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Antidiabetic, cytotoxic, antioxidant and antitrematodal medicinal efficacy of polar and non-polar phytochemicals of *Balanites aegyptiaca* Del.

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ABSTRACT

Objective: To investigate the biological activities of polar and non-polar extracts of *Balanites aegyptiaca* fruits.**Methods:** Antihyperglycemic activity using alloxan induced diabetic rats was evaluated. *In vitro* cytotoxicity against human carcinoma cell lines activity in addition to antioxidant and antitrematodal effects were investigated. Phytochemicals were determined using chromatographic and spectral analyses including TLC, GC and LC/MS/MS methods.**Results:** The reduction in blood glucose level reached 64.13%, 69.07% and 77.01% for hexane, chloroform and methanol extract, respectively. Isolated organs and histopathological examination illustrated improvement in treated animal's pancreas which is the master gland in controlling glucose level. The highest *in vitro* free radical scavenging capacity was achieved by chloroform extract (75.72%). Meanwhile, hexane and methanol extracts exhibited 44.01% and 41.77% scavenging capacity, respectively. Cytotoxic activity against human carcinoma cell lines illustrated efficacious influence against brain, liver, lung, breast and lymphoblastic leukemia cell lines. Brain cell line was the most susceptible cell line by both chloroform and methanol extracts. *In vitro* antitrematodal effectiveness showed that 100% mortality of *Schistosoma* worms was induced at the 3rd and 5th days for methanol and chloroform extracts. Meanwhile, both extracts exhibited antifasciolosis activity with LC₅₀ of 63.19 and 55.15 mg/L, respectively.**Conclusions:** The current results illustrated that *Balanites aegyptiaca* phytochemicals induced potent hypoglycemic activity and potent to moderate antioxidant and cytotoxic effects. Chloroform and methanol extracts were found to have antitrematodal efficacy against *Schistosoma mansoni* and *Fasciola gigantica* hepatic worms.

1. Introduction

During the last decades, drug resistance has been emerged as major problem opposing synthetic drugs. This challenge stimulated researchers all over the world to search for other alternatives. Medicinal plants represented the first and most important target for study. Ethnopharmacological survey is the key factor in studying the treasure of natural resources. *Balanites aegyptiaca* Del. (Family Balantiaceae) (*B. aegyptiaca*), to the greatest extent is one of the most substantial plants that have been used traditionally. It is reported that *B. aegyptiaca* has been used remarkably in ancient Egypt and in Egyptian traditional medicine as well as in Sahara of Africa; the fruits are used as antihyperglycemic medication[1,2].

The fruits are used orally to treat hyperglycemia. Besides, the seeds are utilized as anthelmintic and purgative; seed oil is used as tumors and wounds remediation and bark is used as curative of syphilis and round worm contagion[3]. Furthermore, these ailments are combined with serious problems. Diabetes is incorporated with cardiac and renovascular complications[4]. Diagnoses of bilharziasis implicate gastroenteritis, hepatitis A, B, and C, HIV, salmonellosis, and urinary tract infection[5]. Fascioliasis may lead to liver lesions due to liver parenchyma penetration by fasciola larvae and secretion of larvae in bile ducts[6]. Cancer and its treatment include serious involvements such as endocrine abnormalities, hematologic disorders and malignant effusions, which are common problems[7]. Therefore, there is an urgent need for natural treatments development based on the present ethnopharmacological knowledge.

Consequently, the aim of the current work is to verify the biological activities of the polar and non-polar constituents of *B. aegyptiaca* as a promising medicinal plant for producing natural medications for such risky diseases especially after synthetic drugs hazards awareness.

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2. Materials and methods

2.1. Plant material

B. aegyptiaca fruits were derived from Al-Wahat, Egypt. The plant material was identified by Prof. Salwa Kwashty, Department of Phytochemistry and Plant Systematic, National Research Center (NRC), Egypt. Sample plant is deposited at herbarium of National Research Centre. Registration number is CAIRC 3665.

2.2. Chemicals and tools

Cisplatin injectable grade (98%–102%) was purchased from Merck Co. (Germany). Doxorubicin injectable grade (98%–102%) was from Pharmacia Co. Taxol injectable grade (98%–102%) was from Mayne Pharm Co. Standard fatty acids ready-made mixture (in the form of methyl ester derivatives) and sterols ($\geq 97\%$) all were from Sigma–Aldrich Co. Bergapten, marmesin, quercetin, isorhamnetin, yamogenin and diosgenin were from Sigma Co. Flavonoids glycosides were gifts from Dr. Mohamed El-Raiey and Dr. Neven Ghaly, NRC. DPPH[•] was purchased from Sigma Co. (Switzerland). Vitamin C (> 99.5%) was from Fluka. Praziquantel (97.5%–102%) was from Alexandria Co. (Egypt). Alloxan ($\geq 98.0\%$ TLC) was from Sigma–Aldrich. Daonil (glibenclamide) was from Sanofi–Aventis (Egypt). RPMI medium, streptomycin, penicillin, gentamycin antibiotics and glutamine amino acid were all from Merck Co.

Mass spectrometry was LC-MS/MS4000 QTRAP, AB SCIEX from Foster City, USA.

Eliza plate reader was Asys, Hitech, GmbH, Austria, Model; Expert plus UV GC For sap was Hewlett HP6890 series Packard GC system, oven initial temperature was adjusted to 70 °C raised to 220 °C with flow rate 4 °C/min. Inlet temperature was 250 °C and detector temperature 300 °C (FID). Column was capillary (BP \times 70) L = 60 m, D = 0.320 m, film thickness = 0.25 m. The carrier gas was N₂ 30 mL/min, H₂ 30 mL/min, air 300 mL/min. GC for unsap was Hewlett HP5 series with oven initial temperature 80 °C raised to 250 °C with flow rate 4 °C/min. Inlet temperature was adjusted to 250 °C, detector temperature 300 °C (FID). Column L = 30 m, D = 0.53 m.

Light microscope was Olympus and from Saitama, Japan, with eye piece: 25 \times , oil, objective: 100 \times . Accu-Check: Active, Roche, UK.

