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Phytochemical study and antihyperglycemic effects of *Balanites aegyptiaca* kernel extract on alloxan induced diabetic male rat

Nabila Helmy Shafik*[1], Reham Ezzat Shafek[1], Helana Naguib Michael[1] and Emad Fawzy Eskander[2]

[1]*Chemistry of Tanning Materials and Leather Technology Department, National Research Centre, Dokki, Cairo 12311, Egypt*
[2]*Hormones Department, National Research Centre, Dokki, Cairo 12311, Egypt*

ABSTRACT

Phytochemical investigations of the aqueous ethanolic extract of Balanites aegyptiaca kernel (BE) afforded the presence of 9 natural flavonol compounds which were isolated and identified as:- isorhamnetin 3-rutinoside (1), 3-robinobioside (2), 3-O-glucoside (3), 3-O-galactoside (4), 3,7-diglucoside (5), quercetin 3-glucoside (6), 3-rutinoside (7) beside two aglycones quercetin (8) and isorhamnetin (9). Elucidation of their chemical structures was determined by different spectroscopic methods in addition to the chemical and physical methods of analysis. This extract was assessed for its biological activity on alloxan diabetic rats. Oral administration of (BE) at a dose of 50 mg/kg b. wt showed significant antihyperglycemic and antilipid peroxidative effects as well as increased the activities of enzymatic antioxidants and levels of non enzymatic antioxidants. We also noticed that the antihyperglycemic effect of plant drug (BE) was comparable to that of the reference drug glibenclamide.

Key words: *Balanites aegyptiaca* kernel, Balanitaceae, Antihyperglycemic effects, Flavonol, NMR spectroscopy

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INTRODUCTION

Diabetes mellitus is considered as one of the five leading causes of death in the world [1]. In Egypt diabetes affects approximately 57 million people by the year 2025[2,3]. So it has been an increased interest in various natural products isolated from plants as a source of antidiabetic compounds including polyphenolics especially flavonoids [4,5,6,7]. One among the promising plant is the *Balanites aegyptiaca* (L.) del which belongs to the family Balanitaceae. It is spiny shrub or tree up to l0 m tall, widely distributed in dry land areas of Africa and South Asia [8,9]. Previous phytochemical studies proved that its leaves contained saponin, furanocoumarine and flavonoid [10,11]. The mesocarp of its fruit contains protein, sugars, organic acids, other constituents like 3-rutinoside and 3-rhamnogalactoside of isorhamnetin[12], diosgenin [13], it also contain a mixture of 22R and 22S epimers of 26-(O-β-D-glucopyranosyl)-3-β-[4-O-(β-D-glucopyranosyl)-2-O-(α-L-rhamnopyranosyl)-β-D-lucopyranosyloxy]-22,26-dihydroxyfurost-5-ene. Its kernel contained the xylopyranosyl derivative of above saponin present in mesocarp [14] while nine saponins have been reported from kernel cake of *B. aegyptiaca* [15]. The leaves and fruit kernels of *B. aegyptiaca* L. were found to contain six diosgenin glucosides including di-, tri-, and tetraglucosides [16]. Its bark contain furanocoumarin bergapten and dihydrofuranocumarin D- marmesin [17, 18]. It also contains beta-sitosterol, bergapten, marmesin, and beta-sitosterol glucoside [19,20,21,22,23] balanitin-1,-2, and -3; balanitin-1 for example possesses a yamogenin aglycone with a branched glucose and rhamnose side chain [24]. Almost all the parts of *B. aegyptiaca* plant are traditionally used in several folk medicines. It has potent wound-healing activity and it possess potent antioxidant activity by inhibiting lipid peroxidation, bleaching DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, and protecting against oxidant injury to fibroblast cells [25]. The aqueous extract of *B. aegyptiaca* also has molluscicidal agent to juvenile and adult *Bulinus globosus* and *Bulinus truncates* [26]. Seed is used as expectorant, antibacterial, antifungal, febrifuge [27] and as laxative, treatment of hemorrhoid, stomach aches, jaundice, yellow

