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The Flavonoids and biological activity of *Cleome africana* growing in Egypt.

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ABSTRACT

Cleome africana is a medicinal plant used in Egyptian traditional medicine as antidiabetic. The flavonoidal constituents of the plant from both ethyl acetate and butanol fractions of aqueous methanolic extract were isolated and identified. The compounds were identified as: Vinyl rosmarinate, Kaempherol, Quercetin, Isorhamnetin, Quercetin-3,7-*O*-dirhamnoside, Kaempferol -3,7-*O*-dirhamnoside, kaempferol -7-*O*-glucoside and 6-hydroxy kaempferol-3-*O*-glucoside. Their structure were established through chromatographic and spectroscopic measurements(UV, NMR and MS). These compounds were isolated for the first time from this species. The biological evaluationfor the effect of the tested extracts from *Cleome africana* and *Cleome drosefolia* (TM4, TM2, C2, E2 and B2) were carried out on Glucose loading test after 1hr and 2hr, and on fasting glucose level of diabetic groups after 48 hr post streptoozotocine-treatment as well as serum ALT, AST, Uric acid and creatinine levels of diabetic groups after 7 successive days of daily treatment post CCl_4-intoxicated groups. Extract B2 proved to give the best antidiabetic, antiinflammatory effect and hepato-renal protective effect.

Keywords: *Cleome africana*; flavonoids; hypoglycemic activity and anti-inflammatory activity.

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INTRODUCTION:

Cleome africana belongs to *Cleomaceae* family [1]. *Cleome* species are generally used in folk medicine as stomachic, rubefacient and in the treatment of scabies, rheumatic fever and inflammation [2-5]. The dried herb of *C. africana*, locally known as Samwah, is used by herbalists in Egypt as a hypoglycemic agent, and it's decoction is widely used by the Bedouins of the Southern Sinai for the treatment of diabetes [6-7]. It was found that, *Cleome* species exhibited many biological activities as antidiabetic, antiviral, anticancer, anti-diarrheae, analgesic, anti-inflammatory and hepatoprotective. Many studies were carried out to isolate many chemical constituents like, terpenes, flavonoids, glucosinolates and alkaloids. Sharaf et al., in 1997 isolated thirteen flavonoids from the aerial parts of four *Cleome* species which were identified as: kaempferol-7-*O*-rhamnoside , kaempferol-3-*O*-rutinoside, kaempferol-3,7-*O*-dirhamnoside , kaempferol-3-*O*-glucoside-7-*O*-rhamnoside, kaempferol-3-*O*-rhamnoside-7-*O*-glucoside, quercetin-7-*O*-rhamnoside, quercetin-3-*O*-rutinoside, quercetin-7-*O*-rutinoside, quercetin-3,7-*O*-dirhamnoside, quercetin-3-*O*-glucoside-7-*O*-rhamnoside, isorhamnetin-3-*O*-rutinoside, isorhamnetin-3,7-*O*-dirhamnoside, and apigenin- 6,8-di-*C*-glucoside[8], while, Abdel-kader et.al., in 2009 identified 5,3′-dihydroxy-3,6,7,4′, 5′-pentamethoxyflavone, 5-hydroxy-3,6,7,3′,4′, 5′-hexamethoxyflavone, 3′-methoxy-3,5,4′-trihydroxy flavone-7-neohesperidoside and luteolin from *C.droserifolia*[9]. In addition to pinocembrin and quercetin-3-*O*-glucoside-7-*O*-rhamnosidewere isolated from the same plant by Aboushoer et al.,2010 [10].

The present study was carried out to isolate, identify the flavonoids and biological investigation of *C. africana* extracts.

MATERIALS AND METHOD

Plant material

C. africana plant was collected from Matrouh region, Egypt, during spring (May) 2010. Authentication of the plant was kindly confirmed by Prof. Dr. K.H. El-Batanouny, Botany Department, Faculty of Science, Cairo University. The voucher specimen was kept at the herbarium of the national research center. The plant was dried in shade at room temperature. The herb was finely powdered and kept in glass containers under cool dry conditions till extraction.

Chemicals and Reagents

Thin Layer Chromatography (TLC) was performed using Silica gel G (Kieselgel G, type 60 F254 Merck), Poylamide [6D,Riedl-deHaen AG.D-3016 Seelze-1], Sephadex LH20 (Pharmacia Fine Chemicals AB Uppsala, Sweden). Paper chromatography (PC) Whatman No. 1 for detection and Whatman No. 3MM for preparative isolation. Detection was carried out by examining the chromatograms and / or the chromatoplates under UV light at 366 nm before and spraying with 1 % alcoholic $AlCl_3$. Solvents used are petroleum ether (60-80°C), hexane, ether, chloroform, benzene, acetone, ethyl acetate and methanol (El Gomhoreya company for pharmaceutical and chemical industries). EI-MS was performed using ISQ series direct probe Mass spectrophotometer and NMR using Bruker: (^{1}H-NMR, 400 MHz) The NMR spectra were recorded in $CDCl_3$ or dimethylsulphoxide (DMSO-d_6) , the chemical shifts values were recorded in δ ppm.

Extraction and Fractionation of the Flavonoidal Constituents

About 700g of air dried powdered plant of *Cleome africana* were defatted with 2 liter petroleum ether (b.r.40-60ºC) using soxhlet apparatus. The defatted plant material was macerated with 70% methyl alcohol till exhaustion. The alcoholic extract was evaporated *in vacuo* at about 50 ºC (60 g). The residue was dissolved in hot distilled water (600 ml), left over night in the refrigerator and then filtered. The aqueous filtrate was extracted with successive portions of chloroform (3 x 500 ml) followed by ethyl acetate (5 x 500 ml) and finally with n-butanol (5 x 500 ml). The solvents were dried, separately, over sodium sulphate anhydrous and evaporated *in vacuo* at 50ºC, the chloroform, ethyl acetate and n-butanol free solvent residues amounted to 0.3 g, 3.4 g and 5 g respectively.

The ethyl acetate extract (3.4g) was dissolved in a least amount of methanol, then introduced onto the top of glass column (100 × 5 cm) packed with Silica gel G (120 g). Elution was carried out using chloroform

100% followed by gradient with methanol till 70%. Fractions of 100 ml of each were collected. The course of chromatographic fractionation was followed by paper chromatography developed with butanol : acetic acid : water (4 : 1 : 5, upper layer, S1) as the solvent system and alcoholic $AlCl_3$ as a spray reagent. Similar fractions were pooled together , subjected for further purification using different ways like small silica column , Sphadex LH-20 column and PPC giving us four main compounds I, II, III and IV (Vinyl rosmarinate, Kaempherol, Quercetin, and Isorhamnetin, respectively).