2.3. Phytochemicals investigation

B. aegyptiaca fruits were sliced, air dried and then extracted with organic solvents in ascending polarity. The dried material was extracted first, with *n*-hexane followed by chloroform and at last with methanol each for three times maceration until complete drain of the phytochemicals. The extracts were concentrated under vacuum using rotator evaporator. The constituents of each extract were determined by chromatographic and spectral analysis.

Free flavonoids and saponins aglycones, as well as coumarins were identified in the chloroform extract by co-chromatography against standard samples using solvent system CHCl₃: MeOH (10:1 v/v) and FeCl₃/ethanol was used as color reagent for flavonoids and 10% ethanolic KOH for coumarins. Alkaloids were identified on HPTLC silica plates and sprayed with Dragendorff's reagent.

Preliminary screening for flavonoids glycosides was established by co-chromatography against authentic samples using cellulose plates, solvent system Bu: AcOH: H₂O (4:1:5 v/v/v) and the spots were examined under UV lamp and sprayed with FeCl₃/ethanol reagent.

The methanolic extract was tested for saponins glycosides using

HPTLC silica plates with eluting system CHCl₃: MeOH: H₂O (60:30:5 v/v/v) and sprayed with 20% H₂SO₄ acid as visualizing reagent.

Aglycones detection was inspected by acid hydrolysis. The methanol extract was evaporated, 2 mg of the residue was dissolved in 3 mL solution of 2 mol/L hydrochloric acid and methanol (1:1 v/v), then heated under reflux for 2 h.

The methanol was then evaporated and 5 mL distilled water was added to the reaction mixture. The aqueous phase was extracted three times with chloroform, washed with distilled water until acid free and then evaporated. The saponin aglycones were identified by co-chromatography against standards on silica gel (HPTLC) (chloroform: methanol 9:1 v/v) and visualized by vanillin sulfuric acid reagent. The formed aglycones were identified by applying co-chromatography analyses against standard samples.

Fatty acids were analyzed by GC chromatography in the form of methyl esters in the saponifiable portion of *n*-hexane extract while sterols were identified in the unsaponifiable portion[8]. GC data were compared with standard samples[9,10].

LC/MS/MS measurements were carried at Regional Center for Food and Feed, Agricultural Research Centre, Egypt (RCFF, ARC). Each extract was analyzed by adapting both –ve and +ve modes using hybrid triple quadrupole/linear ion trap mass spectrometer. Mobile phase: methanol: water: formic acid (70%: 30%: 0.1%). Spectral range: 100: 1100 (*mz*, Da).

2.4. Antidiabetic evaluation

Male albino wistar rats weighing 120 \pm 10 g were from Cid company animal house, Egypt. Animals were fed with standard pellet diet and water *ad libitum* and acclimatized for at least one week before the experimental session. The experimental procedures were done following the NRC institutional and international guidelines of Care and Use of Laboratory Animals with registration No: 16 446.

The animals were divided into 6 groups each of 6 animals. First group was control non-treated. In second group, rats received alloxan *i.p.* (150 mg/kg) prior to 8 h fasting for diabetes induction. Third group diabetic rats treated with glibenclamide (Daonil) as standard drug at dose 0.18 mg/kg which was equivalent to 10 mg man daily use following the reported table of conversion[11]. Fourth, fifth and sixth groups diabetic rats received orally, plant materials extracted from hexane, chloroform and methanol, respectively each at dose 100 mg/kg body weight after diabetes induction had been confirmed. Rats with blood glucose measurements ≥ 300 mg/dL were considered diabetic. Animal blood glucose levels were measured next to fasting for 8 h by AquaChek strips through taking tail blood samples after two weeks treatment. At the end of experiment, rats were anaesthetized by ether and isolated organs were separated. Isolated liver, testis, lung, pancreas, spleen, heart and kidney were kept in formalin solution and histopathological examination was carried at Eye Research Institute, Egypt. The tissues were sliced and fixed in 10% buffer formalin. Paraffin 4 μ m thick sections were stained by haematoxylin and eosin (H & E). The slides were examined using light microscope ($\times 20$).

2.5. Scavenging capacity

Free radical holding strength of the isolated phytochemicals was performed following the reported method[12]. Isolated extracts were assayed at 50 μ g/mL using 0.1 mmol/L DPPH[•] in methanol. After incubation for 30 min at room temperature in dark, the absorbance

was measured at 517 nm using Eliza reader. DPPH⁺/methanol mixture was utilized as a negative control. Vit C and oleic acid were used as standard agents.

DPPH⁺ capturing capability was achieved according to the following equation:

$$\text{Percentage reduction (\%)} = [1 - (\text{Abs}_{\text{sample}}/\text{Abs}_{\text{control}})] \times 100$$

where Abs_{sample} is the absorbance of sample and Abs_{control} is the absorbance of the negative control.

2.6. Cytotoxic estimation

The isolated extracts were examined for their cytotoxic activity against Lung (H460), liver (Hepg2-ATCCHB-8065), brain (U251-NIBIO1F050288) and breast (MCF7-HTB-22) human carcinoma cell lines at the National Cancer Institute, Egypt, according to the reported method[13]. The activity against lymphoblastic leukemia (L301) was performed at the National Research Center, Egypt, applying MTT assay[14]. Cytotoxicity against each line was examined in triplicates. Negative control groups were also performed. Cisplatin, doxorubicin and taxol were used as standard drugs. IC₅₀ values (µg/mL) were determined for both tested materials and standards.

2.7. Antitrematodal performance

In vitro vermifugal activity of the isolated extracts was tested against two hepatic worms; *Schistosoma mansoni* (*S. mansoni*) and *Fasciola gigantica* (*F. gigantica*) worms at Theodor Belharz Research Institute, Egypt. The experiments were performed in triplicates using RPMI media contained antibiotics[15]. Phosphate buffer (pH 7.4) was used for rinsing the worms prior hosting in the media; two worms of *S. mansoni* were hosted in 1 mL media and 2 worms of *F. gigantica* were hosted in 5 mL media. L-glutamine amino acid in addition to antibiotics; 300 mg streptomycin, 300 units/mL penicillin and 160 mg gentamycin were added to RPMI medium containing plates. Tested materials were also added at concentrations (40–200 mg/L). Praziquantel at concentration of 100 mg/L was used as standard. Negative control group analysis was performed along with the experiment. Mortality detection was carried out using inverted microscope, after 24, 48, 72 and 96 h for *S. mansoni* and after 24 h for *F. gigantica*. Antiparasitic analyses were estimated using probit analysis and LC₅₀ and LC₉₀ were determined at 95% confidence limit[16].

