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Original Article

CHEMO-PROTECTIVE EFFECT ON HEPATO-RENAL TOXCICITY AND CYTOTOXIC ACTIVITY OF LIPOIDAL MATTER OF *ATRIPLEX LINDLEYI* MOQ

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ABSTRACT

Objective: Bromobenzene (BB) is frequently encountered in table-ready foods as contaminant residues. Therefore, the present study is designed to evaluate the petroleum ether extract of *Atriplex lindleyi* to attenuate the hepato-renal injury induced by BB exposure and study its cytotoxic activity against different human cell line as well as to describe the chemical composition of the petroleum ether extract.

Methods: The phytochemical study of petroleum ether extract was implemented using both GC/MS and column chromatography analysis. The isolated compounds were identified using different spectroscopic analysis.

Hepato-renal assay, rats were intraperitonealy injected bromobenzene at a dose 460 mg/kg BW. The petroleum ether extract as well as Hepaticum were administrated orally twice a week for three consecutive weeks with a dose 150 & 100 mg/kg body weight, respectively. Liver marker enzymes, liver function indices and kidney function tests were estimated.

The cytotoxic activity of, petroleum ether extract was assessed by the mitochondrial dependent reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide).

Results: Forty two compounds as well as sixteen fatty acids were identified in unsaponifiable and saponifiable fractions, respectively. Unsaponifiable fraction constituted of hydrocarbons (73.39% of total unsaponifiable matter), alcoholic (0.88%) and steroidal compounds (2.22%). Furthermore, column chromatography of petroleum ether extract afforded nonsterol tritrepenoids; olean-12-en-3,11-dione (1), *β*-amyrenone (2), erythrodiol I (3), Lupeol (4) as well as sterol triterpenoids; cholesterol (5) and mixture of *β*-sitosterol and stigmasterol (6). Compounds 1, 3 and 5 are first reported from *Atriplex lindleyi.* In addition, GC/MS analysis of the main fraction isolated from column chromatography revealed phytol as a major component.

Drastic changes were observed after BB intoxication in liver function parameters; kidney disorder indices and certain oxidative stress markers. Treatment with petroleum ether extract improved all biochemical parameters under investigation as well as the histopathology of liver and kidney. Petroleum ether extract showed growth inhibition of HepG2 and MCF7 human cells by 44.8 and 29.9%, respectively at 100 µg/ml.

Conclusion: The petroleum ether extract of *A. lindleyi* contains bioactive compounds exhibiting hepato-renal protection and cytotoxic activity.

Keywords: *Atriplex lindleyi*, Petroleum ether extract, Oleanane skeleton, Fatty acids, Hepato-renal and cytotoxic activity.

INTRODUCTION

The genus *Atriplex* belongs to the family Chenopodiaceae (Goosefoot) and includes 225 species. They are valuable sources of vitamins and are used in spring (instead of spinach) as a green leafy vegetable [1]. Several species of *Atriplex* have been investigated for their chemical constituents. The presence of triterpenes [2-4], saponins [5, 6], alkaloids [7,8], flavonoids [9], carbohydrate [8] and protein [10] have been reported in various *Atriplex* species. Some triterpene as *β*-amyrenone, lupeol, sitosterol mixture, *β*-sitosterol glucoside and 3*β*, 23-dihydroxy betulin, in addition, 20-hydroxyecdysone have been isolated from *Atriplex lindleyi* [9,11]. Various species of *Atriplex* have been used for their important medicinal values such as *Atriplex semibacata* and *A. vestita* have been used as an antifungal agent and in the treatment of bronchitis [12]. The total ethanolic extract of *A. inflata* exhibited antihepatotoxic, liver protection and anti-inflammatory activities [11]. Also, *A. inflata* extract was found to be effective against *Herpes simplex* [13]. However, the hexane extract of *A. lindleyi* showed potent antimicrobial activities against *Bacillus subtilis* and *Pseudomonas aeruginosa* [9]. Also, petroleum ether extract of *A. inflat* had potent antifungal activity against *Botrytis cinerea* and *Fusarium oxysporum* [12].

Bromobenzene (BB) has been detected at low frequencies and at low concentrations in samples of food, air, and water [14]. The metabolites of BB are highly hepatotoxic, while secondary metabolites are highly nephrotoxic [15]. The severity and number of people affected by hepatic diseases and renal failure is an Egyptian problem with thousands of people affected annually. In the course of our search for treating these diseases with natural products from Egyptian plants. The work aimed the detailed description of the lipoidal profile as well as hepato-renal protective effect of petroleum ether extract of *Atriplex lindleyi* aerial parts against BB intoxication in rats and its cytotoxic activity.

MATERIALS AND METHODS

General

Melting points (uncorrected) were determined on an electrothermal melting point apparatus. GC/MS analysis was carried out using a Agilent HP6890 GC/MS spectrophotometer equipped with library software Wiley 138 and NBS 75 under the following conditions: Thermo scientific, TR-5MS (5% phenyl polysil phenylene siloxane), capillary column, 30 m in length, 0.25 mm i.d. and 0.25 µm film thickness, carrier gas, helium at a flow rate of 1ml/min, temperature programmed 60-290^{0}C at a rate of 4°C/min (unsaponifiable matters), 60-280°C at a rate of 5°C/min (fatty acids methyl ester derivatives), chart speed: 0.5 cm/min, ionization voltage 70 eV, detector: mass. NMR spectra was measured on JEOL EX-500 spectrometer (Tokyo, Japan) (500 MHz for ^{1}HNMR and 125 MHz for ^{13}CNMR)) using $CDCl_3$ as internal reference.

Plant Material

The aerial parts of *Atriplex lindleyi* Moq. Subsp. *Inflata*, (syn.: *Atriplex inflata*, *Blackiella inflata*), common name Lindley's saltbush, were harvested in March from Road of Suez, Egypt. The plant samples

were dried in the shade and conserved for authentication. Voucher specimen (nº 954) was authenticated by Prof. S. A. Kawashty, taxonomist in the herbarium of National Research Centre [CAIRC], Department of chemotaxonomy.

Investigation of Lipoidal Matter

The dried powdered aerial parts of *Atriplex lindeyl* (1 kg) were extracted with petroleum ether (40-60 ˚C) (SDFCI, India) using a Soxhlet apparatus.

The solvent was evaporated under vacuum at 40 ˚C by Buchi Rotavapor R-200 to dryness. Quantitative estimation of the total steroidal and triterpenoidal contents in the petroleum ether extract was performed according to Swift [16].

Isolation of unsaponifiable matter and fatty acids

The unsaponifiable and saponifiable matter as well as methyl ester of fatty acid was prepared as mentioned in [17], then subjected to GC/MS analysis.