fever, syphilis, and epilepsy [28]. It also used as anthelmintic and purgative. The seed oil is used to treat tumors and wounds [29]. The plant acts as antioxidant [30] and the Balanitin-7 which isolated from aqueous extract of *B. aegyptiaca* seed is reported as anthelmintic agent [31]. In Egyptian folk medicine, water extract *balanite* fruit have been reported as hypoglycemic [32,33]. It also reported that it inhibit *Escherichia coli* growth in rats [34], it have antidiabetic effect in streptozotocin-induced diabetic mice [35]. The fruits are used as an oral hypoglycemic[36] and an antidiabetic; an aqueous extract of the fruit mesocarp is used in Sudanese folk medicine in the treatment of jaundice [37] and in food preparations and herbal medicine, especially in Africa and some developing Countries [38] it used as tooth brush [39] and treat dysentery and constipation. Fruit is used in whooping cough, also in leucoderma and other skin diseases, A fruit is used to treat liver disease and as a purgative, and sucked by school children as a confectionary in some countries [40,41] . It is reported that whole and extracted pulp of *B. aegyptiaca* fruits reported a hypocholesterolemic effect when tested on adult albino rats [42]. Bark is used as spasmolytic [43] and in the treatment of syphilis, round worm infections, and as a fish poison. The aqueous leaf extract and saponins isolated from its kernel cakes have antibacterial activity [44,45]. It is reported that bark aqueous extract of *B. aegyptiaca* used in treatment of both AIDS and Leukemia. An oral administration of the aqueous extract (30% w/v given at 100 ml every 8 hours for 30 days) for the treatment of HIV patients have shown excellent results. The same was given to patients with leukemia and a good increase in platelets and a normal blood differential reading after one month was noted [46] . The acetone and methanolic extracts of stem bark of plant has reported an antivenin activity [47] . The ethanol and petroleum ether extracts showed a greater anti-inflammatory and analgesic effects comparative with the standard drugs, indomethacin and diclofenac sodium, respectively. It also indicated that the ethanolic extract of *B. aegyptiaca* exhibited more significant activity than petroleum ether in the treatment of pain and inflammation [48]. Root extracts have proved 'slightly effective' against experimental malaria [49,50] In Kenya, a root infusion is used as an emetic [51]. The ethanol and methanolic extract of leaves of *B. aegyptiaca* has diuretic effect [52]. Its aqueous and organic extracts were reported to have antibacterial effect against *Salmonella typhi* isolated from blood clot culture using the disc diffusion method [53]. The present study has been designed to isolate the natural flavanoid compounds and evaluate the antihyperglycemic effects of the aqueous ethanolic kernel extract of *Balanites aegyptiaca* (BE) in streptozotocin induced diabetic rats.

EXPERIMENTAL SECTION

Plant material: Seeds of *Balanites aegyptiaca* were collected from the tree growing at Giza Zoo in Egypt and it were botanically authenticated and deposited in the Department of hormones, National Research Centre, Cairo.

General methods:
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR: Jeol spectrometer (Kyoto, Japan) in DMSO-d_6; UV: Shimadzu spectrophotometer model UV-240 (Kyoto, Japan); Polyamide 6S (Riedel, De Häen), Cellulose (Merck) and Sephadex LH-20 (Pharmacia); paper chromatography (PC): Whatman No. 1 and preparative (PPC) on 3 MM paper using the following solvent systems: (1) BAW (n-BuOH/AcOH/H_2O, 6:1:2); (2) H_2O; (3) 15 % AcOH (AcOH/H_2O, 15:85), (4) 6% AcOH (AcOH/H_2O, 06:94) and (5) Forestal (AcOH/ Conc. HCl/ H_2O, 30:3:10), NADH (nicotine amide adenine dinucleotide), NADHP [nicotine amide adenine dinucleotide phosphate (Reduced)].

