: Rhamnosyl, Gal: Galactosyl, Glu: glucosyl

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

Treatment	Control	Leaf ext.	Glycoalkaloids	Daonil	diabetic mice	Leaf ext. treated diabetic mice	glycoalkaloids treated diabetic mice	Daonil treated diabetic mice
Total lipid (mg/dl)	330.45 ± 20.6	327.10 ± 11.35	325.75 ± 4.82	322.63 ± 3.47	650.26 ± 22.06 [a]	408.662 ± 19 [ab]	388.87 ± 17.55 [ab]	411.63 ± 7.89 [ab]
Triglycerides (mg/dl)	72.42 ± 14.98	70.3 ± 2.78	73.35 ± 2.64	69.6 ± 5.6	296.7 ± 5.95 [a]	135 ± 4.87 [ab]	129.82 ± 8.64 [ab]	130.5 ± 4.61 [ab]
T. cholesterol (mg/dl)	94.89 ± 13.24	93.30 ± 4.68	87.95 ± 3.31	92.5 ± 4.7	306.55 ± 11.01 [a]	145.3 ± 6.55 [ab]	138.42 ± 6.84 [ab]	161.85 ± 3.71 [ab]
HDL-cholesterol (mg/dl)	44.64 ± 8.35	39.6 ± 4.71	40.62 ± 4.57	42.48 ± 2.52	81.57 ± 0.35 [a]	65.5 ± 3.41 [ab]	55.92 ± 0.46 [ab]	67.34 ± 4.27 [ab]
LDL-cholesterol (mg/dl)	36.51 ± 7.21	35.78 ± 4.63	37.07 ± 0.32	37.62 ± 3.8	165.75 ± 6.18 [a]	53.25 ± 0.21 [ab]	60.27 ± 0.86 [ab]	69.83 ± 6.79 [ab]
VLDL-cholesterol (mg/dl)	14.37 ± 4.57	13.9 ± 2.50	14.20 ± 1.34	14.12 ± 2.32	59.65 ± 1.21 [a]	27.9 ± 0.50 [ab]	24.67 ± 0.38 [ab]	31.88 ± 1.42 [ab]
B. Glucose (mg/dl)	79.33 ± 11.12	77.54 ± 5.34	75.49 ± 3.86	76.9 ± 3.1	460.33 ± 10.12 [a]	190.55 ± 5.71 [ab]	124.92 ± 8.21 [ab]	109.63 ± 5.81 [ab]
Hb A1c %	5 ± 0.4	4.9 ± 0.1	4.79 ± 0.2	4.8 ± 0.6	13.5 ± 0.04 [a]	7.3 ± 0.05 [ab]	6.9 ± 0.24 [ab]	7.83 ± 0.38 [ab]
Hb %	13.8 ± 0.6	13.9 ± 0.4	13.7 ± 0.8	13.6 ± 0.62	10.6 ± 0.8 [a]	13.1 ± 0.28 [b]	12 ± 0.31 [ab]	11.8 ± 0.4 [ab]

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**).

Treatments	Total Abnormal Metaphases			No. of Different Types of Abnormal Metaphases				Inhibitory Index Excluding Gaps (II)
	No.	Mean (%) ± SE Including Gaps	Excluding Gaps	G.	Frag. and/or Br.	Del.	Polyp.	
I. Control	19	3.80 ± 0.55	2.00 ± 0.50	9	7	3	0	-
II. DM	76	15.20 ± 0.65 [a]	12.20 ± 0.48 [a]	16	54	5	2	-
III. Solanum a) Leaf extract	25	5.0 ± 0.55	3.0 ± 0.60	10	13	2	0	-
b) glycoalkaloids	27	5.40 ± 0.40	3.60 ± 0.72	9	14	4	0	-
IV. Daonil	33	6.60 ± 0.70[ab]	4.20 ± 0.75 [ab]	12	18	3	0	-
V. Solanum + DM a) leaf extract	51	10.20 ± 0.58 [ab]	7.60 ± 0.45 [ab]	13	31	6	1	45
b) glycoalkaloids	60	12.00 ± 0.48[ab]	8.80 ± 0.72[ab]	16	37	4	3	33
VI. Daonil + DM	65	13.00 ± 0.55[a]	10.60 ± 0.50[a]	12	46	7	0	16

Number of examined metaphases= 500 (100 metaphase/animal, 5 animals/group); G.: Gap; Frag.: Fragments; Br.: Breaks; Del.: Deletions; Polyp: Polyploidy. a: Significant compared to -ve control (p < 0.01); b: Significant compared to diabetic group (p < 0.01); t-test.