About 5.2 grams of butanol fraction were dissolved in a minimum amount of aqueous methanol, 10 ml mixed with 10 g polyamide and evaporated *in vacuo* till dryness . The obtained dried powder was applied on to the top of a glass column (120 x 4 cm) packed with polyamide in water. Elution was carried out using water 100% followed by decreasing of polarity water / methanol mixtures till using 100% methanol. Fractions of 100 ml of each were collected. The course of chromatographic fractionation was followed by paper chromatography and applying S1 as the solvent system and alcoholic $AlCl_3$ as spraying reagent. The fractions 41-50, 51-60 and 61-80 were further purified with different methods as before to afford four main compounds V, VI, VII and VIII (quercetin-3,7-*O*-dirhamnoside, kaempferol-3,7-*O*-dirhamnoside, kaempferol-7-*O*-glucoside and 6-hydroxy kaempferol-3-*O*-glucoside, respectively).

Preparation of plant extracts for biological study was carried out as follows:

Methanolic extract

About 100 g of each of air dried powdered plant of *Cleome africana* (Matrouh) (TM2) and *Cleome droserifolia* (St. Cathrine) (TM4) was extracted in microwave in successive portions of methanol : water (80:20) till exhaustion, the methanolic extract was evaporated under reduced pressure to obtain a semi-solid residue of TM2 and TM4.

Chloroform fraction

The semisolid residue obtained from the methanolic extract of *C. africana* was suspended in the least amount of distilled water, then shaking with successive portions of chloroform till exhaustion, the chloroform extract was evaporated under reduced pressure to obtain the residue (C2).

Ethyl acetate fraction

The semisolid residue obtained from the methanolic extract of *C. africana* was suspended in the least amount of distilled water, then shaking with successive portions of ethyl acetate till exhaustion, the ethyl acetate extract was evaporated under reduced pressure to obtain the residue (E2).

n-Butanol fraction

The semisolid residue obtained from the methanolic extract of *C. africana* was suspended in the least amount of distilled water, then shaking with successive portions of butanol till exhaustion, the butanol extract was evaporated under reduced pressure to obtain the residue (B2).

Chemicals and Reagents

Were purchased from Riedel-de Haën, Germany, Biomerieux diagnostic kits, France, Orgenium laboratories, Finland, and Biodiagnostic, Cairo, Egypt. Kits used for biochemical analysis were purchased from Biodiagnostic, Inc., Egypt. Amaryl**,** Streptozotocin**,** Citrate buffer

- Glucose diagnostic kit
- Gum acacia
- Carbon tetrachloride
- Liquid parafin
- AST And ALT diagnostic kit
- Creatinine diagnostic kit

- Uric acid diagnostic kit
- Sodium Chloride (NaCl) (Sigma Co.)

Animals

Sprague Dawley rats of both sexes weighing 150 –170 gm were used throughout the experiments. Animals were housed under standard environmental conditions (23 ± 1 ^{o}C, 55 ± 5% humidity and a 12-h light: 12-h dark cycle) and maintained with free access to water and a standard laboratory diet *ad libitum*. Animal care and the experimental protocols were approved by the National Research Centre Animal Care and Use Committee in accordance with the guidelines of the International Association for the Study of Pain Committee for Research and Ethical Issues.

Experimental design for hypoglycemic effect

Glucose loading test [11]

Fourty mature rats were divided into 8 equal groups (5 rats each). Rats administered amaryl drug (0.03 mg/kg) or the different tested extracts (100 mg/ kg) before 45 min. of glucose loading test (1 gm /kg) given to groups 2-8.

Diabetic rats

Another confirmatory antidiabetic experiment was done using streptoozotocine(STZ) induced hyperglycemia in rats. Fourty mature rats were divided into 8 equal groups (5 rats each). Group 1 are kept as normal while the other nine groups were injected with a single dose of STZ 65 mg / kg) freshly prepared and dissolved in citrate buffer pH 4.5 to induce diabetes [12]. Diabetes was confirmed after 48 h of STZ injection, blood samples were collected via retro-orbital venous plexus and serum glucose levels were estimated by diagnostic kit method [13]. The rats with serum glucose level above 200 mg / dl were selected and used for the present study. The same previous treatments were given to groups from 3-8 and fasted for 8 hrs. for determining serum glucose level, serum prostaglandin, serum interleukin 1B, serum total antioxidant activity and serum malonaldehyde according to method in refrence [14].

Hepatoprotective effect of different extracts

Thirty five adult rats were divided into 7equal groups; group1 received the vehicle (gum acacia 2.5 %) ; groups 2 - 7 received oral doses of saline (control positive) and 100 mg/ kg ofTM2, TM4, B2, E2 and C2for 7 days, respectively. On the 8th day of treatment, all groups were orally administrated carbon tetra choloride 1.5 ml / Kg b.w.(50% CCl_4 v/v in liquid paraffin) to induce hepato-renal injury according to the method of Yadav and Dixit, 2003[15].

BIOCHEMICAL MEASUREMENTS

After 48 hr post CCl_4 administration, blood samples were collected from retro-orbital venus plexus from all animals in plain test tubes. Serum was prepared for biochemical analysis of aspartate and alanine aminotransferase (AST and ALT) activities according to the method of Reitman and Frankel,1957 [16], Creatinine Thefeld et al., 1974 [17] and uric acid Haisman and Miller, 1997 [18].

Serum Tumor Necrosis Factor-α (TNF- α) was determined according to the method described by Seriolo et al. 2006,[19] by Enzyme-linked Immuno-Sorbent Assay (ELISA) using TNF- α kits. Interleukin 1 beta (IL- 1β) was determined with a rat ELISA (Endogen Inc, USA) according to the method of Safieh-Garabedian et al. 1995 [20]

RESULTS AND DISCUSSION

Compound-I (vinyl rosmarinate):This compound was isolated as white powder in a small amount (4mg) and it appears as a blue spot changed to faint blue with $AlCl_3$ under UV light. The UV absorption spectrum in methanol showed a characteristic peak at 291 nm with two shoulders at 238nm and 265nm.The

EI-MS of compound -I showed a molecular ion peak M^+ at m/z = 386 which corresponds to the molecular formula $C_{20}H_{18}O_8$. The fragmentation pattern of the compound displayed another important peaks at m/z=385, 367, 326, 272, 244, 224 and 164 due to loss of H, ($H+H_2O$), ($CH\equiv CH+2^-OH$), ($CH_2=CHO+3H_2O+^-OH$), ($CH_2=CHO+3H_2O+^-OH$ +CO). These data were in agreement with that reported by Evangelos, *et al* in 2007[21], where they identified methyl rosmarinate from *Hypericum perforatum* by LC/SPE/NMR and/or LC/MS.