The identification of the composition of nonsaponifiable matter and fatty acid methyl esters were performed, depending on the fragmentation pattern and comparing with those of available authentic material such as mixture hydrocarbon, *α*- and *β*- amyrin, lupeol, oleanolic acid, urosolic acid, squalene, cholesterol, campesterol, fucosterol, stigmasterol, and *β*- sitosterol (Sigma-Aldrich Chemie GmbH, Germany) or published data **[18-21]** and a library database Wiley and NIST **[22]**. Quantitative determination was carried out based on peak area measurements of the GC chromatograms.

Column Chromatography

Petroleum ether extract (10 g) was chromatographed over activated aluminium oxide (BDH, England) column and eluted with a mixture of solvents with increasing polarity, including *n*- hexane (SD Fine Chem, Mumbai), diethyl ether (SDFCI, India), chloroform (RFCI, India) and methanol (Fisher Scientific, UK). Fractions (100 ml each) were successively collected and those exhibiting similar TLC profiles, were combined. Further, purification by PTLC [Merck GF_{254}, 0.5 mm, benzene: ethyl acetate (6:2 v/v)] was performed using sulfuric acid (H_2SO_4) and vanillin/ H_2SO_4 reagents.

Fraction I was eluted by 100% *n*- hexane and subjected to GC/MS analysis. Compounds 1-6 were eluted with 10 % ether in hexane responded positively to Salkowski and Lieberman-Büchard reactions for triterpenes [23].

Compound 1: White powder (21.32 mg), m. p. 241-244 °C, isolated from fraction II eluted with 10% ether in hexane. MS (70 eV), *m/z* (rel. int) 438 $[M]^+$ ($C_{30}H_{46}O_2$) (30), 423 (35), 410 (50), 273 (65), 232 (100), 217 (40), 135 (25), 69 (32) and 55 (46). ^{1}H-NMR ($CDCl_3$, 500 MHz), *δ* 2.39 (1H, s, H-9), 5.60 (1H, s, H-12), 1.04(3H, s, H-23), 1.07(3H, s, H-24), 1.24(3H, s, H-25), 1.14 (3H, s, H-26), 1.34 (3H, s, H-27), 0.84 (3H, s, H-28), 0.90 (3H, s, H-29), 0.87 (3H, s, H-30). ^{13}C-NMR ($CDCl_3$, 125 MHz), *δ* 40.1 (C-1), 34.5 (C-2), 217.1 (C-3), 47.8 (C-4), 55.3 (C-5), 18.7 (C-6), 31.9 (C-7), 45.1 (C-8), 61.0 (C-9), 35.6 (C-10), 199.6 (C-11), 127.9 (C-12), 171.1(C-13), 43.5 (C-14), 26.4 (C-15), 26.3 (C-16), 32.2 (C-17), 47.6 (C-18), 45.1 (C-19), 30.9 (C-20), 34.3 (C-21), 36.1 (C-22), 22.0 (C-23), 26.4 (C-24), 15.7 (C-25), 18.5 (C-26), 23.4 (C-27), 28.4 (C-28), 23.5 (C-29), 32.9 (C-30).

Compound 2: White crystals (52.4 mg), m. p. (168 °C) [24], isolated from fraction II eluted with 10% ether in hexane. MS (70 eV), *m/z* (rel. int) 424 $[M]^+$ ($C_{30}H_{48}O$) (16), 409 (8), 355 (20), 327 (16), 281(40), 218(100), 205(24), 203(59), 189(20), 163(14), 133 (24), 119 (22) and 55(60). ^{1}H-NMR ($CDCl_3$, 500 MHz), *δ* 2.33 (1H, m, H-9), 5.27 (1H, dd, J= 3.7, 3.4 Hz, H-12), 1.04(3H, s, H-23), 1.07(3H, s, H-24), 1.01(3H, s, H-25), 0.99 (3H, s, H-26), 1.24 (3H, s, H-27), 0.84 (3H, s, H-28), 0.90 (3H, s, H-29), 0.86 (3H, s, H-30). ^{13}C-NMR ($CDCl_3$, 125 MHz), *δ* 39.8 (C-1), 34.5 (C-2), 217.2 (C-3), 47.6 (C-4), 55.3 (C-5), 18.8 (C-6), 33.2 (C-7), 40.1 (C-8), 47.7 (C-9), 36.6 (C-10), 23.7 (C-11), 122.4 (C-12), 144.1(C-13), 42.5 (C-14), 26.2 (C-15), 26.9 (C-16), 32.5 (C-17), 47.2 (C-18), 47.1 (C-19), 31.0 (C-20), 34.6 (C-21), 36.9 (C-22), 26.4 (C-23), 22.1 (C-24), 15.6 (C-25), 16.8 (C-26), 25.9 (C-27), 28.6 (C-28), 23.7 (C-29), 32.8 (C-30).

Compound 3: White crystals (19.3 mg) of m.p. 228 °C isolated from fraction eluted with 10% ether in hexane. MS (70 eV), *m/z* (rel. int) 442 $[M]^+$ ($C_{30}H_{50}O_2$) (8), 409 (9), 234 (28), 216 (10), 207 (11), 204 (26), 203 (100), 189 (13), 107 (9), 95 (9), 81 (15) and 69 (17). ^{1}H-NMR ($CDCl_3$, 500 MHz), *δ* 3.17 (1H, dd, *J*= 11.2, 5.2 Hz, H-3), 5.11 (1H, t, *J* = 3.1Hz, H-12), 3.53 (1H, d, *J*= 10.8Hz, H_b-28), 3.20 (1H, d, *J*= 10.8Hz, H_a-28), 0.91 (3H, s, H-23), 0.77(3H, s, H-24), 0.86 (3H, s, H-25), 0.87 (3H, s, H-26), 0.99 (3H, s, H-27), 0.92 (3H, s, H-29), 1.14 (3H, s, H-30). ^{13}C-NMR ($CDCl_3$, 125 MHz), *δ* 38.6 (C-1), 27.3 (C-2), 79.1 (C-3), 38.8 (C-4), 55.1 (C-5), 18.4 (C-6), 32.6 (C-7), 39.8 (C-8), 46.7 (C-9), 36.9 (C-10), 23.6 (C-11), 122.7 (C-12), 144.2 (C-13), 41.8 (C-14), 25.6 (C-15), 22.3 (C-16), 36.9 (C-17), 42.6 (C-18), 46.6 (C-19), 30.9 (C-20), 34.1 (C-21), 31.1 (C-22), 28.1 (C-23), 15.5 (C-24), 15.6 (C-25), 16.8 (C-26), 25.9 (C-27), 56.5 (C-28), 23.6 (C-29), 33.2 (C-30).