3. Results

3.1. Phytochemical investigation

B. aegyptiaca dry fruits (1 kg) yielded 480.20, 94.74, 421.50 g (w/w) of hexane, chloroform and methanol extracts that represents 48.02%, 9.47% and 42.15%, respectively. The detected compounds were identified based on chromatographic methods and LC/MS/MS analyses by comparing fragmentation including (M+H), (M-H), (M+Na) with reported ones and average molecular weights (MW) are recorded. Saturated and unsaturated fatty acids in addition to sterols constituents of the hexane extract were analyzed and detected by gas chromatography. The main detected fatty acids were lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic, arachidic, arachidonic, behnic and erucic. While the detected hydrocarbons were campesterol, stigmaterol and β-sitosterol.

LC/MS/MS analysis of hexane extract disclosed the presence of essential and non essential amino acids in addition to vitamins. The

detected essential amino acids (MW is expressed in g/mol) were valine (117), threonine (119), leucine (131), lysine (146), methionine (149), histidine (155), phenylalanine (165). The non essential amino acids were serine (105), proline (115), cysteine (121), aspartic acid (133), glutamic acid (147), arginine (174), tyrosine (181). The detected vitamins (MW in Da) were vitamin C (176), tocopherol (430) and β-carotene (536).

Concerning chloroform extract, preliminary TLC screening and LC/MS/MS analysis (MW in g/mol) revealed the presence of tyramine (137) and feruloyltyramine (313) alkaloids. Coumarins were also detected in the chloroform extract via HPTLC comparison with standard and LC/MS/MS analysis (MW in g/mol). The detected coumarins were bergapten (216) and marmesin (246). Co-chromatography against standard and LC/MS/MS investigation (MW in g/mol) have illustrated the presence of flavonoids; quercetin (302) and isorhamnetin (316) aglycones. High performance TLC co-chromatography comparison with standard and LC/MS/MS revealed the presence of diosgenin (414) and/or yamogenin (414) saponin aglycones.

With respect to methanol extract, HPTLC comparison with authentic and LC/MS/MS investigation (MW in g/mol) illustrated the presence of flavonoid glycosides: quercetin-3-o glucoside (464), quercetin-3-o rutinoside (610), isorhamnetin-3-o glucoside (478), isorhamnetin-3-o rutinoside (624), isorhamnetin-3, 7-diglucoside (640). Hydrolysis of the methanolic extract indicated the presence of quercetin and isorhamnetin aglycones by comparing with standards. The methanol extract also, gave positive tests characteristics of saponins. Hydrolysis of methanol extract demonstrated the presence of diosgenin and/or yamogenin comparing with authentic. Data of LC/MS/MS analysis comparing with reported ones elucidated the presence of two diosgenin saponin glycoside: saponin1; 26 - O - P- glucopyranosyl - 25 - furost - 5. ene, -3P, 22, 26 - triol 3 - O[a- rhamnopyranosyl - (1→2)] - [a-rhamnopyranosyl - (1→4)] P- glucoryranoside (1048) and its methoxy derivative saponin2; 22 methoxy - 26 - O - P-glucopyranosyl - 25 - furost - 5. ene, -3P, 26 - diol 3 O[a-rhamnopyranosyl - (1→2)]-[a-rhamnopyranosyl - (1→4)] P- glucoryranoside (1078). The current LC/MS/MS results also disclosed the presence of three yamogenin saponin glycosides. The detected saponins were: saponin3; yamogenin 0-α-L-(1→2) - B - D - glucopyranosyl - (1→4) - 1 α - L - rhamnopyranosyl -(1→2) - β - glucopyranosid (1030), saponin 4; yamogenin 0-β-D-xylopyranosyl-(1→6) - β -D-glucopyranosyl - (1→3) - [α - Lrhamnopyranosyl - (1→2)]-glucopyranoside (1016) and saponin5; yamogenin o - α - L - rhamnopyranosyl - (1→2) - β - D - glucopyranosyl - (1→4) - β - D - glucopyranoside (884).

3.2. Biological examination

Concerning antidiabetic activity study, the present results illustrated that alloxan administration significantly increased blood glucose levels and induced partial to severe damage to different rat organs. Isolated organs of normal, diabetic and treated rats are shown in Figures 1 and 2. While, histopathological examination of diabetic rats' organs is illustrated in Figure 3. Histopathological investigation of normal, diabetic and treated rats' pancreases is demonstrated in Figure 4. Isolated organs showed atrophied pancreas while histopathological examination illustrated that some pancreatic acini showed flattened cells and engorged lumens (Figures 1 and 4D). Regarding liver, isolated organs showed mild to severe congestion, histopathological investigation demonstrated that sinusoids were dilated and congested (Figures 1 and 3A). Concerning testis, isolated organs showed atrophied testis. Histopathological investigation

illustrated that the seminiferous tubules showed partial degeneration with maturation arrest at spermatid stage. In addition, congested blood vessels were observed (Figures 1 and 3B). With respect to lungs, isolated organs showed atrophied congested lungs, histo examination illustrated that alveolar wall was ruptured (disrupted), and aggregation of inflammatory cells were found (Figures 1 and 3C). Concerning spleen, the splenic red pulp was oedematous and congested (Figures 1 and 3D). As regard to heart, isolated organs showed congestion whereas histo examination illustrated that myocardial muscles showed intramyocardial congestion (Figures 1 and 3E). With respect to kidney, mild congestion was seen in isolated organs examination while histopathological investigation showed that few glomeruli were shrunken with diminished mesangium size. Some of them appeared atrophied. The epithelium of cortical tubules was disrupted (Figures 1 and 3F).