Compound-II (kaempferol):This compound was isolated as yellow amorphous powder (15mg). It is an aglycone in nature according to it's chromatographic behavior on PC in different solvent systems (R_f = 0.85, in S1 and R_f= 0.11 in 15 % acetic acid, S2). The UV absorption spectra of the compound displayed band-I at λ_{max} (MeOH)=366 nm which prove the flavonol nature of this compound. Also, the other data with shift reagents proved that, the compound have free OH groups at C4', C-7 with no *Ortho*-dihydroxy system in ring-B [22] .The EI –MS of the compound exhibited a molecular ion peak M^+ at m/z =286 which related to the molecular formula $C_{15}H_{10}O_6$. The fragmentation pattern displayed another important peaks at m/z= 165 and 121 and retero Diel's Alder fragmentation (RDAR) giving rise to A_1 fragment at m/z=153 and B_1 fragment at m/z= 133. In addition to the peaks at m/z=285, 267 and 228 due to loss of (H, (H_2O+H) and 2CHO,) respectively. The ^{1}H nmr (DMSO) showed the characteristic signals for the aromatic protons of the B- ring as two doublets at δ 8.03, δ 6.93 with *J* = 8.5 Hz due to ortho coupling of H-2', 6' and H-3', 5' respectively, two aromatic protons of the A-ring were assigned as two doublet at δ 6.41and δ 6.17 with *J* = 2.0 Hz due to meta coupling of H-6 and H-8 respectively. These data coincided with that stated by Tsukasa *et al* in 2012[23] which permit us to elucidate the structure of compound -II as kaempferol.

Compound-III (Quercetin): This compound was isolated as yellow needle-like crystals in an amount of 12mg. It is an aglycone in nature according to it's chromatographic behavior on PC in different solvent systems (R_f = 0.69, in S1 and R_f= 0.21 in S2). The UV absorption spectra of the compound displayed band-I at λ_{max} (MeOH)=357 nm which prove the flavonol nature of this compound. Also, the other data with shift reagents proved that, the compound have free OH group at C4', C-5, C-7 with an *Ortho*-dihydroxy system in ring -B[22].

The EI –MS of compound showed a molecular ion peak M^+ at m/z =302 which is compatible with the molecular formula $C_{15}H_{10}O_7$. The fragmentation pattern displayed important peaks at m/z= 164 and 138 in addition to RDAR fragments (A_1 fragment at m/z=154 and B_1 fragment at m/z= 148) [24-25]. Other peaks at m/z=301, 274 and 246 are due to loss of (H, CO, and 2CO) respectively. The ^{1}H nmr (DMSO) showed the characteristic signals for aromatic proton of the B- ring as doublet at δ 7.69, *J* = 2.1 Hz of H-2' due to meta coupling of H-6'anddoublet of doublet atδ 7.57, *J* = 2.1 Hz and 8.4 Hz of H-6' due to meta coupling with H-2'and ortho- coupling with H-5' respectively, a doublet at (δ 6.9, *J* = 8.4 Hz) for H-5' due to an ortho coupling with H-6' was observed , two aromatic proton of the A-ring showed as two doublet at δ 6.2 and δ 6.42with *J* = 1.8 Hz of each proton due to meta coupling of H-6 and H-8 respectively. The previous measurements allow us to identify compound -III as Quercetin which coincided with that stated by Guvenalp, Z *et al* 2005 [26].

Compound-IV (Isorhamnetin): This compound was isolated as yellowish white powder (17mg) and it appear as a dark brown spot changed to yellow with $AlCl_3$ under UV light. It is an aglycone in nature according to it's chromatographic behavior on PC in different solvent systems (R_f = 0.54, in S1 and R_f=0.25 in S2). The UV absorption spectra of the compound proved it's flavonol nature where it displayed band-I at λ_{max} (MeOH)=370 nm. The other data with various shift reagents showed that the presence of a free OH group at C4' and C-7 with absence of an *Ortho*-dihydroxy system[22].The EI-MS of compound exhibited a molecular ion peak M^+ at m/z =316 which fit with the molecular formula $C_{16}H_{12}O_7$. The fragmentation pattern of compound showed peaks at m/z= 164 and 152 in addition RDAR fragmentation giving rise to A_1 fragment at m/z=152 and B_1 fragment at m/z= 164[24]. Also the fragmentation pattern displayed another peaks at m/z=285, 272 and 257 due to loss of (OCH_3, $COCH_3$ and $OCOCH_3$) respectively. The ^{1}H-nmr spectrum (CD_3OD) showed signals at δ=8.1(d,1H, H-2'), 7.83(dd, 1H, H-6'), 6.88(d,1H, H-5'), 6.43(d, 1H, H-8), 6.2(d,1H, H-6), 3.6(s , OCH_3). So the above chromatographic and spectroscopic data, coincides with that stated by Dong-Mei W et al., 2012 [27] which permit us to identify the structure of compound IV as Isorhamnetin.

Compound- V (Quercetin-3,7-O-dirhamnoside):The compound appeared as a dark brown spot (11mg) under the UV light and not changed with ammonia and it has R_f value in both S2 and S1 (0.75 and 0.23 respectively) which indicates that , it may be a diglycosidic flavonoidal compound. The UV absorption spectra of the compound in methanol gave band –I at 356 nm which indicated that, it is a flavonol type structure. Also the other data with shift reagents proved that the compound have free OH group at C4' and C-3' and absence

of free OH group at C-7[22]. The EI -MS showed a small molecular ion peak at m/z =594. It was found that, this peak correspond to the molecular formula $C_{27}H_{30}O_{15}$. The peaks at m/z =448 corresponding to [M^+ - Deoxysugar moiety (146)] and m/z =302 corresponding to [M^+ - two Deoxysugar moieties (146x2)] showing that, the M^+ for the aglycone is 302. Additional peaks at m/z =148 and 154 which proves the fragmentation pattern of RDAR. Other peaks at m/z=272 are due to loss of two deoxysugar moieties with CH_2O group. The ^{1}H-nmr spectrum (CD_3OD) showed signals at δ=7.34 (d, 1H, H-2') 7.15(dd, 1H, H-6'), 6.9(d, 1H, H-5'), 6.7(d ,1H, H-8), 6.45(d,1H, H-6'), 5.5(s, 1H, H-1'''), 5.3(s,1H,H-1''), 1.3(s, 3H, CH_3 of rhamnose at C-7) , and 0.9(s,3H, CH_3 of rhamnose at C-3).

Acid Hydrolysis

About 2 mg of compound-V were dissolved in 10 ml methanol, mixed with 10 % HCl and refluxed on a boiling water bath for 3 hours. The solution was diluted with distilled water and extracted with ether (3 x 25 ml). The aqueous acidic solution was neutralized with barium carbonate, filtered through a centered glass funnel. The clear filtrate was evaporated till dryness. The residue was dissolved in redistilled pyridine, filtered, and evaporated *in vacuo* at 70 ºC. The residue was dissolved in 10 % isopropanol and subjected to paper chromatography. using EtOAc : pyridine : H_2O (12 : 5 : 4, S3) [22]. The chromatogram was visualized by spraying with aniline phthalate, and heated at 105 ºC for few minutes. rhamnose the only sugar was detected in the hydrolyzate by using authentic sugar samples . (Mabry,T.J. *et al* ., 1970). The UV and MS data of the obtained aglycone of the compound are in agreement with Quercetin, so that, compound-V could be identified as Quercetin-3,7-*O*-dirhamnoside which agree with that stated by Sharaf M. *et al* in 1997 [8].

Compound-VI (Kaempferol-3,7-O-dirhamnoside): A yellowish amorphous powder in an amount of 14mg which appears as a purple spot under UV light at R_f 0.70 in S2. The characteristic features of it's absorption spectra in UV are , band –I at 350 nm which shifted to 412 nm on addition of NaOMe. So, it is a flavonol type structure with free OH group at C4$^-$,C-5 and absence of free OH group at C-7 and *Ortho*-dihydroxy system [22].