Compound 4: Colorless crystals (40 mg), m. p. 212-214 °C. MS (70 eV), *m/z* (rel. int) 426 $[M]^+$ ($C_{30}H_{50}O$) (22), 411 (15), 408 (30), 393 (35), 384 (15), 220 (80), 218 (90), 207 (25), 189 (38), 139 (70) and 55 (100). ^{1}H-NMR ($CDCl_3$, 500 MHz), 3.18 (1H, dd, *J* = 11.2, 5.2 Hz, H-3), 2.34 (1H, m, H-19), 0.79 (3H, s, Me-23), 0.83 (3H, s, Me-24), 0.94 (3H, s, Me-25), 0.96 (3H, s, Me-26), 1.03 (3H, s, Me-27), 0.75 (3H, s, Me-28), δ 4.66 (1H, br s, H_a-29), 4.55 (1H, br s, H^b-29), 1.66 (3H, s, Me-30).

Compound 5: Colorless needles (47.2 mg), m. p. 148-149 °C [25]. MS (70 eV), *m/z* (rel. int) 386 $[M]^+$ ($C_{27}H_{46}O$) (22), 371(7), 368(9), 353(4), 273(25), 255(42), 231(15), 213 (20), 147 (39) and 55(100).

Compound 6: White needles (76 mg), m. p. 136-142 °C [26]. MS (70 eV), *m/z* (rel. int) 414 $[M]^+$ ($C_{29}H_{50}O$) (26), 412 (18), 399 (7), 329 (3), 314 (7), 301(9), 300 (5), 281(4), 273 (15), 271(25), 255(21), 231 (12), 213 (15) and 55(100).

Hepatorenal toxicity studies

***In vivo* study**

Animals

Male Wistar albino rats (100-120g) were selected for this study. They were obtained from the Animal House, National Research Center, Egypt. All animals were kept in the controlled environment of air and temperature with the access of water and diet.

Ethics

Anesthetic procedures and handling with animals were complied with the ethical guidelines of the Medical Ethical Committee of the National Research Centre in Egypt and performed for being sure that the animals do not suffer at any stage of the experiment.

Toxicity study

Animals were subdivided into 4 subgroups (8 rats each). All groups received one oral dose of 100, 300, 500 and 1000 mg of petroleum ether extract/kg body weight. After 24 hours, there were no dead animals; representing the safety of the extract.

Doses

The administration regimens was twice a week for three consecutive weeks. The petroleum ether extract was administrated orally with a dose 150 mg/kg body weight. The dose was selected according the toxicity study. The selected dose was confirmed by [27].

Bromobenzene was intraperitonealy administrated at a dose 460mg/kg BW (1:10 w/v corn oil) [15]. Hepaticum as a reference herbal drug (active constituent; silymarin) was orally given at a dose 100mg/kg BW [5].

Experimental design

Six animals groups (6 rats each) were classified as follows:

Group 1: Normal healthy rats. Group 2: orally administered with plant extracts. Group 3: i.p. injected with bromobenzene. Group 4: forced with petroleum ether extract and BB at the same time and for the same duration. Group 5: forced with hepaticum as reference drug and BB as group 4. Group 6: orally received Hepaticum drug only.

Sample preparations

Serum sample: Blood collected from each animal by puncture the sub-lingual vein in a clean and dry test tube, left 10 min to clot and centrifuged at 3000 g for 10 min at 4°C for serum separation. The separated serum was stored at -80 °C for further determinations of liver and kidney functions tests and serum protein.

Liver tissues were homogenized in normal physiological saline solution (0.9% NaCl) (1:9 w/v). The homogenate was centrifuged at 3000 rpm for 10 min at 4°C. The supernatant was used for estimation of hepatic antioxidant and marker enzymes.

Biochemical assays

Cell organelle marker enzymes; hepatic succinate dehydrogenase [28], lactate dehydrogenase [28], acid phosphatase [30], glucose-6-phosphatase [31], 5`-nucleotidase [32], were carried out. Liver function indices; serum aspartate & alanine aminotransferase [33], alkaline phosphatase [34], gamma glutamyl transferase [35], and bilirubin were estimated. Kidney function tests; serum cratinine [36], urea [37] and total protein [38] were done. Antioxidant parameters; liver glutathione [39], lipid peroxides [40] and superoxide dismutase [41] were estimated.

Histopathological analysis

Liver and kidney sections of all groups were stained with haematoxyline & eosin as well as Masson's Trichome to detect changes in cells and different degree of fibrosis [42].

Cytotoxic Assay

Cytotoxic effect was accomplished on human cell line (HePG2 – MCF7 – HT29 and A549). Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan [43].

Procedure: All the following procedures were performed in a sterile area using a Laminar flow cabinet Biosafety class II level (Baker, SG403INT, Sanford, ME, USA). The method was carried out according to Thabrew [44]. Cells were batch cultured for 10 days, then seeded at concentration of $10x10^3$ cells/well in fresh complete growth medium in 96-Well Microtiter plastic plates at 37 °C for 24 h under 5% CO_2 using a Water jacket CO_2 incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations of sample to give a final concentration of (100, 50, 25, 12.5 and 6.25 µg/ml).

Cells HePG2 & HT29 were suspended in Roswell Park Memorial Institute 1640 medium and MCF7 & A549 were suspended in Dulbecco's modified Eagles Medium, supplemented with 1% antibiotic-antimycotic mixture (10,000U/ml Potassium Penicillin, 10,000µg/ml Streptomycin Sulfate and 25µg/ml Amphotericin B) and 1% L-glutamine (all purchased from Lonza, Belgium) in 96-well flat bottom microplate at 37 °C under 5% CO_2. After 48 h of incubation, the medium was aspirated, 40ul MTT salt (2.5µg/ml) were added to each well and incubated for a further four hours at 37°C under 5% CO_2.

To stop the reaction and dissolving the formed crystals, 200µL of 10% Sodium Dodecyl Sulphate (SDS) in deionized water was added to each well and incubated overnight at 37°C. A positive control which composed of 100µg/ml of *Annona cherimolia* leaves extract was used as a known cytotoxic natural agent which gives 100% lethality under the same conditions. The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595nm and a reference wavelength of 620nm. Adriamycin ® (Doxorubicin) 10 mg vials (Pharmacia, Sweden) was used as the reference drug.

Statistical analysis

For hepato-renal assay: All data were expressed as mean ± S.D. of six rats in each group. Statistical analysis was carried out by one-way analysis of variance (ANOVA), Costat Software Computer Program.

For cytotoxicity assay: All values were expressed as the mean ± SEM of the three replicates for each treatment. Data were subjected to paired- samples SPSS Statistical Software Package (version 8.0). $P<0.005$ was regarded as significant.