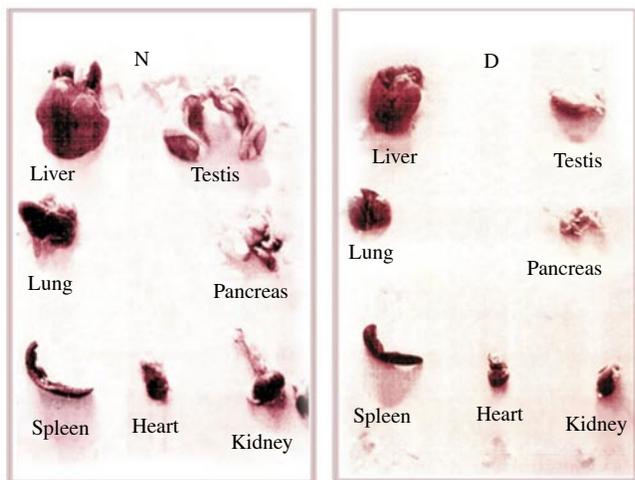


Figure 1. Isolated normal rat's organs (N) and diabetic rat's organs (D) showing atrophied pancreas.

Treatment of diabetic rats with *B. aegyptiaca* phytochemicals from hexane, chloroform and methanol isolated extracts showed hypertrophy in pancreas mass in isolated organs while histopathological study showed normal pancreas; the endocrine

components of pancreas consists of normally closely packed secretory acini and the endocrine tissues forming islets of langerhans were present in all treated groups (Figures 2 and 4: T1, T2 and T3).

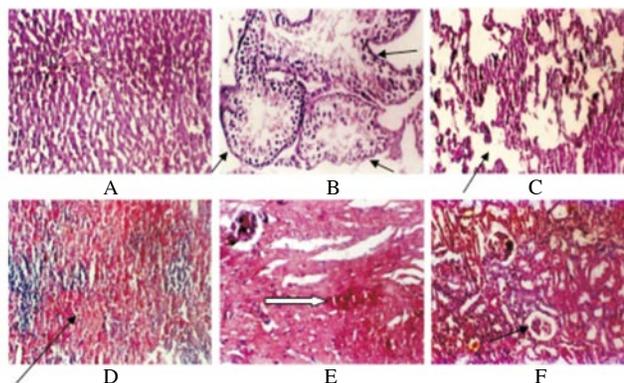


Figure 3. Histopathology of diabetic rat's organs (H & E, 20 ×).

A: Liver with dilated and congested sinusoids; B: Testis showing partial degenerated seminiferous tubules; C: Lung showing ruptured alveolar wall; D: Spleen with congested oedematous splenic red pulp; E: Heart showing intramyocardial congestion; F: Kidney illustrating shrunken glomeruli with diminished mesangium size.

The results also revealed significant reduction of blood glucose levels in various grades. The present results illustrated that blood glucose levels of diabetic rats were significantly elevated comparing to normal ones. Meanwhile, after treatment with the extracted phytochemicals, significant reduction in blood glucose measurements was achieved. Treated rats with Daonil, methanol, chloroform and hexane extracts exhibited no significant difference in glucose levels comparing to normal untreated group. Active constituents extracted by methanol showed the highest reduction in blood glucose levels (77.01%) followed by that of chloroform extract (69.07%) and finally hexane extract constituents (64.13%) comparing with glibenclamide (Daonil) that induced 73.14% reduction (Figure 5).

Referring to antioxidant capacity, the current results indicated that chloroform extract exhibited potent free radical scavenging power (75.72%), while hexane and methanol extracts showed moderate

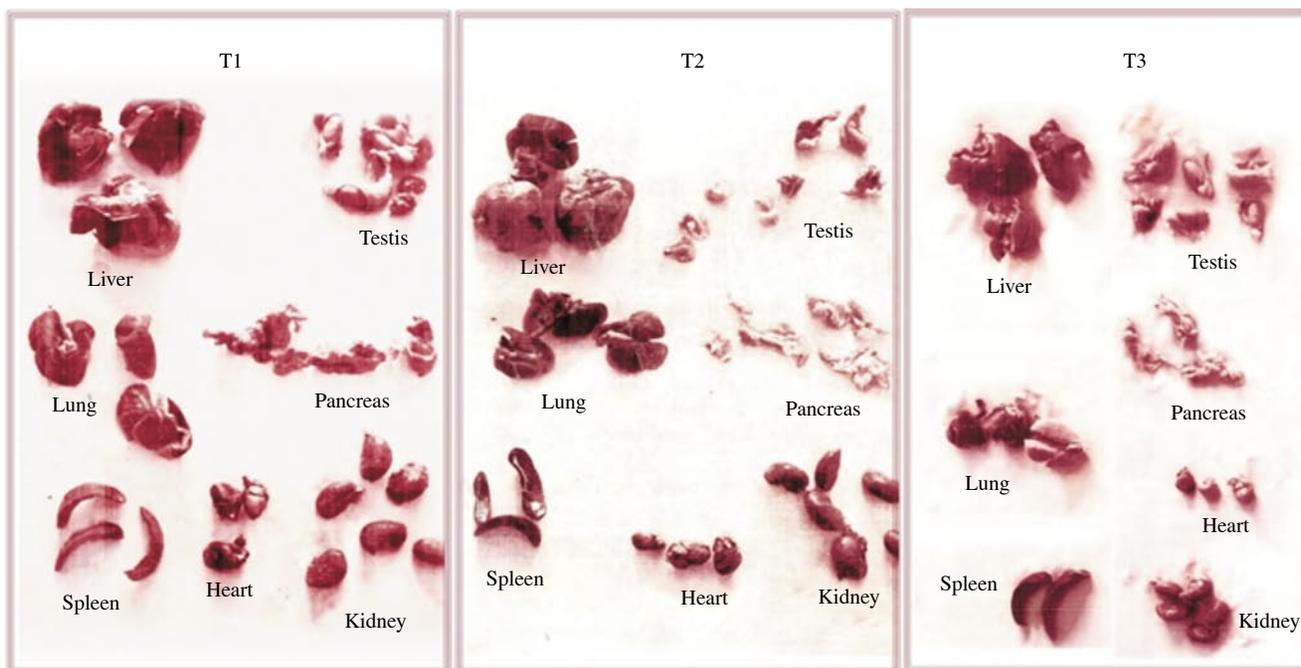


Figure 2. Isolated organs of treated rats with hexane extract (T1); chloroform extract (T2) and methanol extract (T3) showing pancreas hypertrophy.

activities (44.01% and 41.77%, respectively). Moreover, the results clarified that hypoglycemic activity is not directly proportional to antioxidant activity as the extract bearing the highest antioxidant activity is not that responsible for the highest reduction in blood glucose level (Figure 6). For antioxidant capacity examination, vitamin C was used as standard and was found to have 99.60% antioxidant effect.