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

Treatments	Total Abnormal Metaphases		No. of different types of abnormal metaphases				Inhibitory index (II)
	No.	Mean (%) ± SE	XY-uni.	Auto.uni.	XY-uni.+ Auto.uni.	Chain (IV)	
I. Control	14	2.80 ± 0.40	9	5	0	0	0
II. DM	65	13.00 ± 0.45[a]	41	21	2	3	0
III. *Solanum* a) Total extract	19	3.60 ± 0.52	13	6	0	0	0
b)glycoalkaloids	26	5.20 ± 0.48	15	9	0	0	0
IV. Doanil	32	6.40 ± 0.55[a]	20	12	0	0	0
V. *Solanum* + DM a)Total extract	43	8.60 ± 0.50[ab]	26	15	1	1	48
b)glycoalkaloids	48	9.60 ± 0.58[ab]	31	17	0	0	39
VI. Daonil + DM	50	10.00 ± 0.42[ab]	32	15	3	0	36

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;
a: Significant compared to -ve control (p < 0.01); b: Significant compared to diabetic group (p < 0.01); t-test.

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$^{\bullet}$

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 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 mg/kg b.wt induced statistically significant inhibition in aberrations comparing to diabetic group

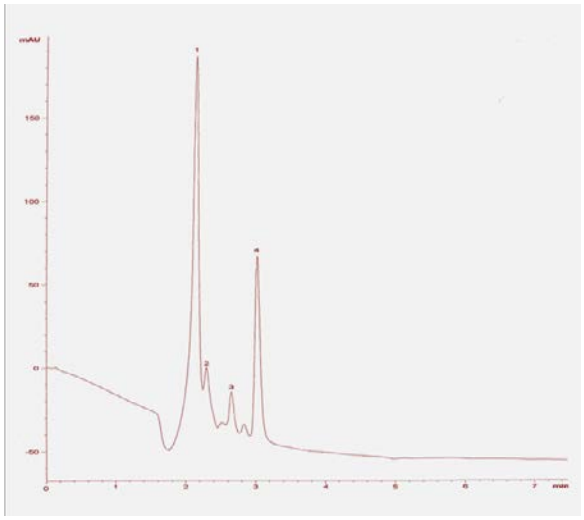

Figure 1. HPLC chromatogram of *S. torvum* where 1: solasonine, 2: solanine, 3: solamargine and 4: solasodine.

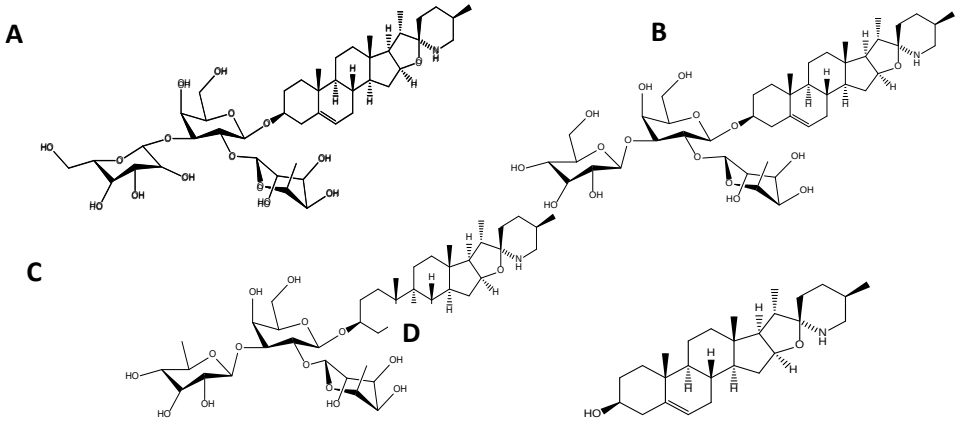

Figure 2. Glycoalkaloids detected by LC/MS/MS of *S. torvum* leaf methanol extract where: solasonine (A), solanine(B), solamargine (C) and solasodine (D).