RESULTS AND DISCUSSION

The yield of petroleum ether extract from *Atriplex Lindleyi* aerial parts was 6.57%. It showed positive Liebermann-Bürchard and Salkowski tests indicating the presence of triterpenes and sterols. The total steroidal and triterpenoidal contents were quantitatively estimated and were found 2.8%w/w as stigmasterol and 3.55%w/w as *α*-amyrin, respectively. Saponification of lipoidal matter afforded 74.80 % of unsaponifiable matter and 18.36 % of the fatty acids. GC/MS analysis of unsaponifiable matter led to identify 42 compounds summarized in Table (1), accounting for 84.27%. The unsaponifiable matter was composed of hydrocarbons (73.39% of total unsaponifiable matter), alcoholic hydrocarbons (0.88 %) and sterols (2.22%). While, GC/MS analysis of fatty acid methyl ester derivatives revealed 16 fatty acids presented in Table (2). The saturated fatty acids represent 85.10 % of total identified fatty acid, whereas unsaturated fatty acids represent 14.90%. The Cerotic acid (20.30%) and palmitic acid (15.70 %) were major fatty acids. GC/MS analysis of unsaponifiable matter didn't reveal triterpenes compounds that led us to fractionate the pet. ether extract over column chromatography of alumina oxide. The main fraction I (1.34 g) eluted with 100% *n*-hexane and further subjected to GC/MS. The GC/MS analysis led to identify 22 compounds representing 87.09% and illustrated in Table (3). Phytol (26.17%) and 2,6-*bis* (1,1-dimethylethyl)-4-methyl phenol (18.23%) represent the main components of fraction I. Whilst, phytol wasn't detected in unsaponifiable matter. In addition, four nonsterol tritrepenoids; 12-oleanene- 3,11-dione (1), 12-oleanene-3-one (2), olean-12-ene-3*β*, 28*β*-diol (3), 5*α*-lup-20(29)-en-3*β*-ol (4) as well as three sterol tritrepenoids; cholesterol (5), mixture of *β*-sitosterol (24-ethylcholest-5-en-3*β*-ol) and stigmasterol (24-ethylcholesta-5,22-diene-3*β*-ol)(6) were isolated and identified by comparing their physical and spectroscopic data with those reported in the literature and by comparison with authenticated samples.

Compound 1 was obtained as white powder of m. p. 241~245 °C and the electron impact mass (EI-MS) $[M]^+$ peak at *m/z* 438 corresponding to the molecular formula $C_{30}H_{46}O_2$. Mass spectrum showed prominent peaks at *m/z* 423$[M-15]^+$, 273 $[C_{16}H_{24}O]^+$, 135$[C_{16}H_{24}O]^+$, 232$[C_{16}H_{24}O]^+$ retro-Diels-Alder (RDA) fragments characteristic for Δ 12-oleane series with diketones [45] and are confirmed by the appearance of ketone groups at *δ* 217.1 & 199.6 in ^{13}C NMR. The intense peak at *m/z* 273 indicated the presence of a carbonyl group in rings A-C and that no other groups were present in these rings. Further, the appearance of two singlet signals at *δ* 5.60 & 2.39 in ^1H NMR and signal at 127.9 (C-12) & 60.0 (C-9) in ^{13}C NMR prove that the one ketone was present at C-11. Comparing the characteristic features of compound 1 with that of the reported of compound 12-oleanene-3,11-dione [46-48] emphasized that compound 1 is 12-oleanene-3,11-dione and is recorded here for the first time isolated from *Atriplex* species.

Compound 2 was isolated as white crystals of m. p. 168 °C and (EI-MS) $[M]^+$ peak at *m/z* 424 corresponding to the molecular formula $C_{30}H_{48}O$. The spectroscopic analysis of this compound was closely similar to the reported for *β*- amyrenone (12-oleanene-3-one) isolated previously from *Atriplex inflate* [11]. The ^1H NMR spectra revealed the presence of a doublet of doublet signal at 5.31 with *J*=3. 7, 3.4 Hz for olefinic proton and eight methyl groups H-23, 24, 25, 26, 27, 29, 28, 30 appeared in *δ* 1.04, 1.07, 1.01, 0.99, 1.24, 0.84, 0.90, 0.86, respectively, together with mass fragments at *m/z* 409 $[M-CH_3]^+$, 218[RDA], 203 $[218-CH_3]^+$, 205 $[C_{14}H_{21}O]^+$ and 189 $[218-CH_2CH_3]^+$ implied the compound 2 is typical for pentacyclic triterpene with Δ 12-oleane skeleton. Further, the ^{13}C NMR spectrum showed characterstic signal at *δ* 217.2 for carbonyl group of C-3. The mass spectra, physical and NMR spectral data are in agreement with those reported in the literatures of *β*- amyrenone [18,49].

Table 1: GC/MS analysis of unsaponifiable matter isolated from the aerial parts of *Atriplex lindleyi*

Compounds	Rt	%	BP *m/z*	MWt *m/z*	Main fragments (*m/z*)
I- Hydrocarbon					
n-Undecane	12.13	0.27	57	156	71, 85, 99, 113, 127,140
5-Methylundecane	14.22	0.35	57	170	71, 44, 85, 112, 69
4-Methylundecane	14.32	0.28	43	170	71, 41, 57, 85, 126, 127, 98
2-Methylundecane	14.42	0.59	43	170	57, 71, 41, 85, 99, 126,127
3-Methylundecane	14.59	0.60	57	170	71, 85, 43, 99, 113, 141
1-Methyl, 3-pentylcyclohexane	15.44	0.68	97	168	55, 69, 81, 96, 125, 139
Cyclododecane	15.53	0.50	69	182	55, 83, 97, 111,168, 139
***n*-Dodecane**	16.10	4.69	57	170	43, 71, 85, 99, 113, 127
2, 6- Dimethylundecane	16.31	1.31	57	184	43,71, 98, 85, 113, 141
1-Hexylcyclohexane	17.40	0.35	83	168	82, 55, 41, 67, 97
1-Tetradecene	22.51	1.49	55	196	41, 43, 83, 57, 69, 97, 168
n-Tetradecane	23.01	0.17	57	198	43, 71, 85, 99, 113
7-Hexadecene	29.00	2.40	55	224	43, 69, 83, 97, 111,140
n-Hexadecane	29.08	0.19	57	226	43, 71, 85, 99, 127, 113
1-Octadecene	34.30	2.81	57	252	43, 55, 83, 97, 69, 111, 125
Octadecane	34.36	0.19	57	254	43, 71, 85, 99, 113, 127
Cubitene	36.24	1.45	67	272	121,107,133,257,189,215,177, 161, 229
Dolabradiene	37.32	1.16	93	272	81, 107, 121, 135, 67, 147, 257, 243, 215, 201
5-Eicosene	39.28	2.66	55	280	41, 97, 83, 69, 111, 125, 139, 154, 252
n-Heneicosane	41.52	0.42	57	296	71, 43, 85, 99, 113, 127, 141, 169
1-Docosene	44.06	2.67	55	308	57, 43, 97, 83, 69, 111, 125
n-Tricosane	46.11	1.46	57	324	71, 85, 99, 113, 127, 141
Cyclotertacosane	48.11	2.13	83	336	97, 55, 69, 43, 111, 210,308
n -Pentacosane	50.12	7.17	57	352	71, 85, 99, 113, 127, 141
1-Hexacosene	52.20	1.83	57	364	83, 97, 69, 55, 111, 125, 139
5- Methyl, 5-ethyltetracosane	53.11	0.19	57	380	71, 85, 99, 113, 365, 337
n -Heptacosane	53.56	5.90	57	380	71, 85, 99, 113, 127
n -Octacosane	55.38	1.55	57	394	71, 85, 99, 113, 127
Squalene	55.55	0.54	69	410	81, 95, 123, 137, 55, 109, 121
5-Methyl, 5-ethylhexacosane	56.40	1.14	57	408	71, 85, 99, 365, 393
n -Nonacosane	57.27	14.34	57	408	43, 71, 85, 99, 113
n -Triacontane	58.57	1.62	57	422	71, 43, 85, 99, 113
n -Hentriacontane	60.38	10.29	57	436	71,85, 99, 113,43
Alcoholic Hydrocarbon:					
3-Nonacosanol	58.34	0.48	59	424	57,97, 83, 41, 111, 125, 407
3-Triacontanol	61.53	0.40	59	452	57, 97, 83, 41, 111, 125, 435
Ester:					
Tributyl acetylcitrate	45.38	0.61	185	402	259, 129, 57, 157, 329, 213, 273, 301
sterols:					
Cholesterol	58.16	1.55	43	386	371, 355, 341, 313, 301, 267, 147, 97
β- Sitosterol	59.45	0.67	55	414	399, 396, 381, 329, 303, 273, 213, 161, 145, 136, 119, 107, 95
Miscellaneous					
1,1- Dioxidetetrahydrothiophene	18.41	3.06	41	120	56, 55, 60, 64, 76, 90
2,6-Di-(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	25.30	2.32	165	236	180, 57, 137, 193, 205, 221
2,6-bis(1,1-dimethylethyl)-4-methyl phenol	26.27	0.69	205	220	57, 204, 145, 177, 105, 189
7,9-Di-tert-butyl-1oxaspiro[4.5] deca-6, 9-diene-2, 8-di-one	32.42	1.10	57	276	205, 175, 217, 189, 232, 261