Extraction and isolation:
500 g of *Balanites aegyptiaca* seeds kernel were dried, powdered and then soaked in 1500 ml of 95% ethanol over night. After filtration, the residue obtained was again resuspended in equal volume of 95% ethanol for 48 hrs and filtered again. The above two filtrates were mixed and the solvent was evaporated in a rotavapour at 40-50^{0}C under reduced pressure. A light greenish yellow material obtained was stored at 0-4^{0}C until used. A known volume of the residual extract is suspended in distilled water and was orally administered to the animals during the experimental period. The TDPC of the extract using the solvent systems (1) and (3), respectively, revealed the presence of many components of polyphenolic nature. The concentrated ethanolic extract (113 g) was chromatographed on a polyamide column; elution being performed with water followed by water-ethanol mixtures to give six fractions which were further purified on subcolumns of Sephadex LH-20 and/or PPC to give rise to 9 pure compounds. The two Compounds **(1,** 70 mg**)** and **(2,** 88 mg)) were isolated from fraction **I** (eluted with H_2O) and fraction **II** (eluted with 20% EtOH), respectively, and purified using Sephadex LH-20 column using EtOH/H_2O (decreasing polarity) for elution. Applying the third fraction (40% EtOH) on Sephadex LH-20 column and eluted by 50% ethanol to obtain the two pure natural compounds (**3**, 38 mg; **4**, 47mg). From the fourth fraction (60% EtOH), compounds (**5**, 40 mg and **6**, 37 mg) were separated in a pure form by PPC using 40% EtOH as eluent. The compound **7 (**62mg), was separated from fraction 5 and purified on PPC using the solvent system BAW for elution. Finally, the two aglycones **8** (50 mg), **9** (41 mg) were obtained from fraction **VI** using ethanol as eluent and purified on a cellulose column eluted with ethanol. Their chemical structure have been established by conventional methods of chemical and physical analysis and confirmed by ^{1}H and ^{13}C NMR spectroscopy.

Drugs and chemicals:-
Streptozotocin was purchased from Sigma Aldrich Chemicals, Cairo.

Experimental animals:-
Albino wistar male rats weighing 120-150g were used for the present study. The animals were obtained from Central animal house, National Research Centre, Giza and were maintained in the central animal house with 12 h light and 12 h dark cycles. Standard pellets were used as a basal diet during the experiment. The control and experimental animals were provided food and drinking water *ad libitum*

Induction of diabetes mellitus:
Diabetes mellitus was induced in wistar rats by single intraperitoneal injection of streptozotocin (50mg/kg) dissolved in 0.1M-citrate buffer (pH 4.5) after overnight fasting for 12 h [54]. The diabetes was assessed by determining the blood glucose concentration within 48 hours after injection of streptozotocin. The rats with blood glucose level above 400 mg/dl were selected for the experimental studies.

Experimental design:
In the experiment a total number of 24 rats (18 diabetic rats, 6 normal rats) were used. The rats were divided into 4 groups of six each.
Group I: Control rats
Group II: Diabetic control (streptozotocin 50mg/kg b. wt)
Group III: Diabetic rats orally receiving (BE) (50 mg/kg b. wt)
Group IV: Diabetic rats orally receiving glibenclamide (600 ng/kg b. wt)
After the experimental period, all animals were sacrificed by cervical dislocation and biochemical studies were conducted on blood, plasma, erythrocytes, erythrocyte membranes and liver of control and experimental animals in each group.