The EI- MS of this compound displayed a molecular ion peak M^+ at m/z =578, it was found that it is compatible with the molecular formula $C_{22}H_{30}O_{14}$ .The ion peak at m/z =432 refers to M^+ -Deoxyhexose moiety(rhamnose) and the peak at m/z=286 correspond to M^+ - 2Deoxyhexose moeities)] . The peak at m/z =153 and 133 confirm the fragmentation pattern, where it undegoes RDAR. Another fragmnet is 256 which is due to loss of CH_2O.The ^{1}H-nmr spectrum (CD_3OD) showed signals at δ=7.77(dd, 1H, H-6', H-2'), 6.9(dd, 1H, H-5', H-3'), 6.7(d , 1H, H-8), 6.45(d,1H, H-6) , 5.5(s, 1H, H-1'''), 5.3(s,1H,H-1''), 1.3(s, 3H, CH_3 of rhamnose at C-7) and 0.9(s,3H, CH_3 of rhamnose at C-3).The C^{13}-nmr spectrum (DMSO) showed signals at δ=178.44 (C4) , 162.19 (C7), 161.67 (C5, C4'), 158.60 (C2), 156.74 (C9), 135.05 (C3), 130.65 (C2', C6'), 120.69 (C1'), 115.44 (C3', C5'), 106.20 (C10), 102.15 (C6), 99.19 (C1"), 98.50(C1'''), 94.26 (C8), 72.24 (C4", C4'''), 70.75 (C3",C3'''), 70.70 (C2", C5", C2''', C5'''), 16.72 (C6"), 16.32 (C6'''). The acid hydrolysis resulted in kaempferol as an aglycone which identified by co-chromatography on TLC and PC using an authentic sample and the sugar moieties were identified as rhamnose by using PC Whatman no.1 with S3 [22]. The UV and MS data of the aglycone of this compound are in agreement with those reported for Kaempferol, so that compound could be identified as Kaempferol-3,7-*O*-dirhamnoside which was coincided with data stated by Mustafa K in 2000 [28].

*Compound – VII (*kaempferol -7-*O*-glucoside*)*: The chromatographic behavior of the compound proved that, it is a glycosidic in nature where it's R_f in different solvent systems are (0.35 and 0.62) in S1 and S2 respectively. The UV absorption spectra of the compound with different shift reagents showed that, it is a flavonol type structure where it displayed band- I at 358 nm in methanol having a free OH group at C-4', C-5 and the absence of *Ortho*-dihydroxy system and the OH group at C-7 is occupied. The EI –MS displayed a molecular ion peak at 448 which are corresponding to the molecular formula $C_{21}H_{20}O_{11}$.The peak at m/z =286 attributed to M^+-162 which confirm the presence of a hexose moiety as a sugar. Also the peak at m/z =116 confirm the fragmentation pattern, where it undergoes RDAR (133- $^-$OH=116). The ^{1}H- nmr (CD_3OD) spectrum displayed signals at δ in ppm =7.8 (d, 2H, H-2', H-6'), 6.9 (d, 2H, H-5', H-3'), 6.71 (d,1 H, H-8), 6.46 (d, 1H, H-6), 5.4 (d, 1H, H-1") .

The acid hydrolysis gave kaempferol as an aglycone which identified by co-chromatography on TLC and PC using an authentic samples and the sugar moieties were identified as glucose by using PC Whatman

no.1 , S3[22]. From all the previous data, compound VII could be identified as Kaempferol -7-*O*-glucoside which coincided with data stated by Cristiane *et al*.,in 2012 [29].

*Compound -VIII (6-hydroxy kaempferol-3-O-glucoside):*The chromatographic behavior of the compound proved that it is a glycosidic in nature where its R_f in different solvent systems are (0.39 and 0.59) in S1 upper layer and S2 respectively . The UV absorption spectra of the compound with different shift reagents showed that, it is a flavonol type structure occupied at C-3, where it displayed band- I at 344 nm in methanol. Also it has free OH groups at C-4', C5, C7 and the absence of *Ortho*-dihydroxy system. The EI-MS displayed a molecular ion peak at 464 which are corresponding to the molecular formula $C_{21}H_{20}O_{12}$ .The peak at m/z =302 attributed to M^+-162 which confirm the presence of a hexose moiety. Also the peak at m/z =169 confirm the fragmentation pattern due to RDAR [24] .

The ^{1}H- nmr (DMSO) spectrum displayed signals at δ in ppm =7.89 (d, 2H, H-2', H-6'), 6.9 (d, 2H, H-5', H-3'), 6.41 (s,1H, H-8), 5.7 (d, 1H, H-1") .
The acid hydrolysis resulted in 6-hydroxy Kaempferol as an aglycone which identified by co-chromatography on TLC and PC and the sugar moieties were identified as glucose as in reference[22]. From the previous data, compound VIII could be identified as 6-hydroxy Kaempherol -3-*O*-glucoside which are approved by that obtained by Nazemiyeh et al., in 2009[30].

Antidiabetic effect

The obtained results from the biological evalution (tables 1 & 2, fig.1) as hypoglycemic of the tested extracts, was illustrated in glucose loading test 1 hr after treatment showed significant decrease in glucose level in groups treated with B2 > E2> Amaryl and C2 > TM4 > TM2. While, after 2 hrs , glucose loading test was normalized by B2 treatment, and significantly decreased than diabetic non-treated group (gr2) as follows: E2 >amaryl, C2 , TM4 > TM2..

Moreover, glucose level significantly reduced in the fasting diabetic rats treated with B2> E2>amaryl> TM2 > TM4 as shown in table 3 and fig.1.

Herbal products may contain several active constituents or compounds that can act by several modes of action to influence multiple biological pathways and to alleviate the diabetic symptoms, providing thereby multifaceted benefits [31]. Moreover, the low cost of these compounds and the minimal side effects are other reasons behind the hunt for effective natural agents to be used as complementary and/or alternative medicine.

The potential antidiabetic activity of five extracts (TM2, TM4, B2, E2 and C2) varies significantly. The resultant hypoglycemic effect due to several hypothesis; herbs inhibit glucose absorption from intestine, increasing insulin secretion from the pancreas, inhibiting glucose production from hepatocytes, or enhancing glucose uptake into the peripheral tissue via the glucose transporters (GLUT) [32] .

Antiinflammatory effect

The data presented in table 4, fig. 2 showed significant inhibition in serum PGE2 than normal control in groups given B2, E2,TM2 and . While, TM4, C2, and amaryl normalized PGE2 level comparing with diabetic non-treated rats.

Serum IL1B value significantly reduced by all treatment groups as following manner: B2> E2, >C2 , amaryl> TM2, TM4 when compared with diabetic control group (table 5 , Fig 2).

Furthermore, serum TAC significantly reduced in diabetic control than any treated rats. All tested extracts significantly increased TAC level than control group in variable degrees (B2 >E2, TM2> C2 >TM4>amaryl as shown in table 6 and fig 2 .