With regard to cytotoxic efficacy, the present study illustrated that hexane extract possessed cytotoxic effect against two new examined cell lines; breast and lymphoblastic leukemia carcinoma cell lines. IC₅₀ values were 26.68 µg/mL and 278.40 µg/mL, respectively. Cytotoxicity against brain, liver and lung cell lines was examined in previous study.

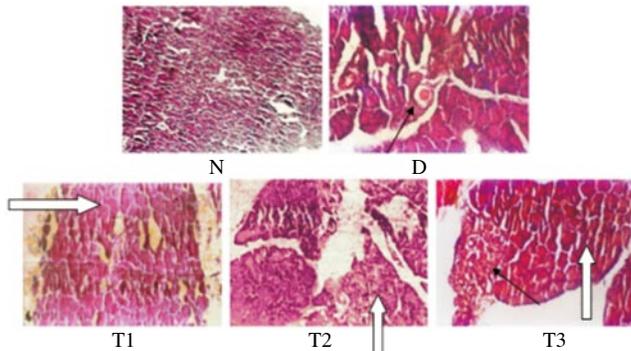


Figure 4. Histopathology of diabetic and treated rat's pancreases (H & E, 20×)

N: Normal rat's pancreas; D: Diabetic rat's pancreas where some pancreatic acini showed flattened cells and engorged lumens; T1, T2 and T3: Rat's pancreases treated with hexane, chloroform and methanol extracts. The exocrine components of pancreas consist of normally closely packed secretory acini (white arrow) and the endocrine tissues forming islets of langerhans (black arrow) were present in all treated groups.

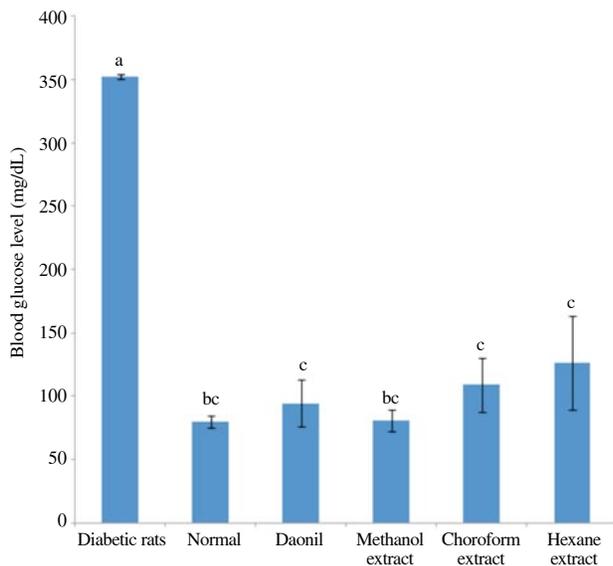


Figure 5. Blood glucose levels of normal, diabetic and treated rats. Treated groups (c) are significantly different at $P < 0.01$ comparing to diabetic group (a) whereas groups (b) are significantly different at $P < 0.001$ comparing to (a). Treated groups of Daonil, methanol, chloroform and hexane extracts are not significantly different at $P < 0.05$ comparing to normal group.

The present data showed that both chloroform and methanol extracted constituents displayed high cytotoxicity against human carcinoma of brain, lung, breast, liver and lymphoblastic leukemia

cell lines (Figure 7). The activity was higher than standard drugs doxorubicin, cisplatin and taxol with much lower IC₅₀ regarding lung, brain, liver and breast cell lines. The results showed that brain carcinoma cells were the most liable cells to the chloroform and methanol extracts. In addition, lymphoblastic leukemia cells were highly affected by methanol extract rather than hexane and chloroform extracts (Table 1).

Table1

Cytotoxicity of phytochemicals of methanol and chloroform extracts of *B. aegyptiaca*.

Cell line	IC ₅₀ (µg/mL)				
	Ch. ext.	Me. ext.	Cis.	Dox.	Tax.
Lung (H460)	0.60	2.04	4.77		
Brain (U251)	0.43	0.34	2.30		
Liver (Hepg2)	0.77	1.01	5.99		
Lymphoblastic leukemia	350.50	4.46			0.60
Breast (MCF7)	4.83	2.64		6.71	

Ch. ext.: Chloroform extract; Me. ext.: Methanol extract; Cis.: Cisplatin; Dox.: Doxorubicin; Tax.: Taxol.

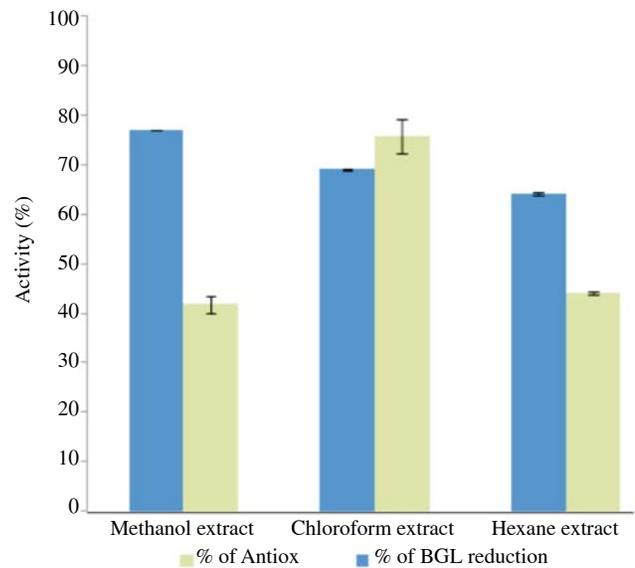


Figure 6. Percentage (%) of blood glucose level reduction (BGL reduction) of *B. aegyptiaca* extracts comparing with antioxidant (antiox) activity.