Biochemical studies:-
According to Eskander *et al* the blood glucose level was determine [55] using *o*-toluidine reagent. Hexokinase activity was assayed according to Brandstrup *et al* method [56]. The method is based on the phosphorylation of glucose by the enzyme hexokinase (tissue homogenate) and the residual glucose in the supernatant was determined by the method of Sasaki *et al* [55]. Glucose-6-phosphatase was assayed according to the method of Koida and Oda [57]. The method is based on the estimation of inorganic phosphorus liberated from the glucose-6-phosphate present in the incubation mixture (glucose-6-phosphate, tissue homogenate and malic acid buffer). The liberated inorganic phosphorus was estimated by the method of Fiske and Subbarow [58]. TBARS (thiobarbituric acid reactive substances) in plasma was assayed by the method of Yagi [59]. Plasma was deproteinised with 10% phosphotungstic acid and the precipitate was treated with thiobarbituric acid at 90°C for 1 hour. After cooling, 5.0 ml of n-butanol was added and the mixture was shaken vigorously and centrifuged at 1000g for 15 minutes. The pink colour formed gives a measure of TBARS which its presence in erythrocytes and erythrocyte membranes were assayed according to the method of Donnan [60]. The erythrocytes and erythrocyte membranes were deproteinized with 10% TCA (trichloroacetic acid) and then treated with TBA (thiobarbituric acid). The above mixture was heated in a boiling water bath for 15 minutes. It was cooled at room temperature and pink color that developed was measured at 535nm. The activity of glutathione peroxidase was estimated according to the method of Rotruck *et al* [61]. The enzyme preparation was allowed to react with H_2O_2 in the presence of reduced glutathione for a specified time period, and then the remaining reduced glutathione content was assayed by the method of Beutler and Kelley [62]. Superoxide dismutase (SOD) activity was assayed by the method of Kakkar *et al* [63]. The assay of superoxide dismutase was based on the inhibition of formation of NADH-phenazine methosulphate nitroblue tetrazolium formazan. The colour formed at the end of the reaction was extracted into butanol layer and measured at 520nm. Catalase activity was assayed using the method of Sinha [64]. The method is based on the utilization of H_2O_2 by the enzyme. The color developed was read at 620nm. Reduced glutathione was measured according to the method of Beutler and Kelley [62]. The technique involved in protein precipitation by *m*- phosphoric acid and spectrophotometric assay at 412nm of the yellow derivative obtained by the reaction of supernatant with 5, 5'-dithio-bis-2-nitrobenzoic acid (DTNB). Vitamin E was estimated by the method of Desai [65] based on the classical Emmerie Engle reaction. The lipid residue obtained using redistilled ethanol and petroleum ether was redissolved in absolute ethanol. To this solution, ferric chloride, orthophosphoric acid and bathophenanthroline reagents were added. Vitamin E present in the lipid residue reduces ferric ion to ferrous ions and forms a pink colored complex with bathophenanthroline orthophosphoric acid. Absorption due to the pink complex was measured at 536nm. Ascorbic acid level was estimated by the method of Omaye *et al* [66]. Copper to form dehydroascorbic acid and diketoglutaric acid oxidizes ascorbic acid. These products when treated with 2, 4-dinitrophenylhydrazine (DNPH) form the derivatives bis-2,4-dinitrophenylhydrazone which undergoes rearrangement to form a product with an absorption maximum at 520nm.

Statistical analysis
The data are expressed as mean ± SE. Statistical comparisons were performed by one-way analysis of variance, followed by Student's *t*-test. The results were considered statistically significant if the *P* values were 0.05 or less.

RESULTS AND DISCUSSION

Diabetes have been treated with several medicinal plants for a long time, whereby the medicinal plant extracts were found to improve the diabetic control and meanwhile reduce associated side effects than the synthetic ones [67,68] . Therefore, the search for more effective and safer antidiabetic agents has become an area of active research.

Flavonoids are well-known for their multi-directional biological activities including anti-diabetic efficacy [69,70,71,72].

Phytochemical investigation of the ethanolic extract of *Balanites aegyptiaca* using column and preparative paper chromatography resulted in 9 compounds: isorhamnetin 3-rutinoside (1), 3-robinobioside (2), 3-*O*-glucoside (3), 3-*O*-galactoside (4), 3,7-diglucoside (5), quercetin 3-glucoside (6), 3-rutinoside (7) beside two aglycones quercetin (8) and isorhamnetin (9). Figure 1. The structure of these compounds was determined by their chromatographic behaviors as well as spectroscopic analysis whereby compounds (**2**), (**5**) and (**7**) were isolated from the fruits kernel for the first time. Thus, their spectral data is represented as follow:

Isorhamnetin 3-robinobioside (2) UV (λmax, nm) MeOH: 255, 268sh, 302sh, 358; +NaOMe: 274, 327, 416 + NaOAc: 272, 318, 388; +NaOAc/H_3BO_3: 257, 268sh, 308sh, 361; + $AlCl_3$: 268, 299 sh, 365, 407; + HCl : 268, 298sh, 358, 402; ^{1}H-NMR (500 MHz, DMSO) δ: 8.08 (d, *J* = 2.5 Hz, H-2'), 7.55(dd, *J*=8.5, 2.5 Hz, H6'), 6.83 (d, *J* = 8.5 Hz, H-5`), 6.55 (d, *J*= 2.5 Hz, H-8), 6.25 (d, *J*=2.5 Hz, H-6), 3.73 (s, OMe-3`),