The tested antidiabetic agents herein have antioxidant properties as they significantly decreased MDA and increased TAC in serum of treated diabetic groups.

Serum MDA value significantly elevated in diabetic non-treated group than control and the other treated animals. B2 treatment significantly decreased MDA level than normal control (table 7 and Fig.2). Our results revealed that all treatment groups inhibited PGE2 and IL1B levels comparing with the diabetic control group in different degrees. It was noticed that diabetes increases inflammatory cytokines as discussed by El-Abhar *et al*., 2014[33] where was concluded that cyclooxygenase and lipoxygenase play an important role as inflammatory mediators. Flavonoids inhibits both cyclooxygenase and lipoxygenase activities along with eicosanoid biosynthesis, thus diminishing the formation of these inflammatory metabolites [34-36]. Flavonoids also inhibit both cytosolic and membranal tyrosine kinase [37].

Hepato-renal protective Effects

CCl_4-intoxicated rats showed significant elevation in ALT, AST, creatinine, and uric acid when compared with any group in the experiment (tables8-11).

Only B2 treatment significantly decreased ALT, AST and uric acid; and normalized the increased creatinine value. The other treatments significantly decreased ALT and AST values; and decreased UA and creatinine in variable degree as follows: E2> C2> TM2 and TM4 comparing with CCl_4-treatment group (Tables 8-11 and Fig.3). This effect of flavonoids as a preserved hepato-renal damages induced by CCl4 may be due to their capacity in preventing free radical formation [38].

Table 1: Effect of different Herbal plant extracts (100 mg/kg, per Os) on glucose loading test (2 gm/kg) after 1 hours of treatments versus the standard drug Amaryl (0.03 mg/kg). (n= 5; means ± standard errors of the means)

Groups	Normal Control	Non treated	Amaryl	TM2	TM4	B2	E2	C2
	A	B	C	B	D	E	F	C
Mean	70.0	181.0	130.0	172.0	150.0	96.0	120.0	135.0
Std. Error	± 1.40	± 1.50	± 1.40	± 1.60	± 1.80	± 1.70	± 1.30	± 2.90

ANOVA one way at p< 0.05, followed by Tukey comparing all pairs of groups using Graph Pad Prism ver. 5 program.
The similar capital litters are insignificantly different with each other.

Table 2: Effect of different Herbal plant extracts (100 mg/kg, per Os) on glucose loading test (2 gm/kg) after 2 hours of treatments versus the standard drug Amaryl (0.03 mg/kg). (n= 5; means ± standard errors of the means)

Groups	Normal Control	Non treated	Amaryl	TM2	TM4	B2	E2	C2
	A	B	C	CD	D	A	E	F
Mean	69.22	140.1	110.1	124.4	118.9	75.22	103.4	113.9
Std. Error	± 1.269	± 2.684	± 2.619	± 4.051	± 3.567	± 2.473	± 3.408	± 1.704

ANOVA one way at p< 0.05, followed by Tukey comparing all pairs of groups using Graph Pad Prism ver. 5 program.
The similar capital litters are insignificantly different with each other.

Table 3: Effect of different treatments Herbal plant extracts (100 mg/kg, per Os) on fasting serum glucose level in streptoozotocine diabetic rats versus the standard drug Amaryl (0.03 mg/kg). (n= 5; means ± standard errors of the means)

Groups	Normal Control	Non treated	Amaryl	TM2	TM4	B2	E2	C2
	A	B	C	D	E	F	G	H
Mean	69.22	276.4	138.2	147.0	151.8	76.60	106.2	124.0
Std. Error	± 1.45	± 1.61	±1.83	± 1.41	± 1.77	± 1.43	± 1.54	±1.16

ANOVA one way at p< 0.05, followed by Tukey comparing all pairs of groups using Graph Pad Prism ver. 5 program.
The similar capital litters are insignificantly different with each other.

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Table 4: Effect of different treatments Herbal plant extracts (100 mg/kg, per Os) on serum Prostaglandine E2 (PGE2; ng/L) level in streptoozotocine diabetic rats versus the standard drug Amaryl (0.03 mg/kg). (n= 5; means ± standard errors of the means)

Groups	Normal Control	Non treated	Amaryl	TM2	TM4	B2	E2	C2
	A	B	A	C	A	D	D	AC
Mean	67.40	90.23	63.80	56.40	66.39	48.50	54.21	61.70
Std. Error	± 1.2	± 1.04	± 1.95	± 1.95	± 1.25	± 1.34	± 1.78	± 1.52

ANOVA one way at p< 0.05, followed by Tukey comparing all pairs of groups using Graph Pad Prism ver. 5 program.
The similar capital litters are insignificantly different with each other.

Table 5: Effect of different treatments Herbal plant extracts (100 mg/kg, per Os) on serum interleukin 1B (IL1B; pg/ml) level in streptoozotocine diabetic rats versus the standard drug Amaryl (0.03 mg/kg). (n= 5; means ± standard errors of the means)

Groups	Normal Control	Non treated	Amaryl	TM2	TM4	B2	E2	C2
	A	B	CE	BC	BC	AD	DE	E
Mean	2.500	19.77	13.21	15.82	16.22	5.430	8.310	10.32
Std. Error	± 0.130	± 1.210	± 1.120	± 1.240	± 1.760	± 0.210	± 0.410	± 1.04

ANOVA one way at p< 0.05, followed by Tukey comparing all pairs of groups using Graph Pad Prism ver. 5 program.

Table 6: Effect of different treatments Herbal plant extracts (100 mg/kg, per Os) on serum total antioxidant capacity(TAC; mmol/L) level in streptoozotocine diabetic rats versus the standard drug Amaryl (0.03 mg/kg). (n= 5; means ± standard errors of the means)

Groups	Normal Control	Non -treated	Amaryl	TM2	TM4	B2	E2	C2
	A	B	C	D	E	F	G	D
Mean	1.260	0.8800	1.540	2.140	1.830	4.210	2.940	2.110
Std. Error	± 0.040	± 0.0110	± 0.0500	± 0.0760	± 0.063	± 0.055	± 0.0730	± 0.0380

ANOVA one way at p< 0.05, followed by Tukey comparing all pairs of groups using Graph Pad Prism ver. 5 program.
The similar capital litters are insignificantly different with each other.

Table 7: Effect of different treatments Herbal plant extracts (100 mg/kg, per Os) on serum malonaldehyde (MDA; nmol/ml) level in streptoozotocine diabetic rats versus the standard drug Amaryl (0.03 mg/kg). (n= 5; means ± standard errors of the means)

Groups	Normal Control	Non – treated	Amaryl	TM2	TM4	B2	E2	C2
	A	B	C	D	E	F	G	H
Mean	3.27	6.80	5.39	4.63	4.92	2.85	3.89	4.31
Std. Error	± 0.027	± 0.021	± 0.095	± 0.054	± 0.055	± 0.068	± 0.047	± 0.075

ANOVA one way at p< 0.05, followed by Tukey comparing all pairs of groups using Graph Pad Prism ver. 5 program.
The similar capital litters are insignificantly different with each other.