Table 2

Anti schistosomal activity of phytochemicals of methanol and chloroform extracts (mg/L).

Time	Chloroform extract		Methanol extract	
	LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀
Day 1	-	-	133.0	161.1
Day 2	760.8	1240.0	67.1	139.1
Day 3	601.7	1146.0	100% mortality	
Day 4	238.0	380.7	-	
Day 5	100% mortality		-	

Related to antitrepatodal activity, the current data displayed that chloroform extract manifested higher fascioliasis activity with less LC₅₀ and LC₉₀ (55.15 and 106.65 mg/L) comparing with methanol extracted phytochemicals (63.19 and 119.98 mg/L), respectively after 24 h. Meanwhile, for antibilharziasis efficiency the methanolic constituents were more efficient. Complete eradication of schistosoma worms was achieved at the 5th day after treatment with secondary metabolites constituents of the chloroform extract while phytochemicals of the methanolic extract induced 100% eradication of the worms at the 3rd day (Table 2). Praziquantel as standard drug

induced 100% mortality after 24 h for both schistosoma and fasciola worms.

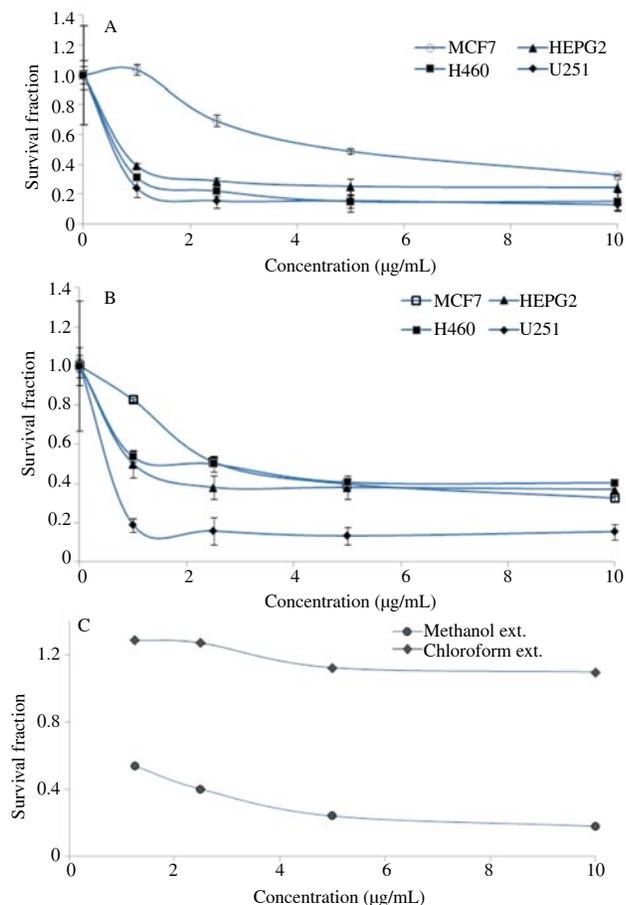


Figure 7. Cytotoxic activity of *B. aegyptiaca* extracts against human carcinoma cell lines of Lung (H460), liver (Hepg2), brain (U251) and breast (MCF7).

A: Cytotoxicity of chloroform extract; B: Cytotoxicity of methanol extract; C: Cytotoxicity against lymphoblastic leukemia (1301).

4. Discussion

The results showed that, administration of alloxan induced moderate to severe damage in rat's organs. Isolated organs and histopathological examination (Figures 1 and 4D) revealed atrophied congested pancreas in diabetic rats, indicating decrease of functional β -cells that might lead to relative deficiency of insulin secretion and or insulin effect, and thus could contribute to the elevated blood sugar. This in agreement with studies illustrated that diabetes is endocrine disorder which is resulted from mal or absences of insulin secretion and or action[17]. In addition, the present results also showed that diabetic rats suffered from damage with different degrees in all organs including liver, testis, lung, pancreas, spleen, heart and kidney (Figures 1 and 3). This is in consistence with previous finding of connecting different complications with diabetes syndrome[4]. In the present study, treatment of diabetic rats with secondary metabolites of *B. aegyptiaca* hexane, chloroform and methanol extracts showed significant decrease in blood glucose levels comparing to diabetic ones and non significant glucose levels comparing to normal rats (Figure 5). In the current research, the effect of *B. aegyptiaca* extracts on diabetic rats' pancreases was investigated. The effect of the extracted phytochemicals on other organs will be discussed in upcoming study. Isolated organs of treated rats showed hypertrophy of rat pancreas with normal

histological pattern (Figures 2 and 4), that might lead to restoring functional β -cells and increasing insulin secretion thus, could explain the significant decrease of elevated blood sugar in treated rats. This result is in close consistence with previous study which showed that pancreas hypertrophy was observed in insulin resistance rats, and consequently, rat pancreas adapted itself to increase β -cell mass and reproduction, leading to betterment of its function[18]. The author also reported that enhanced pancreatic islet mass was associated with enhancement of insulin receptor substrate activation. Thus, in the present study, diabetes may result from mal function, maladaptation and decrease of β -cells rather than complete apoptosis or destruction (which appeared from histological examination) and probably may be related to type 2 diabetes. This is in consistence with reported results which illustrated that β -cell reduction is an important factor contributing in the initiation and progression of type 2 diabetes[19]. While, treatment with different *B. aegyptiaca* isolated phytochemicals in the current research might exert hypoglycemic activity via enhanced functional β -cells. The current results are compatible with previous results correlated between reduction in blood glucose levels in hyperglycemic mice and increase in β -cell induction as pancreatic β -cell is considered the main player in both type 1 and type 2 pathogenesis where recently, a unifying classification of diabetes is proposed[20]. Maladaptation of β -cells might also attribute to diabetes incidence and could be a virtue of multipurpose factors as glucose, dyslipidemia, autoimmunity. The genetic factor also, has a crucial function in susceptibility to glucose-induced β -cell apoptosis. This sensitivity difference in glucose may explain why hyperglycemia increases rates of apoptosis in animals genetically predisposed to diabetes. Probably, such differences also occur between humans with different genetic predispositions[21].