Rt = Retention time BP= Base Peak MWt= Molecular Weight

was obtained as white crystals of m. p. 228 °C and EI-MS revealed a molecular ion peak at *m/z* 442 corresponding to the molecular formula $C_{30}H_{50}O_2$. The mass spectrum of compound 3 displayed retro-Diels-Alder fragment peak at *m/z* 234, characteristic for erythrodiol [50] analog with other fragments at *m/z* 411 ($[M]^+$-CH_2OH), 203, 189, 175, 133, indicating a C-12/C-13 double bond that suggested an oleanane structure substituted by two hydroxyl groups (OH), one located at A/B rings and one D/E rings.

These data confirmed by the presence resonances of tertiary (C-3) and secondary alcoholic carbon (C-28) at 79.1 & 56.5 in ^{13}C NMR and also appeared as a double doublet signal at 3.17 (*J*= 11.2, 5.2 Hz) and two doublet signals at 3.20 (*J*= 10.8Hz) and 3.53 (*J*= 10.8Hz), respectively, in ^1H NMR. Further, seven tertiary methyl groups resonating at *δ* 0.91, 0.77, 0.86, 0.87, 0.99, 0.92 and 1.14, an olefinic proton resonating at *δ* 5.11 (t, *J*=3.1 Hz) were observed in the ^1H NMR spectrum of **3**. These spectral data agreed with those published for erythrodiol [51].

Compound 4

was isolated as white crystals of m. p. 212-214 °C and (EI-MS) $[M]^+$ peak at *m/z* 426 corresponding to the molecular formula $C_{30}H_{50}O$. The ^1H NMR spectrum showed seven tertiary methyl singlet signals and one secondary hydroxyl group as a doublet of doublets at *δ* 3.18.

Also, it showed two olefinic protons at *δ* 4.68 and 4.56 representing the exocyclic double bond.

The physical and spectral data of this compound were closely similar to the reported data of lupeol in the literature [52] and was confirmed by co-TLC with authentic samples. Lupeol was isolated previously from *Atriplex inflate* [11].

Table 2: Fatty acid methyl ester derivatives identified by GC/MS from aerial parts of *Atriplex lindleyi*

	Fatty Acid	RRT	Bp *m/z*	Mwt *m/z*	Relative percentage of total fatty acids
	Saturated Fatty acids:				
14:0	Myristic acid (Tetradecanoic acid)	0.873	74	242	2.24
16:0	Palmitic acid (Hexadecanoic acid)	1.000	74	270	15.70
18:0	Stearic acid (Octadecanoic acid)	1.115	74	298	1.87
13:0	Tridecanedioic acid	1.190	98	284	4.54
20:0	Arachidic acid (Eicosanoic acid)	1.221	74	326	1.51
22:0	Behenic acid (Docosanoic acid)	1.319	74	354	2.30
23:0	Tricosylic acid (Tricosanoic acid)	1.364	74	368	1.51
24:0	Lignoceric acid (Tetracosanoic acid)	1.384	74	382	8.82
25:0	Pentacosylic acid (Pentacosanoic acid)	1.409	74	396	2.47
26:0	Cerotic acid (Hexacosanoic acid)	1.451	74	410	20.30
26:0	24-Methylhexacosanoic acid	1.493	74	424	2.47
28:0	Montanic acid (Octacosanoic acid)	1.535	74	438	12.33
32:0	Dotriacontanoic acid	1.635	74	494	9.04
	Total				**85.10**
	Unsaturated Fatty Acids:				
18:1	Oleic acid (9- Octadecenoic acid)	1.002	55	296	2.53
18:2	Linoleic (9,11-Octadecadienoic acid)	1.098	67	294	4.44
18:3	Linolenic acid (9,12, 15-Octadecadienoic acid)	1.103	79	292	7.93
	Total				**14.90**

RRt = Relative Retention time BP= Base Peak MWt= Molecular Weight

Table 3: GC/MS analysis of fraction I isolated from column chromatography of pet. ether extract of *Atriplex lindleyi*