sugar moiety: δ: 5.59 (d, *J* = 7.5 Hz, H-1`` of glucose), 4.4 (d, J = 2.5 Hz, H-1`` of rhamnose), 0.98 (d, J = 6 Hz, rhamnose methyl protons, ^{13}C-NMR (DMSO-d_6): aglycone moiety: (ppm) 156.4 (C–2); 133.3 (C-3); 160.3 (C-5); 98.5 (C-6); 163.4 (C-7); 93.5 (C-8); 157.3 (C-9); 104.1 (C-10); 120.4 (C-1'); 114.0 (C-2'); 149.1 (C-3'); 147.4 (C-4'); 114.9 (C-5'); 122.4 (C-6'); Sugar moieties: (ppm) 101.7 (C-1''); 71.3 (C-2''); 68.4 (C-3''); 68.0 (C-4''); 73.7 (C-5''); 65.8 (C-6''); 100.3 (C-1'''); 68.2 (C-2'''); 70.8 (C-3'''); 72.0 (C-4'''); 68.3 (C-5'''); 18.1 (C-6''').

Isorhamnetin 3,7-diglucoside (5)
UV (λmax, nm) MeOH: 254, 267sh, 370; +NaOMe: 274, 327sh, 435+ NaOAc: 271, 325, 395; +NaOAc/H_3BO_3: 256, 273sh, 375; + $AlCl_3$: 264, 300 sh, 365sh, 430; + HCl : 266, 271sh, 302sh, 358, 427; ^{1}H-NMR (500 MHz, DMSO) δ: 7.66 (d, *J* = 2.5 Hz, H-2'), 7.85(dd, *J*=8.5, 2.5 Hz, H6'), 6.96 (d, *J* = 8.5 Hz, H-5`), 6.78 (d, *J*= 2.5 Hz, H-8), 6.45 (d, *J* = 2.5 Hz, H-6), 3.6 (s, OMe-3`), sugar moiety: δ: 5.52 (d, *J* = 7.5 Hz, H-1`` of glucose at position 3), 5.3 (d, J = 7.5 Hz, H-1`` of sec glucose at position 7), ^{13}C NMR (DMSO, 125 MHz) δ: 157.1 (C-2), 133.3 (C-3), 177.6 (C-4), 160.7 (C-5), 98.7 (C-6), 161.80 (C-7), 94.5 (C-8), 155.9 (C-9), 105.4 (C-10), 121.8 (C-1'), 113.9 (C-2'), 149.60 (C-3'), 149.50 (C-4'), 115.1 (C-5'), 122.2 (C-6'), 101.3 (C-1''), 74.3 (C-2''), 76.60 (C-3''), 70.2 (C-4''), 77.3 (C-5''), 61.0 (C-6''), 100.3 (C-1'''), 73.4 (C-2'''), 76.7 (C-3'''), 70.0 (C-4'''), 77.2 (C-5'''), 61.1 (C-6''').

Quercetin 3-rutinoside (7) UV (λmax, nm) MeOH: 268, 267sh, 298sh, 361; +NaOMe: 273, 327,415; + NaOAc: 272, 325, 390; +NaOAc/H_3BO_3: 261, 297, 385; + $AlCl_3$: 272, 303sh, 432; + HCl : 270, 298sh, 364sh, 402;^{1}H-NMR (500 MHz, DMSO) 7.6 (dd, J = 2.0, 8.0 Hz, H6'), δ: 7.6 (d, J = 2.0 Hz, H-2'), 6.80 (d, J = 8.0 Hz, H-5'), 6.42 (d, J = 2.5 Hz, H-8), 6.22 (d, J = 2.0 Hz, H-6), 5.83 (d, J = 7.5 Hz, H-1''), 4.4 (d, J = 2.0 Hz, H-1'''),3.0-3.8 (m, rutinose protons); 0.98 (d, J = 6 Hz, CH_3 of rhamnosyl). ^{13}C NMR (DMSO, 125 MHz) δ: 158.1 (C-2), 134.2 (C-3), 177.63 (C-4), 161.7 (C-5), 100.1 (C-6), 164.80 (C-7), 95.0 (C-8), 156.59 (C-9), 105.3 (C-10), 122.0 (C-1'), 115.53 (C-2'), 145.10 (C-3'), 148.47 (C-4'), 116.30 (C-5'), 122.20 (C-6'), 101.72 (C-1''), 74.93 (C-2''), 76.40 (C-3''), 70.62 (C-4''), 75.372 (C-5''), 66.73 (C-6''), 100.5 (C-1'''), 70.46 (C-2'''), 70.7 (C-3'''), 72.0 (C-4'''), 68.09 (C-5'''), 17.35 (C-6''').