In concern with glucose, it is considered as the physiological key regulator of insulin secretion by regulating β -cell turnover and subsequently, which regulates long-term insulin production adaptation[21]. The pathogenic effect of glucose, is also interceded through oxidative stress and may affect insulin sensitivity, insulin secretion, and also play a role in diabetes secondary complications evolution. These effects are at most stimulated by the generation of reactive free radicals species, which eventually activate stress-induced pathways[22]. Therefore, the examined antioxidant activities of different *B. aegyptiaca* extracts in the current study, may account partially to their hypoglycemic activity. The present results pointed out that antioxidants might oppose reactive free radicals species, which ultimately stimulates stress-induced pathways and hence hinders the pathogenic effect of glucose. In spite that chloroform extract exhibited the highest scavenging activity due to its phenolics content, which is compatible with previous reports[3], the methanolic extract, which is moderately active as antioxidant exhibited the highest antidiabetic activity (Figure 6). The results indicated that the antidiabetic activity is not directly proportion to the free radical scavenging activity. Therefore, the antioxidant effect is not the sole limiting factor controlling the antidiabetic activity, which is multifactorial process as mentioned above. This is also in close agreement with reports manifested that other factors such as antiinflammatory response, increase glucose tolerance, β -cell turnover and peripheral insulin regulating effects are influential elements in diabetes control[21].

With respect to inflammation, the current study illustrated that congestion was observed in diabetic rat's liver in both isolated organs and histopathological examination (Figures 1 and 3A). This is in agreement with studies reported evidence that nuclear factor NF- κ B signaling is associated with low-grade inflammation that

occurs in the liver of type 2 diabetic models, leading to insulin resistance[23]. Besides, inflammatory response is common in most factors that affect both the regulation of β -cell secretory function and cell turnover.

As regards to the effect of active constituents on blood glucose levels, the present study proved that treating diabetic rats with extracted *B. aegyptiaca* phytochemicals revealed significant reduction of blood glucose levels regarding all the examined extracts (Figure 5).

LC/MS/MS analyses have elucidated the presence of diosgenin and yamogenin saponin glycosides in the methanol extract that are compatible with earlier reported ones[1,24,25]. The methanol extract also gave positive tests characteristic for saponins[26]. Presence of saponins may attribute to the reduction of blood glucose levels. It was reported that saponins could be considered as perfect antidiabetic therapy[17]. Besides, triterpenoid saponins isolated from *Momordica cymbalaria* were found to enhance both β -cells and insulin release[27]. Furthermore, the presence of quercetin and rutinoid flavonoid glycosides disclosed by LC/MS chromatogram is conformable with informed literature data[24], and could also account for the resultant antidiabetic effect. Quercetin-3-O-glucoside and quercetin-3-O-galactoside, isolated from berry *Vaccinium vitis* extract, stimulated insulin-independent glucose uptake[28].

In the present study, phytochemicals extracted by chloroform; tyramine and feruloyl-tyramine alkaloids, are in agreement with those previously acquainted[29]. Alkaloids might explicate the antidiabetic performance in the running study. *N-trans*-feruloyl-tyramine alkaloid isolated from *Smilax aristolochiifolia*, showed hypoglycemic and hypotensive activities and insulin resistance nearly reduced by 40%[30]. In the same line, different classes of alkaloids were found to enhance glucose uptake with credence of an increment in insulin-mediated glucose elimination[31]. Alkaloids from *Catharanthus roseus* displayed hypoglycemic activity, with promoted glucose uptake in pancreatic and myoblast cells. The authors also, illustrated that alkaloids showed good antioxidant prospect by relieving H_2O_2 -induced oxidative suggesting their therapeutic prospect contra type 2 diabetes[32]. In addition, diosgenin and yamogenin saponins revealed by LC/MS/MS in the chloroform extract are matched to saponins reported before[24,25] and could also account for the decrease in blood glucose as they were reported to have lowering hyperglycemia activity[33]. As well, diosgenin found in fenugreek modulates glucose metabolism by enhancing adipocyte differentiation and suppressing inflammation in fat tissues[34]. While, yamogenin and its diastereomer diosgenin inhibit liver X receptor which is activated in diabetic syndrome[35].

Coumarins constituents of the chloroform extract, in the present investigation, are congruent with that mentioned before[24]. They could participate in lowering glucose level and its complications. It was reported that total coumarins fraction remedy from *Urtica dentate* could be virtually, renoprotective in diabetic nephropathy, which is one of most critical entanglement of diabetes and is tight with end-stage renal disease[36]. Likewise, scopolamine, a derivative of coumarins, was documented to reduce blood glucose levels[37]. In the same line, 7-methoxycoumarin from the bark of marine plant *Rhizophora mucronata* promoted antidiabetic efficacy[38].

With respect to hexane extract, fatty acids constituents that detected in this work were the same as previous finding[8] and may explain the hypoglycemic effect. Previous studies stated that free fatty acid receptors intermediate essential metabolic functions, as peptide hormone secretion and inflammation, and herewith participate to

energy homeostasis[39]. So, fatty acids are considered to be important medication targets to metabolic disorders, such as obesity and type 2 diabetes that result from imponderables in energy homeostasis. The authors declared that free fatty acid receptors are activated by long, medium and short chain fatty acids[39]. Furthermore, intake of omega-3 polyunsaturated α -linolenic derived fatty acids is an approach for repressing inflammation and consequently diabetes control as inflammation stimulates insulin resistance and triggers diabetes mellitus[40]. The identified amino acids and vitamins in the current hexane extract were formerly reported[41] and could share in the observed hypoglycemic effect. Oral amino acids intake may decrease plasma glucose and improve insulin sensitivity[42]. Likewise, vitamins in the hexane moiety, could also participate in lowering elevated blood glucose in diabetic mice. This is in close agreements with the reported significant decrease in the enzymatic independence antioxidants as vitamins E and C in diabetic rats that render normal after treating with *Passiflora ligularis* fruit extract[43].