Compounds	Rt	%	BP *m/z*	MWt *m/z*	Main fragments (*m/z*)
1,1- Dioxidetetrahydrothiophene	18.25	7.03	41	120	56, 55, 60, 64, 76, 90
1,1- Diethoxypentane	23.04	1.39	103	160	75, 83, 55, 129, 89
2,6-di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	25.31	1.26	165	236	57, 180, 137, 123, 205, 221, 193
2,6-bis(1,1-dimethylethyl)-4-methylphenol	26.40	18.23	205	220	145, 177, 105, 161, 189
5, 6, 7, 7a- Tetrahydro 4, 4, 7a-tri -2-(4H)- benzofuranone	28.31	1.53	111	180	109, 137, 67, 55, 124, 152, 165
1-Hexadecene	28.58	0.95	43	224	55, 69, 83, 97,111
7,9- Benzo-1,5-dithiecane	34.06	0.61	104	210	103, 105, 211, 135, 149, 212
1-Octadecene	34.28	1.83	57	252	55, 83, 97, 69, 111, 125
6, 10, 14-Trimethyl-2-pentadecanone	35.57	1.33	58	268	71, 85, 95, 109, 124, 210
5-Eicosene	39.26	1.79	55	280	69, 83, 97,111
n-Heneicosane	41.50	0.54	57	296	71, 85, 99, 113, 252
Phytol	42.25	26.17	71	296	123, 81, 57, 81, 278
1-Decosene	43.59	1.05	55	308	43, 57, 69, 83, 97, 111
n-Docosane	44.02	0.62	57	310	43, 71, 85, 99, 113
n-Tricosane	46.10	1.21	57	324	71, 43, 85, 99, 113
4,8,12,16-Tetramethylheptadecan-4-olide	47.46	0.95	99	324	43, 55, 69, 114, 126, 83, 151, 166, 196
n-Tetracosane	48.12	1.39	57	338	57, 43, 71, 85, 99, 113
Dioctyl hexanedioate	48.20	0.77	129	370	57, 70, 112, 147, 241, 259
n-Pentacosane	50.10	3.83	57	352	43, 71, 43, 85, 99, 113
n-Hexacosane	52.02	1.49	57	366	43, 71, 85, 99, 113
n-Heptacosane	53.51	4.77	57	380	43, 71, 85, 99, 113
n-Nonacosane	57.18	8.35	57	408	43, 71, 85, 99, 113

Rt = Retention time BP= Base Peak MWt= Molecular Weight

Compound 5 was obtained as colorless needles of m. p. 148-149 °C and its mass spectrum gave one molecule ion (M^+) at *m/z* 386 corresponding to $C_{27}H_{46}O$ and other prominent peaks at *m/z* 371 (M^+- CH_3), 368 (M^+- HOH), 353 (M^+- (CH_3+HOH), 273 (M^+- side chain), 255 (M^+- side chain+HOH), 231 (M^+- (side chain+ 42)), 213 (M^+-(side chain+ 42+HOH)). These data are typical to Δ 5-cholestane skeleton together with direct comparison (Co-TLC, m.p. and MS) proved to be cholesterol that was also identified in GC/MS analysis. Compound 5 is first reported from *Atriplex lindleyi*, however, it was identified previously in *Atriplex halimus* [8], *Schanginia aegyptiaca* and *Salsola tetrandra* (Chenopodiaceae) [7].

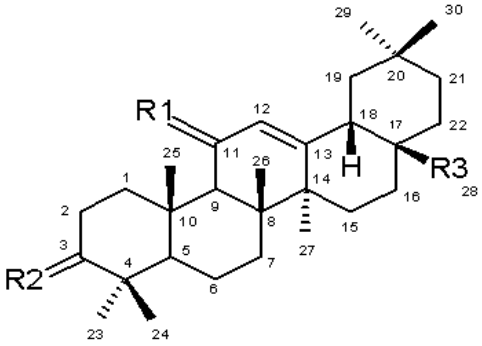
Compound 6 was obtained as colorless needles of m. p 135-140 °C. Two molecular ions given at *m/z* 414 and 412 analog with other ion peaks at *m/z* 271, 273 suggesting 6 was a mixture of two compounds. The ion peaks at *m/z* 271, 273 due to *β* bond cleavage of side chain leading loss of $C_{10}H_{21}$ and $C_{10}H_{23}$, respectively [20]. The fragmentation pattern of compound 6 and direct comparison with authentic samples (Co-TLC, R_f, mp) proved that it was composed of *β*-sitosterol and stigmasterol with molecular weight 414 and 412, respectively. These compounds were identified as mixture previously from *Atriplex inflate* [9, 11].

Indeed, most of the pentacyclic triterpenoids obtained from family Chenopodiaceae belongs to the oleanene skeleton [7]. Salt *et al* (1985) reported that 4-desmethylcholesterols are predominant sterols in the family Chenopodiaceae [57]. Various sterols such as *β*-sitoterol, cholesterol, stigmasterol and 24*ξ*- methyl-5*α*-cholestan-3*β*-ol were previously reported in *Atriplex species* [4, 8]. The sterol profiles of *Atriplex* species were characterized by coexistence of Δ^{7-} and Δ^{5-} sterols in ratios of 0.3:1 to 0.4: 1 and male of *Atriplex* plants contained a higher proportion of Δ^{5-} sterols than female [58].

Biological activity

Drastic changes in all biochemical parameters under investigation after bromobenzene toxicity were recorded (Tables 4-7).

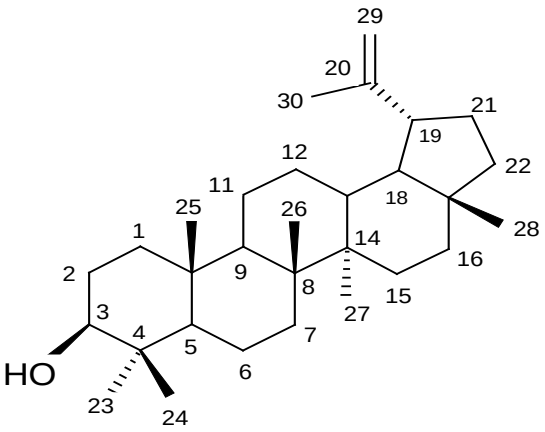
Treatment with petroleum ether extract of *Atriplex lindleyi* showed improvement in liver marker enzymes, liver function indices and kidney function tests with variable degrees. In addition, petroleum ether exerts antioxidant activity (Tables 4-7) through its ameliorative effect recorded in the levels of glutathione, lipid peroxide and superoxide dismutase as compared with the reference drug. Hepaticum as an antioxidant flavonoid complex derived from the herb milk thistle (*Silybum marianum*), has also the ability to scavenge free radicals, chelate metal ions, inhibiting lipid peroxidation and preventing liver glutathione depletion [53].



	1	2	3
R1	O	H, H	H, H
R2	O	O	OH
R3	CH_3	CH_3	CH_2OH

In the oxidative stress process, the free radicals involvement affected the mitochondria, the plasma membrane permeability and the microsomal integrity which lead to leakage of liver enzymes into circulation [54]. The extract role as a free radical scavenger could in turn normalize microsomes, mitochondria and plasma membranes permeability and integrity which lead to restore the hepatic enzymes to normal levels.

BB metabolites are formed in the liver and transported to the kidney, where they exerted the nephrotoxic action [15]. This nephrotoxic action was mediated by the observed disturbance in the urea, creatinine and protein levels. Treatment with *Atriplex lindleyi* petroleum ether extract improved the kidney function parameters which give an additional support that this extract mops up free radicals generated by BB and induces a healthy state of renal cells, suggesting its role as a renal protective agent.