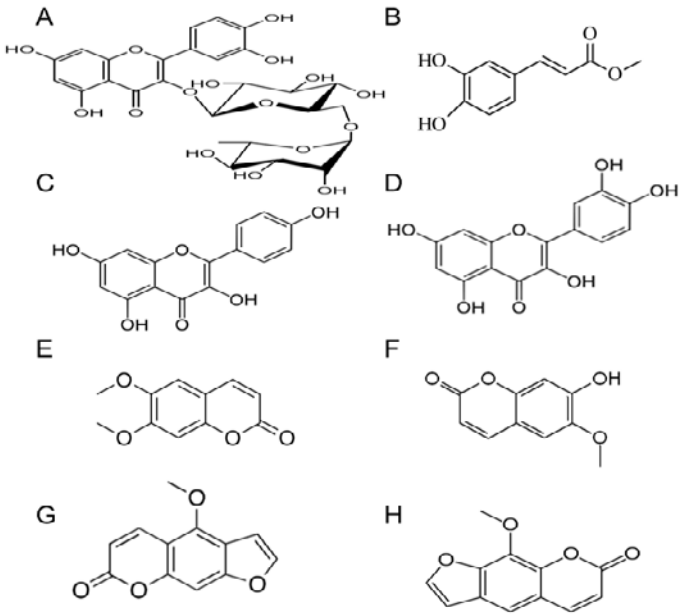

Figure 3. Flavonoids and coumarins phenolics detected by LC/MS/MS of *S. torvum* leaf methanol extract where: rutin (A), methyl caffeate (B), kaempferol (C), quercetin (D), scoparone (E), scopoletin (F), bergapten (G) and xanthotoxin (H)

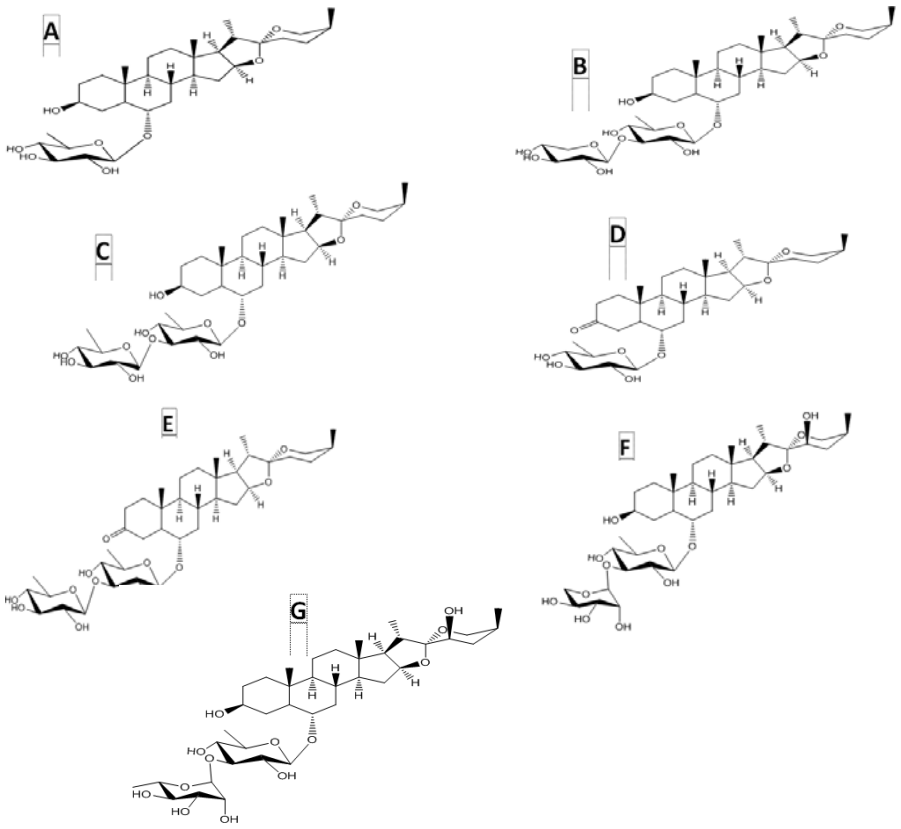

Figure 4. Saponins detected by LC/MS/MS of *S. torvum* leaf methanol extract where:
Saponin 1 (A), Saponin 2 (B), Saponin 3(C) Saponin 4(D), Saponin 5 (E), Saponin 6 (F) and Saponin 7 (G).

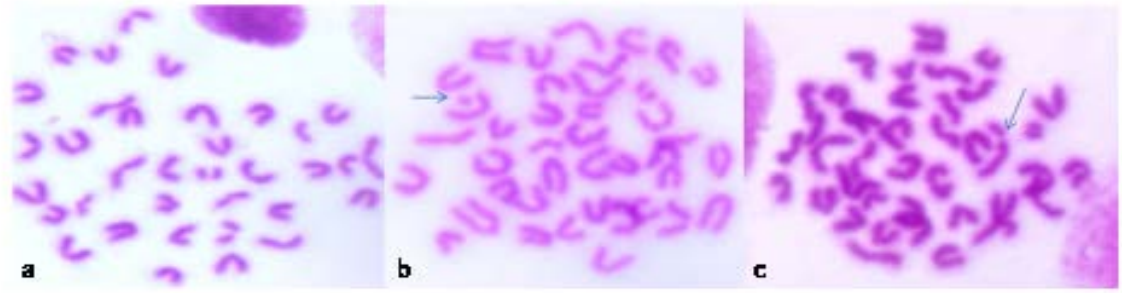

Figure 5. Chromosomal aberrations in bone marrow cells of alloxan induced diabetic mice showing
(a) normal, (b) fragment and (c) break.