Hepatotoxic rats:

Table 8: Effect of different treatments Herbal plant extracts (100 mg/kg, per Os) on serum ALT level in normal rats. (n= 5; means ± standard errors of the means)

Groups	Normal Control	CCl4	TM2	TM4	B2	E2	C2
	A	B	CE	C	D	EF	EF
Mean	25.61	45.98	35.58	39.00	20.26	28.40	30.00
Std. Error	± 1.01	± 1.00	± 0.81	± 0.82	± 0.58	± 0.93	± 0.71

ANOVA one way at p< 0.05, followed by Tukey comparing all pairs of groups using Graph Pad Prism ver. 5 program.
The similar capital litters are insignificantly different with each other.

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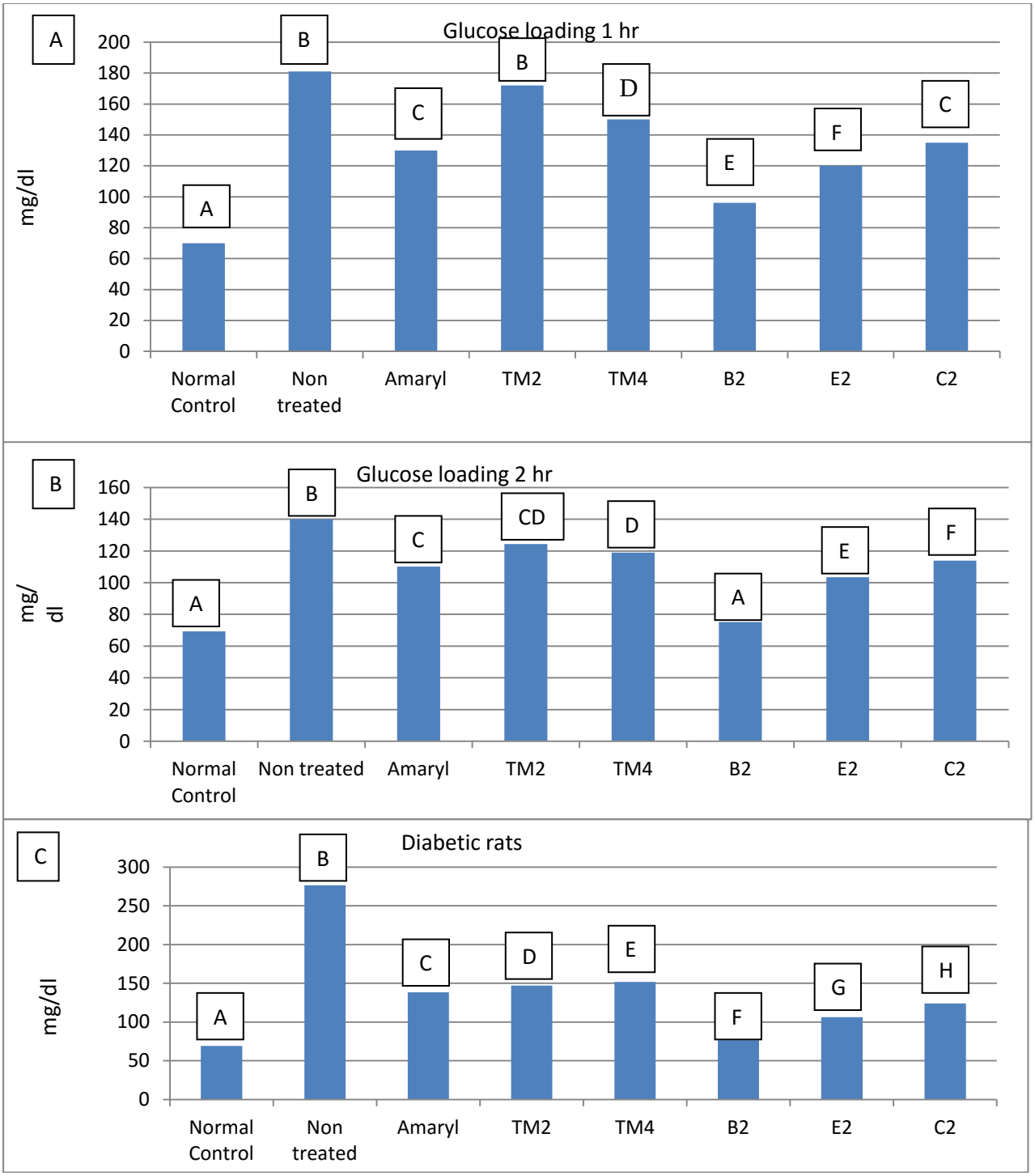

Fig. (1) : Effect of the tested plant extracts on Glucose loading test after 1hr (A) and 2hr (B), and on fasting glucose level of diabetic groups after 48 hr post streptoozotocine-treatment.

ANOVA one way at $p< 0.05$, followed by Tukey comparing all pairs of groups using Graph Pad Prism ver. 5 program. The similar capital litters are insignificantly different with each other.

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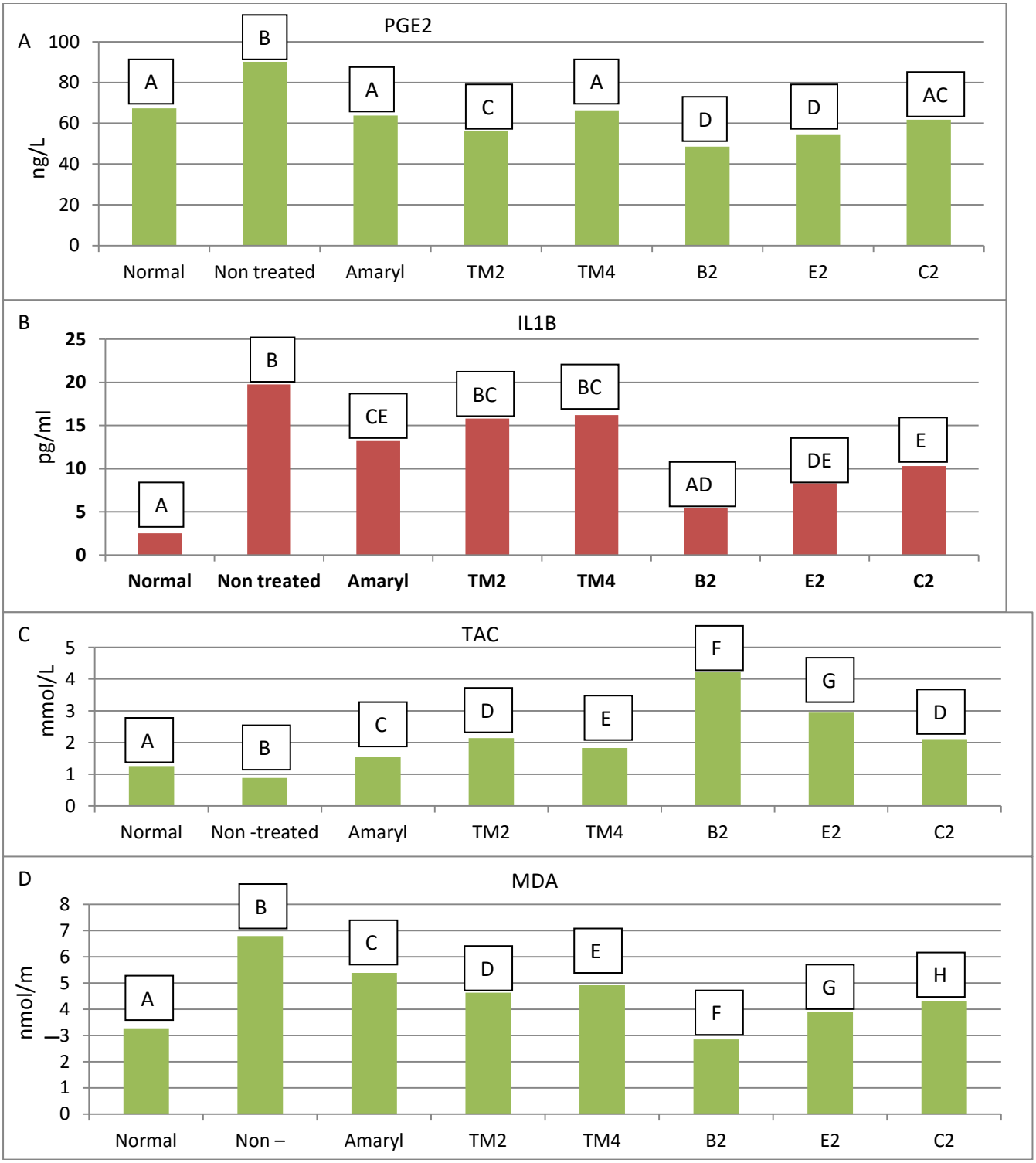