Lupeol

Liver morphological and histopathological observations (Figure 1 a & b) confirmed our biochemical determinations through reduction in fibrotic and infiltration area as well as collagen distribution. Kidney section also showed minimal infiltration area and low dilatation in Bowman capsules (Figure 2).

24-methylenecholesterol and β-sitosterol were found to decrease significantly the cholesterol level in both serum and liver [59]. Rats pre-treated with lupeol had the serum and liver enzyme levels restored to almost normal. Additionally, treatment with lupeol substantially normalized degenerative alterations in hepatocytes with granular cytoplasm. Lupeol also re-established antioxidant enzyme activities in mouse liver affected by 7, 12- dimethylbez(α) anthracene induced oxidative stress [60].

Table 4: The effect of *Atriplex lindleyi* petroleum ether extract on liver marker enzymes of healthy, intoxicated and treated rats

Parameters	Control	Control plant treated	Control drug treated	Intoxicated (BB)	Intoxicated plant treated (a)	Intoxicated drug treated (b)	% of improvement (a)	% of improvement (b)
Succinate dehydreogenase	245.50±6.18^a	240.33±3.14^a (-2.10)	241.33±2.25^a (-1.69)	132.69±11.12^c (-45.95)	206.66±9.97^b (-5.82)	212.55±8.80^b (-13.42)	30.13	32.53
Lactate dehydrogenase	142.03±3.75^a	138.23±3.85^{ab} (-2.67)	136.17±2.78^{ab} (-4.12)	103.81±9.66^c (-26.91)	133.39±9.04^b (-6.08)	135.25±6.72^{ab} (-4.77)	20.83	22.14
Glucose -6- Pase	32.72±1.68^a	32.22±1.56^a (-1.53)	32.16±2.13^a (-1.71)	18.66±1.35^c (-42.97)	24.65±2.46^b (-24.66)	26.10±1.71^b (-20.23)	18.30	22.74
Acid phosphatase	5.27±0.74^b	5.49±0.46^b (+4.17)	5. 18±0.19^b (-1.70)	8.83±0.98^a (+67.55)	6.16±1.16^b (+16.88)	5.66±1.03^b (+7.40)	50.66	60.15
5`-nucleotidase	188.29±5.40^c	190.34±4.65^c (+1.09)	193.66±5.00^c (+2.85)	325.04±9.31^a (+72.63)	230.37±6.73^b (+22.35)	212.83±7.67^{bc} (+13.03)	50.28	59.59

- Data are means ± SD of six rats in each group.
- Data are expressed as µmole/ min/ mg protein.
- Values between brackets are percentages change as compared to control group.
- Unshared superscript letters between groups are the significant values at p< 0.0001.
- Statistical analysis is carried out by one way analysis of variance (ANOVA) - Costat Computer Program.
- % of improvement = $\frac{\text{Mean intoxicated} - \text{mean treated}}{\text{Mean control}} \times 100$

Table 5: Effect of *Atriplex lindleyi* petroleum ether extract on liver function indices of healthy, intoxicated and treated rat serum

Parameters	Control	Control plant treated	Control drug treated	Intoxicated (BB)	Intoxicated plant treated (a)	Intoxicated drug treated (b)	% of improvement (a)	% of improvement (b)
AST	20.48±1.78^{d}	21.22±3.28^{d} (+3.61)	21.50±1.04 ^{d} (+4.98)	32.54±2.68^{a} (+58.88)	28.49±3.60^{b} (+39.11)	24.73±3.21^{c} (+20.75)	17.57	38.13
ALT	19.23±1.02^{d}	19.58±1.20^{d} (+1.82)	19.80±1.04 ^{cd} (+2.96)	26.15±1.73^{a} (+35.98)	23.93±2.07^{b} (+24.44)	21.96±0.86^{c} (+14.19)	11.54	21.78
ALP	7.20±0.87^{c}	7.44±0.78 ^{c} (+3.33)	7.40±0.52^{c} (+2.77)	14.19±0.86^{a} (+97.08)	9.15±1.55^{b} (+27.08)	8.21±0.84^{bc} (+14.02)	70.00	83.05
GGT	3.57±0.38^{c}	3.22±0.36^{c} (-9.80)	3.15±0.18 ^{c} (-11.76)	5.86±0.73^{a} (+64.14)	4.33±0.94^{b} (+21.28)	3.82±0.63^{bc} (+7.00)	42.85	57.14
Total bilirubin	5.30±0.52^{d}	4.77±0.44 ^{d} (-10.00)	4.59±0.55 ^{d} (-13.39)	9.74±0.92^{a} (+83.77)	8.11±0.62^{b} (+53.01)	7.22±0.69^{c} (+36.22)	30.75	47.54

- Data are means ± SD of six rats in each group.
- Data are expressed as unit/L and total bilirubin as µmole/L.
- Values between brackets are percentages change as compared to control group.
- Unshared superscript letters between groups are the significant values at p< 0.0001.
- Statistical analysis is carried out by one way analysis of variance (ANOVA) - Costat Computer Program.
- % of improvement = $\frac{\text{Mean intoxicated – mean treated}}{\text{Mean control}} \times 100$

Table 6: The effect of *Atriplex lindleyi* petroleum ether extract on antioxidant levels of healthy, intoxicated and treated rat liver

Parameters	Control	Control plant treated	Control drug treated	Intoxicated (BB)	Intoxicated plant treated (a)	Intoxicated drug treated (b)	% of improvement (a)	% of improvement (b)
Lipid peroxides	0.92±0.05^{b}	0.91±0.06^{b} (-1.08)	0.86±0.06^{b} (-6.52)	1.72±0.30^{a} (+86.95)	1.03±0.11^{b} (+11.95)	0.97±0.02^{b} (+5.43)	75.00	81.52
Glutathione	67.93±3.24^{a}	62.54±3.83^{bc} (-7.93)	63.00±2.36^{b} (-7.25)	59.47±4.3^{c} (-12.45)	61.98±1.57^{bc} (-8.75)	64.82±1.23^{ab} (-4.57)	3.29	7.87
Superoxide dismutase	16.64±1.48^{bc}	16.94±1.35^{c} (+1.80)	16.90±1.05^{c} (+1.56)	20.70±6.92^{a} (+24.39)	19.64±2.37^{b} (+8.02)	18.80±2.80^{bc} (+12.98)	6.37	11.41

- Data are means ± SD of six rats in each group.
- Data are expressed as µmole/ min/ mg protein for lipid peroxides, µg/ mg protein for glutathione, µmole/ mg protein for superoxide dismutase.
- Values between brackets are percentages change as compared to control group.
- Unshared superscript letters between groups are the significant values at p< 0.0001 for lipid peroxides, superoxide dismutase and p< 0.001 for glutathione.
- Statistical analysis is carried out by one way analysis of variance (ANOVA) - Costat Computer Program.
- % of improvement = $\frac{\text{Mean intoxicated – mean treated}}{\text{Mean control}} \times 100$