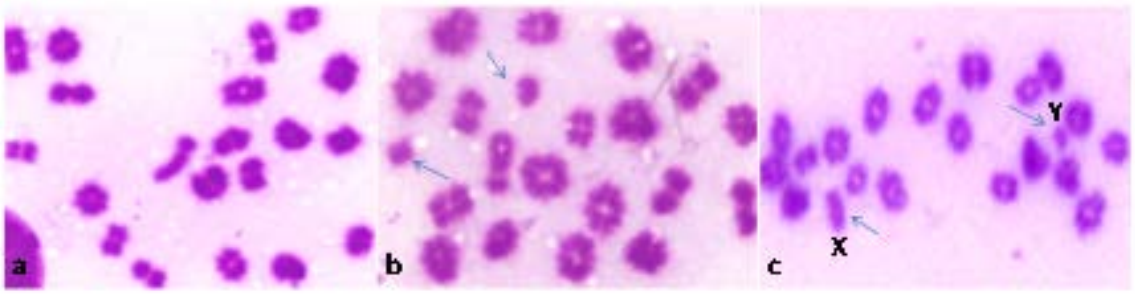

Figure 6. Chromosomal abnormalities in diakinase - metaphase 1 cells in alloxan induced diabetic mice showing (a) normal, (b) autosomal univalent and (c) XY univalent

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 aloxan 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 glycosylated 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 & spermatocyte chromosomes at the tested doses.

Concerning phytochemical investigation, results elucidated from LC/MS/MS and HPLC analyses illustrated presence of characteristic solanum glycoalkaloids [15].The results disclosed presence of flavonoids in agreements with that reported [21,22]. LC/MS/MS data demonstrated the existence of spirostanol 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 $DPPH^{\bullet}$. 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 solasonine, 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. laciniatum* glycoalkaloids were reported as antioxidants due to solanidine 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 antihyperglcemic 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 antihyperglcemic 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 ß 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 solasonine and solamargine 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 antihyperglcemic 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 methanolic 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 cafeate 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. torvum* 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. torvum* fruit accumulates high concentration of iron and is considered as hematinic supplier [46].

Regarding mutation study, the antigenotoxic activity of *S. torvum* 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. torvum* 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 appeared from higher inhibitory indices which was 45,33and 16 for *S.torvum* leaf extract, glycoalkaloids and glibenclamide, respectively for bone marrow aberrations. Meanwhile, for spermatocytes abnormalities, the inhibitory indices were 48, 39 and 36, respectively, The chromosomal amendment covers all examined bone marrow aberrations including gaps, fragments, breaks, deletions and polyploidy. Spermatocyte amelioration included XY- univalent, autosomal univalent; XY-univalent + autosomal univalent and chain abnormalities. The results also indicated that *S. torvum* and glycoalkaloids extracts at 100 mg/kg b.wt at the tested treatment period were not mutagenic (Tables 3 and 4).

Thus our results are compatible with previous findings which illustrated that *S. lycocarpum* contained solasonine and solamargine had no mutagenic activity and exerted significant reduction of doxorubicin induced chromosomal aberrations in mice [50]. The same outcomes were recorded on studying solamarine from *S. tuberosum* that displayed neither mutagenic nor genotoxic effects but exhibited antimutagenic activity against nitro-phenylenediamine direct mutagen [49]. No available datum about antigenotoxic effect of S. torvum extract on DM induced DNA damage was observed. Reports documented only that *S. torvum* has the potency to prevent nephrotoxicity, hepatotoxicity and testicular toxicity induced by doxorubicin [52].The ability of *S. torvum* extract to improve DNA genotoxicity might be due to scavenging capacity of active oxygen species and /or enhancement of antioxidant defense enzymes [52].

The present study has characteristic importance whereas intense glucose management decrease kidney, cardiovascular and eye hazards [28]. Additionally, synthetic drugs as sulfonylureas to which Daonil belongs, might induce severe harms as possibility of higher cancer rate incidence and deterioration of insulin secretion [14, 53].

CONCLUSION

Going through current results and discussion, it is clear that important novel findings were demonstrated including, that both *S. torvum* leaves extract and glycoalkaloids possessed the adequacy to reduce significantly elevated fasting blood glucose level which reached 58.61 and 72.86 % reduction. Tracing the duration effects of the administered doses of *S. torvum* leaf extract and glycoalkaloids that were 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. torvum* 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.torvum* extract and glycoalkaloids induced antimutagenic 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.tor*vum and glycoalkaloids extracts at the tested doses. Built upon these outcomes, effective and secure antidiabetic medication from plant origin could be conducted from *S.torvum* 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, antigenotoxic and antioxidant activities is very important for therapy of complications of chronic diabetes.

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