Fig (2) : Effect of the tested plant extracts on serum PGE2(A), IL1B (B), TAC (C) and MDA (D) levels of diabetic groups after 48 hr post streptoozotocine-treatment.

ANOVA one way at $p< 0.05$, followed by Tukey comparing all pairs of groups using Graph Pad Prism 5 program. The similar capital litters are insignificantly different with each other.

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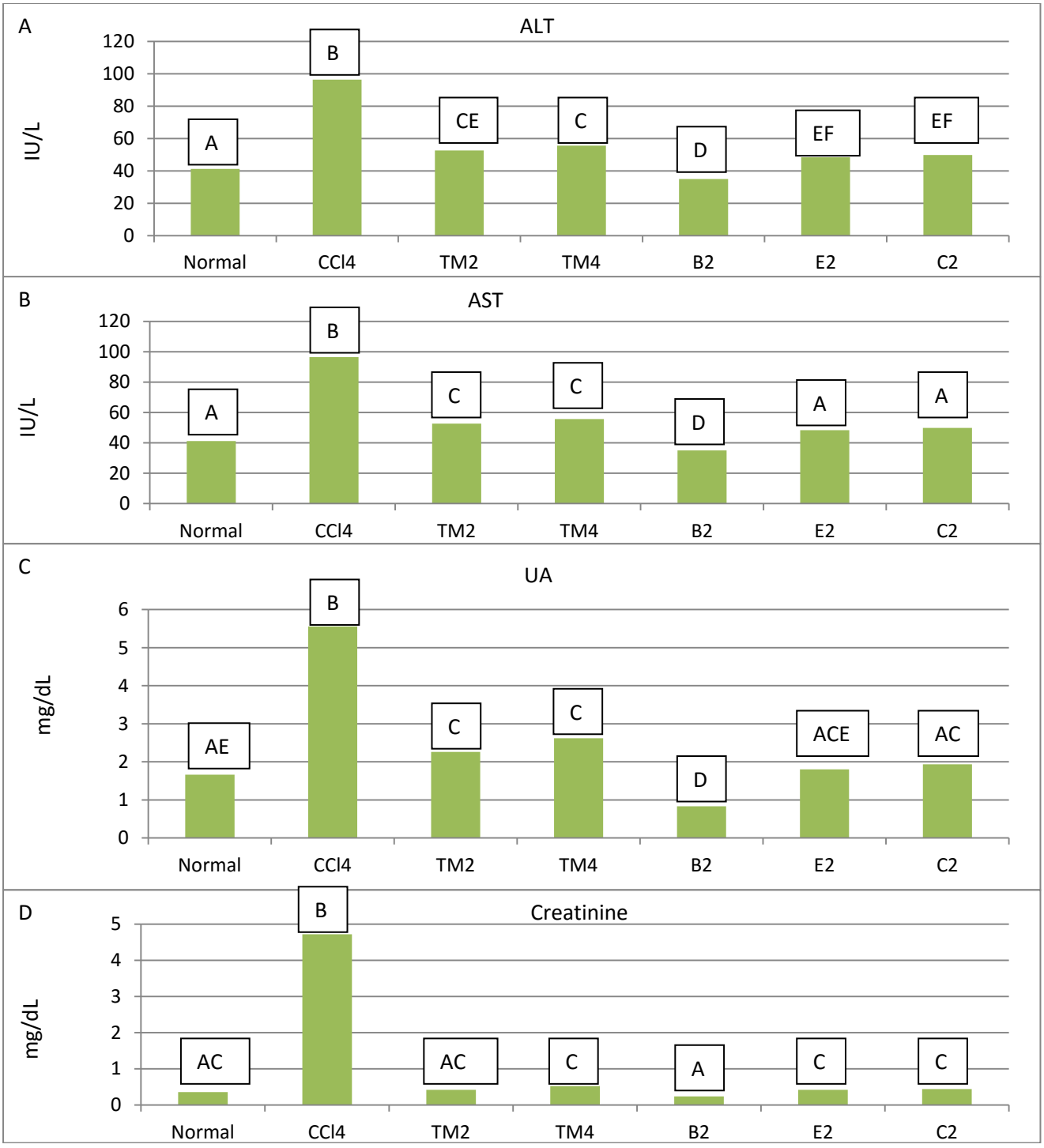

Fig (3) : Effect of the tested plant extracts on serum ALT (A), AST (B) , Uric acid (C) and creatinine (D) levels of diabetic groups after 7 successive days of daily treatment post CCl4-intoxicated groups.

ANOVA one way at $p< 0.05$, followed by Tukey comparing all pairs of groups using Graph Pad Prism ver. 5 program. The similar capital litters are insignificantly different with each other.

Table 9: Effect of different treatments Herbal plant extracts (100 mg/kg, per Os) on serum AST level in normal rats. (n= 5; means ± standard errors of the means)

Groups	Normal Control	CCl4	TM2	TM4	B2	E2	C2
	A	B	C	C	D	A	A
Mean	41.14	96.40	52.60	55.60	35.00	48.40	49.80
Std. Error	± 0.92	± 1.36	± 0.93	± 1.63	± 1.14	± 1.21	± 1.28

ANOVA one way at p< 0.05, followed by Tukey comparing all pairs of groups using Graph Pad Prism ver. 5 program. The similar capital litters are insignificantly different with each other.