Antihyperglycemic activity:-
Ethanolic extract of *Balanites aegyptiaca* has antihyperglycemic activity whereby, Table 1 shows the level of blood glucose in control and experimental animals in each group. The level of blood glucose was significantly increased in streptozotocin alone treated rats (Group II) as compared to control animals (Group I). However, the level of blood glucose was returned to near normal concentrations in diabetic rats treated with Balanites ethanol and glibenclamide. "BE" showed comparable effect to that of glibenclamide.

Table 1 Blood Glucose level in control and experimental animals in each group

Groups	Blood Glucose (mg/dl)
Control	66.3 ± 2.1
Diabetic control	267 ± 2.7 a
Diabetic + alc BE (300 mg/kg bw)	102 ± 8.5 ab
Diabetic + glibenclamide (600 ng/kg bw)	110.0 ± 7.2 abc

Values are given as mean ±SD (n= 6 rats).
a- significantly different from control animals ap<0.001
b- significantly different from diabetic control bp<0.001
c-as compared to diabetic +alc BE treated rats cp-not significant

Table 2:
Indicates the activities of hexokinase and glucose-6-phosphatase in the liver of control and experimental animals in each group. A significant decrease in hexokinase and increase in glucose-6-phosphatase activities were noticed in the liver of diabetic animals as compared to control animals.

Oral administration of "BE" to diabetic animals revert back the enzyme activities to near normal concentrations.

Groups	Hexokinase (U*/mg protein)	Glucose-6-phosphatase (U ** / mg protein)
Normal	0.27 ± 0.03	2.14 ± 0.03
Diabetic control	0.12 ± 0.02a	2.77 ± 0.2 a
Diabetic + alc BE(300mg/kg bw)	0.14 ± 0.06 b	3.2 ± 1.4 a*b
Diabetic+glibenclamide(600ng/kg bw)	0.14 ± 1.0 a*bc	3.4 ± 2.1ab*c

Values are given as mean ±SD (n= 6 rats)
*a-significantly different from control animals ap<0.001, a*p<0.01,a*p<0.05*
b-significantly different from diabetic control bp<0.001
*c-significantly different from diabetic +alc "BE" treated rats cp<0.01, *cp<0.05*
U- Micromoles of glucose phosporylated/ h*
*U** - Micromoles Pi liberated/min*

Table 3:
Table 3 shows the level of plasma, erythrocytes and erythrocyte membranes TBARS in control and experimental animals in each group. The levels of TBARS were significantly increased in diabetic animals as compared to control animals. The levels of TBARS were significantly reduced in diabetic animals treated with "BE".

Levels of TBARS in plasma, erythrocytes and erythrocyte membranes of control and experimental animals in each group

Groups	Plasma (nmol/ml)	Erythrocyte membranes (nmol/mg protein)	Erythrocytes (nmol/mg/Hb)
Normal	1.6 ± 0.18	0.35 ± 0.04	0.65 ± 0. 08
Diabetic control	2.8 ± 0.21a	0.84 ± 0.07a	0.99 ± 0.07a
Diabetic + alc BE (300mg/kgbw)	2.7 ± 1.1a*b	0.52 ± 0.05ab	0.79 ± 0.05 b
Diabetic+ glibenclamide (600ng/kg bw)	2.8 ± 0.24 ab*c	0.66 ± 0.03ab*c	0.84 ± 0. 06 a*b

Values are given as mean ±SD (n= 6 rats) (600 ng/kg bw)
*a- significantly different from control animals ap<0.001, a*p<0.01, a*p<0.05*
b-significantly different from diabetic control bp<0.001
*c-significantly different from diabetic +alc "BE" treated rats *cp<0.05*

Tables 4 & 5:
It shows the levels of non-enzymatic antioxidants and activities of enzymatic antioxidants in plasma and erythrocytes, respectively, in control and experimental animals in each group. The levels of non-enzymatic antioxidants and activities of enzymatic antioxidants were decreased in diabetic animals as compared to control animals. However, oral administration of "BE" at a dose of 50mg/kg bw revert back the levels of non-enzymatic antioxidants and activities of enzymatic antioxidants to near normal range in diabetic animals.