So, the present results are of special interest, as regard to diabetes treatment, it is important to mention that over 300 millions may suffer from diabetes by the year 2025 according to the World Health Organization projections[17]. Besides, side effects of drug in use nowadays make it insistent demand to search for natural antidiabetic agents. *In vitro* study showed that Ca^{2+} -dependent β -cell apoptosis in rodent and individuals might be induced by sulfonylureas tolbutamide and glibenclamide whilst insulin secretion deterioration was observed in patients under sulfonylureas treatment[21].

Regarding cytotoxic activity, the fatty acids and sterols constituents of hexane extract may explain the present cytotoxicity against breast and lymphoblastic leukemia cell lines. These phytochemicals were previously reported to have cytotoxic effect against brain, liver and lung carcinoma[8]. The present results showed potent cytotoxic effect chloroform and methanol phytochemicals with IC_{50} less than 10 μ g/mL. The results also illustrated much higher cytotoxic effect than standard drugs against the selected human carcinoma cell lines of brain, lung, liver and breast. The most susceptible cells were brain carcinoma cells for both extracts, which is in close agreement with previous finding[8].

The current investigated cytotoxic effects are related to the extracted secondary metabolites. Saponins components of the methanol extract may be responsible for the elucidated cytotoxic effectiveness. The present data are in close agreement with reported findings which showed that *B. aegyptiaca* saponin mixture demonstrated cognizable anticancer effects in human A549 non-small-cell lung cancer (IC_{50} 0.09 μ g/mL) and U373 glioblastoma (IC_{50} 0.15 μ g/mL) human carcinoma cell lines[3] comparing with IC_{50} 0.6 and 2.04 μ g/mL against H460 lung cell line of both chloroform and methanol extracts, respectively in the present results. *In vivo* saponin prolonged the survival time of mice bearing murine L1210 leukemia grafts exactly as vincristine[3], which is in good agreements with present finding where methanol fraction displayed remarkable *in vitro* cytotoxic effect against lymphoblastic leukemia (1301) cell line. Moreover, the current data illustrated powerful cytotoxicity of both *Balanites* extracts against liver carcinoma which is in conformity with reports recorded that *Balanites* suppressed Ehrlich ascetic tumors invaded/or affected mice liver and spleen[44]. Cytotoxic activity could also be related to quercetin glycosides present in the methanol extract. Quercetin-3-glycoside was suggested as a prospect antitumor element against liver cancer through possible mechanism of action apoptosis and cell-cycle arrest[45]. As well, epidemiological investigations and meta-analyses propose that

flavonoid-rich diets consuming is inversely related to development of many aging-linked diseases inclusive cancers, cardiovascular disease, diabetes, osteoporosis, and neurodegenerative defects[28].

Concerning chloroform fraction, coumarins might partially account for the current cytotoxicity. The effects of psoralens coumarins on human breast cancer as cell growth, survival and apoptosis was documented while, 5-methoxypsoralen or bergapten was able to affect transductional routes mainly involved in the regulation of cell survival in two hormone-dependent and hormone-independent human mammary tumoral cell lines of breast cancer[46]. In the same study, the authors added that coumarins exhibited immunomodulatory activity and photo activated coumarins are efficient in prohibiting propagation of bladder and mucocarcinoma, mammary cancer cells and human melanoma cell line.

Also, the role of marmesin coumarin in angiogenesis regulation was evidenced[47]. High cost of treatment, current used drugs' side effects, emerge of drug resistance and first of all, the risk hazards of cancer[7], make it paramount necessity to turn to natural drug origin remedy. The present study could be helpful in this approach. Flavonoids and saponin aglycones may also be responsible for the elucidated cytotoxicity. Isorhamnetin, the methyl derivative of quercetin, was reported to have *in vitro* and *in vivo* suppressive effect against lung cancer. Moreover, the binding affinity of diosgenin and yamogenin to ER (estrogen receptor alpha) in hormonal-dependent carcinoma of breast is greater than estradiol and tamoxifen currently in use[48].

As regard to trematodal, even diagnoses may lead to severe problems such as viral and urinary tract infections[5]. Other hazards such as *Schistosoma* induced mutation and increase rate of cancer as liver, bladder and colorectal cancer were also reported. Moreover, most drug in use as antiparasitic including praziquantel were reported to have critical side effects[1]. Consequently, the activity of *B. aegyptiaca* extracted principles as natural antitrematodal agents is of prominent assessment. The activity of hexane extract main phytochemicals as antitrematodal agents was discussed formerly[8]. The current results demonstrated that both methanolic and chloroform extracts exhibited *in vitro* antischistosomiasis and antifasciolosis activities which are in close agreement with previous findings documented the *in vivo* schistocidal activity of saponin rich fraction of *B. aegyptiaca*[1]. The results are also supported by the use of the berries and seeds of the plant by popular that might protect against *Schistosoma*[2,3,49]. In the same line, *B. aegyptiaca* was reported to induce antitrematodal activity against *F. gigantica* adult worms in infected goats at dose 9 g/kg fruit water extract[50]. Meanwhile, the *in vitro* potent activity of the methanolic extract at dose 200 µg/mL on *Paramphistomum microbothrium* tegument was illustrated which might explain its *in vivo* high activity against biologically relative trematode, *F. gigantica*[51].

In conclusion, *B. aegyptiaca* polar and non-polar phytochemicals have powerful effectiveness against different classes of severe maladies due to their wide range of secondary metabolites contents. The study rationalizes the traditional use of the plant. The separated fractions displayed pronounced antihyperglycemic activity that was approximately, as effective as the current drug in use sulfonylurea glibenclamide. The segregated fractions exhibited efficient cytotoxicity against the selected human carcinoma cell lines. The observed antioxidant performance could partially account for the antidiabetic and cytotoxic effects. Outright extirpation of the *Schistosoma* worms was achieved using the plant materials that also could serve as antifasciolosis candidate. The study could

serve as guide for development of natural functional treatment at a time where these illnesses have emerged as causative elements of productivity loss and health risk.

Conflict of interest statement

I declare that I have no conflict of interest.

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