Table 10: Effect of different treatments Herbal plant extracts (100 mg/kg, per Os) on serum Uric acid level in normal rats. (n= 5; means ± standard errors of the means)

Groups	Normal Control	CCl4	TM2	TM4	B2	E2	C2
	AE	B	C	C	D	ACE	AC
Mean	1.66	5.560	2.260	2.620	0.832	1.804	1.936
Std. Error	± 0.15	± 0.157	± 0.121	± 0.102	± 0.027	± 0.080	± 0.052

ANOVA one way at p< 0.05, followed by Tukey comparing all pairs of groups using Graph Pad Prism ver. 5 program. The similar capital litters are insignificantly different with each other.

Table 11: Effect of different treatments Herbal plant extracts (100 mg/kg, per Os) on serum creatinine level in normal rats. (n= 5; means ± standard errors of the means)

Groups	Normal Control	CCl4	TM2	TM4	B2	E2	C2
	AC	B	AC	C	A	C	C
Mean	0.356	4.720	0.416	0.522	0.236	0.416	0.436
Std. Error	± 0.011	± 0.107	± 0.019	± 0.011	± 0.009	± 0.008	± 0.014

ANOVA one way at p< 0.05, followed by Tukey comparing all pairs of groups using Graph Pad Prism ver. 5 program. The similar capital litters are insignificantly different with each other.

CONCLUSION

It could be concluded that the glycosides which are present and isolated from the butanolic fraction (Quercetin-3,7-*O*-dirhamnoside, Kaempferol -3,7-*O*-dirhamnoside, kaempferol -7-*O*-glucoside and 6-hydroxy kaempferol-3-*O*-glucoside) of *Cleome africana* species showed significant decrease in glucose level in fasting diabetic rats, and also inhibited serum PGE2, IL1B, TAC and MDA values giving an anti-inflammatory effect which is a common side effect by diabetes. More over decreased ALT, AST and uric acid; and normalized the increased creatinine value giving a hepato-renal protective effect.

REFERENCES

[1] TäckholmV. Students` Flora of Egypt, 2nd Ed.; Cairo University Press: Cairo, Egypt, 1974: p167.
[2] Boulos L. Flora of Egypt; Al Hadara Publishing: Cairo, Egypt, 1999: p177.
[3] Boulos L. Medicinal Plants of North Africa; Reference Publication Inc.: Michigan, USA, 1983; p 52.
[4] Ghazanfar S.A. Handbook of Arabian Medicinal Plants; CRC Press: Boca Raton-London-Tokyo, 1994: p 76.
[5] Chopra R.N.; Chopra I.C.; Handa K.L. and Kapur L.D. Chopra`s Indigenous Drugs of India; Dhur& Sons, Private Limited: Calcutta, India, 1972: p 321- 669.
[6] Abdel-Hady N.M. M.Sc Thesis, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, 1998.
[7] Ayoub, N.A. M.Sc. Pharm. Thesis, Faculty of Pharmacy, Cairo University 1993.
[8] Sharaf M. , El-Ansari M. A. , and Saleh N. A. M . Biochemical Systematics and Ecology. 1997; 25 (2):161–166.
[9] Abdel-Kader M.S. , Alqasoumi S.I., and Al-Taweel A.M. Chemical and Pharmaceutical Bulletin 2009; 57 (6): 620-624

[10] Aboushoer M.I., Fathy H.M., Abdel-Kader M.S. Goetz, G., and Omar A A. Natural Product Research 2010; 24 (7): 687-696.
[11] Trinder P. J. Clin. Pathol. 1969 ; 22(2): 158-61.
[12] Tuzcu, M. and Baydas G. Eur. J. Pharmacol. 2006; 537: 106-110.
[13] Kuhad, A., Sethi R. and Chopra K. Life Sci. 2008; 83: 128-134.
[14] Shilpa k., Subhashchandrappa M., Mallikarjun C.R., Swapnali. An International Research Journal of Pharmacy and Plant science. 2013; 1(2): 1-7.
[15] Yadav N.P.and Dixit V.K. J. Ethno-pharmacol. 2003; 86(2-3):197-202.
[16] Reitman S., and Frankel S.A., *Am. J. Clin. Pathol.* 1957; 28: 56-58.
[17] Thefeld, W., Hffmiester, H., Busch, E., W.,Koller, P.U. and Volmer,J. Deut. Med. Wochenchr, 1974; 99 (8): 343- 351.
[18] Haisman P, and Muller BR. Glossary of Clinical Chemistry Terms. London, UK: Butterworth 1997.
[19] Seriolo B., Paolino S., Sulli A., and Ann N. Y., Acad Sci. 2006; 1069: 414–19
[20] Safieh- Garabedian B., Poole S., Allchorne A., Winter J., and Woolf C.J. Br. J. Pharmacol., 1995; 115: 1265–1275.
[21] Evangelos C. T., Sjef B., Vassiliki E., Anastassios N. T., Jacques V., and Ioannis P. G., 2007; 68: 383–393.
[22] Mabry T.J., Markham K.R., and Thoms M.B. The systematic identification of flavonoids 1970.
[23] Tsukasa I. and Goro K. Bull. Natl. Mus. Nat. Sci., Ser. B, 2012; 38(2): 63–67
[24] Markham K. R., Techniques of flavonoid identification by Academic Press 1997.
[25] Anibal L.R. Biochemical Systematics and Ecology 2004; 32 (3): p 351-353
[26] Guvenalp, Z. Uhal , Omur L. and Irezer D. Turk. J. Chem., 2005; 29: 163 – 169.
[27] Dong-Mei W., Wen-Jun P., Yong-Hong W., Yu-Juan Z. and Shan-Shan W. Molecules 2012; 17: 4595-4603.
[28] Mustafa K., Islamoglus U., Nurettin Y., Hasan B.S., and Hasan G. Turk. J. Chem., 2000; 24: 191 – 197.
[29] Cristiane P., Cleber B., Barreto J., Ricardo M. K., Naomi K. S., Cassia M. S., Andrea P. and Ludger W. Nova, 2012; 35(6): 1123-1126.
[30] Kar A, Choudhary BK, and Bandyopadhyay NG. J Ethnopharmacol 2003; 84: 105-108
[31] Gao H, Huang YN, Gao B, Li P, Inagaki C, and Kawabata J. Food Chemistry 2008; 108: 965–972
[32] El-Abhar H. S., and Schaalan M. F. World J Diabetes 2014; 5(2): 176-197
[33] Robak J, Gryglewski RJ. Pol J Pharmacol 1996; 48: 555–564.
[34] Kim HP, Mani I, Iversen L, and Ziboh VA. Essent Fatty Acids 1998; 58: 17–24.
[35] Formica JV, and Regelson W. Food Chem Toxicol 1995; 33: 1061–1080.
[36] Damas J, Bourdon V, Remacle-Volon G, and Lecomte J. Prostaglandins Leukot Med 1985;19:11–24.
[37] Hanasaki Y, Ogawa S, and Fukui S. Free Radic Biol Med 1994; 16: 845–850.