Table 4: Enzymatic antioxidants and non- enzymatic antioxidants in the plasma of control and experimental animals in each group

Groups	GSHPx (U*/l)	Vitamin C (mg/dl)	Vitamin E (mg/dl)	Reduced Glutathione (mg/dl)
Control	122.6±11.5	2.1±0.10	1.42±0.07	27.4±2.3
Diabetic control	96.5 ± 10.8 a	1.2±0.07a	0.85±0.06 a	16.5±1.8 a
Diabetic + alc BE (300 mg/kg bw)	128.3± 8.5 a*b	3.3±0.08 a*b	1.22±0.07 ab	22.4±2.1 b
Diabetic +glibenclamide (600 mg/kg bw)	121.4±7.9 ab*c	1.65±0.09 ab	1.00±0.05 ab*c	21.3±1.5 b

Values are given mean ±SD (n=6 rats)
*U*Micromoles of glutathione utilized / min*
*a-significantly different from control animals ap<0.001, a*p<0.05*
b-significantly different from diabetic control bp<0.001
*c-significantly different from diabetic +alc "BE" treated rats*cp<0.05*

Table 5: Enzymatic antioxidants and non- enzymatic antioxidants in erythrocytes and erythrocyte membranes of control and experimental animals in each group

Groups	SOD (Ua/mg Hb)	Catalase (Ub/mg Hb)	GSHPx (Uc/g Hb)	Vitamin E in erythrocyte membranes glutathion (ng/mg protein	Reduced glutathion (Mg/dl)
Control	5.52 ± 0.42	123.5 ± 12.8	12.9±0.22	1.07±0.09	32.5±1.3
Diabetic control	3.59 ± 0.29a	77.17 ± 7.8a	6.2± 1.01 a	0.71±0.06 a	22.3±1.9 a
Diabetic + alc BE	4.85±0. 32a*b	104.21±10.4b	9.9±0.08a*b	0.85±0.06 a*b	30.1±2.3 a*b
Diabetic+glibenclamide(600ng/kg bw)	4.55 ±0.41a*bc	95.22±9.7a*bc	8.7± 0.72a*bc	0.79±0.04 abc	26.2±1.2 a*bc

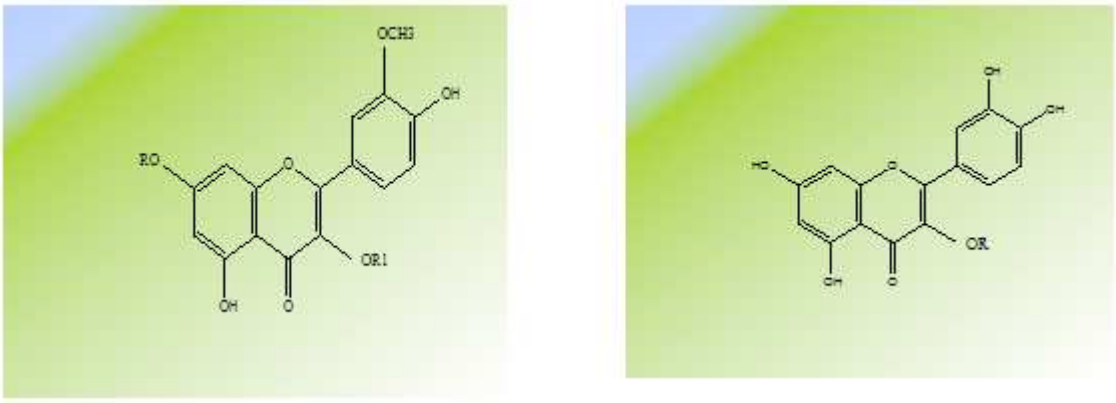
Values are given mean ±SD (n=6 rats)
*a-significantly different from control animals ap<0.001, a*p<0.01, a*p<0.05*
b-significantly different from diabetic control bp<0.001
c-as compared to diabetic +alc BE treated rats cp-not significant
Ua- The amount of enzyme required to inhibit 50% NBT reduction,
Ub- Micromoles of H_2O_2 utilized/ min,
Uc- Micromoles of glutathione utilized / min

DISCUSSION

Diabetes mellitus is a life threatening metabolic disorder and it is estimated that its annual incidence rate will continue to increase in the future worldwide. Hyperglycemia, the primary clinical manifestation of diabetes mellitus, is associated with the development of micro and macro vascular diabetic complications [73]. In the present study, TBARS levels were significantly increased and antioxidants were decreased indiabetic rats. Reactive oxygen species induced oxidative damage has been implicated in the pathogenesis of several disorders including diabetes mellitus [74]. Streptozotocin damages pancreatic b–cells possibly by generating excess reactive oxygen species, and thus widely used for the induction of experimental diabetes mellitus. Streptozotocin generated lipid peroxidation and DNA breaks in pancreatic islet cells have been demonstrated [75]. Prakasam *et al* [76] have reported an elevated lipid peroxidation and lowered antioxidants in streptozotocin induced diabetes mellitus. Measurement of plasma TBARS help to assess the extent of tissue damage [77]. Elevated plasma TBARS observed in the diabetic rats can therefore be related to overproduction of lipid peroxidation byproducts and diffusion from damaged pancreatic tissues. The major thological consequence of free radical induced membrane lipid peroxidation includes increased membrane rigidity, decreased cellular deformability, reduced erythrocyte survival, and lipid fluidity [78]. Hunt *et al* [79] reported that glucose oxidation in the presence of transition metals result in excessive generations of reactive oxygen species, which in turn affect biomembrane structure and function by mediating lipid peroxidation process. Enhanced TBARS and declined antioxidants observed in the erythrocytes of diabetic rats can therefore be attributed to increased biomembrane lipid peroxidation process and thereby contributing to alterations in antioxidants status. Vitamin E is one of the most important free radical scavenging chain-breaking antioxidant within biomembrane [80]. Reduced glutathione, a major endogenous antioxidant, plays a crucial role in the antioxidant defense [81]. Vitamin C, a major extra cellular non-enzymatic antioxidant, has crucial role in scavenging several reactive oxygen species. Enzymatic antioxidants (SOD, CAT (catalase enzyme), GSHPx (glutathione peroxidase defense mechanism)), form the first line of antioxidant defense mechanism to protect the organism from ROS mediated oxidative damage [82]. Several studies have demonstrated lowered non-enzymatic antioxidant levels and enzymatic antioxidant activities in streptozotocin induced diabetic rats [83,84]. Our results lend credibility to these observations. In the present study, orally administered "BE" to diabetic rats at dose of 300 mg/kg bw for 45 days showed significant antihyperglycemic and antilipid peroxidative effects as well as improved antioxidant defense mechanism. The antihyperglycemic activity of "BE" is probably due to stimulation of insulin secretion from remnant pancreatic β-cells, which in turn enhance glucose utilization by peripheral tissues of diabetic rats. The observed increase in hexokinase activity and decrease in glucose-6-phosphatase activity in diabetic rats treated with "BE" suggest its stimulatory effects on glycolysis and inhibitory action on gluconeogenesis in diabetes mellitus. The

observed increase in antioxidant status and decline in TBARS concentration in "BE" treated diabetic rats suggests its potent antilipid peroxidative and antioxidative effects. Furthermore the plant drug was found to be as effective as that of the reference drug glibenclamide.

Figure 1. The structure of the Compounds which are isolated from *Balanites aegyptiaca*



(7)R=glucoside

(1) R = H, R_1 = rutinose (glucose →rhamnose)

(2) R = H, R_1= 3-robinose
(3) R = H, R_1= glucose
(4) R = H, R_1= galactose
(5) R_1= R glucose
(6) R = H, R_1= 3-rhamnogalactoside

(8) R= rutinoside (glucose →rhamnose)

(9) R= H
(10) R = R_1= H

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