Table 7: Effect of *Atriplex lindleyi* petroleum ether extract on kidney functions of healthy, intoxicated and treated rats

Parameters	Control	Control plant treated	Control drug treated	Intoxicated (BB)	Intoxicated plant treated (a)	Intoxicated drug treated (b)	% of improvement (a)	% of improvement (b)
Urea	21.42±1.45^{bc}	20.13±1.37^{c} (6.02)	20.16±1.47 ^{c} (5.88)	18.23±0.64^{d} (14.89)	27.16±1.77^{a} (26.79)	22.35±1.45^{b} (4.34)	41.69	23.90
Creatinine	0.59±0.05^{c}	0.66±0.06^{c} (11.86)	0.57±0.04 ^{c} (3.38)	0.81±0.03^{a} (37.28)	0.68±0.04^{b} (15.25)	0.65±0.03^{b} (10.16)	22.03	42.37
Serum protein	17.03±2.21^{a}	15.97±0.45^{ab} (6.22)	15.57±0.51 ^{ab} (8.57)	14.68±1.20^{b} (13.79)	15.16±1.46^{b} (10.98)	15.83±1.22^{ab} (7.04)	2.81	6.75

- Data are means ± SD of six rats in each group.
- Data are expressed as mg/ Dl for urea and creatinine, mg/ ml for serum protein.
- Values between brackets are percentages change as compared to control group.
- Unshared superscript letters between groups are the significant values at p< 0.0001.
- Statistical analysis is carried out by one way analysis of variance (ANOVA) - Costat Computer Program.
- % of improvement = $\frac{\text{Mean intoxicated – mean treated}}{\text{Mean control}} \times 100$

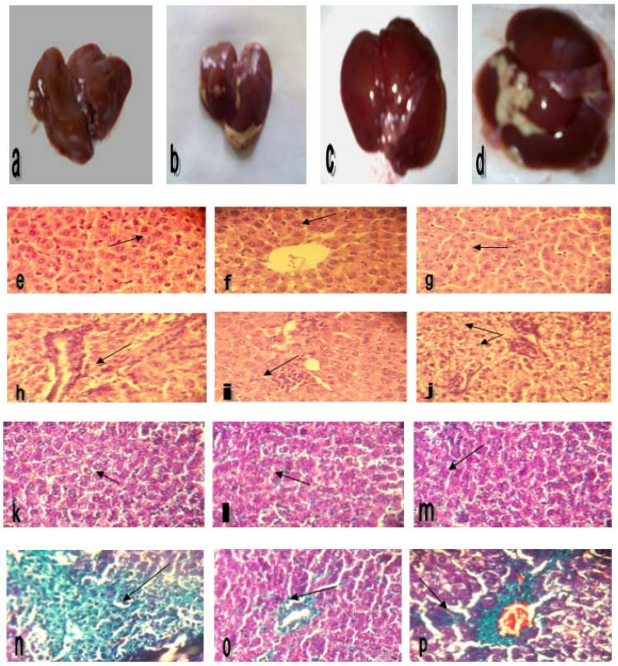


Fig. 1a: Morphological structure of control liver (a), BB intoxicated liver (b), intoxicated liver treated with pet. ether extract (c), intoxicated liver treated with Hepaticum drug (d). b: Heamatoxyline & eosin and Masson's Trichrome stain liver sections of control rat (e, k), control treated with petroleum ether extract of *Atriplex lindleyi* (f, l), control treated with Hepaticum drug (g, m), BB intoxicated (h, n), BB intoxicated rats treated with pet. ether extract (i, o), BB intoxicated rats treated with drug (j, p). Arrows show normal hepatic cells in normal and normal treated liver (e, f, g, k, l, m). Massive fibrosis with collagen deposition in intoxicated liver (h, n). Less fibrotic tissue and collagen deposition were seen in treated liver with pet. ether extract and Hepaticum drug (i, j, o, p).

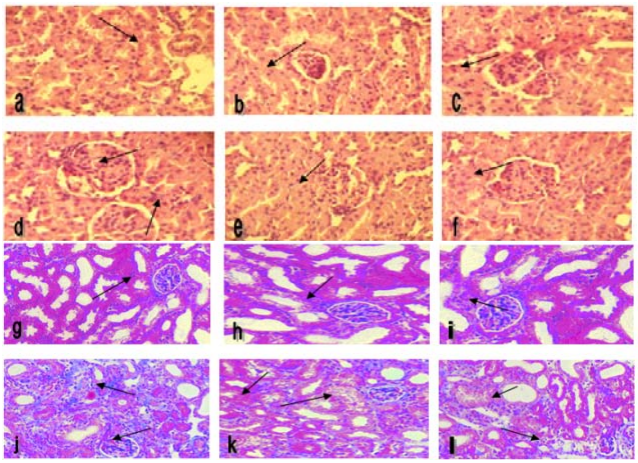


Fig. 2: Heamatoxyline & eosin and Masson's Trichrome stain kidney section (400 x) of control rat (a, g), control treated with petroleum ether extract of *Atriplex lindleyi* (b, h), control treated with Hepaticum drug (c, i), BB intoxicated (d, j), BB intoxicated treated with petroleum ether extract (e,k), BB intoxicated treated with drug (f, l). Arrows show normal glomeruli in normal and normal treated rats (a, b, c, g, h, i). Dilatation in Bowman capsules with massive infiltrations and fibrosis in interstitial space were observed in intoxicated rats (d, j). Normal glomeruli with minimal infiltrations and less fibrosis in interstitial space were seen in intoxicated rats treated with plant extract and Hepaticum drug (e, f, k, l).

Furthermore, the assessment of cytotoxic activity of petroleum ether extract against different human cell line showed growth inhibition on HepG2 and MCF7 human cells to 29.9 and 44.8%, respectively at concentration 100 ppm (Table 8). Capua *et al* (2010) reported that the hexane fraction of *Atriplex confertifolia* reduced MCF7 cell viability by less than 20%.[55]. Whereas, Donaldson (2000) found that the hexane fraction of *Atriplex canescens* showed 48.7% cell inhibition against HeLa cells [56].

Table 8: Cytotoxic activity of petroleum ether extract of *Atriplex lindleyi* in *vitro* on different human cell lines

Cell lines	Conc. µg/ml	% 0f Inhibition ± SEM	
		Petroleum Ether Extract	Doxrubicin
A549	100	13± 1.05*	100± 0.00
	50	3.4± 1.76*	75.63± 1.71
	25	0.0± 0.003*	53.66± 1.55
HCT116	100	0.0± 0.00*	100± 0.00
	50	0.0± 0.02*	65.2± 0.66
HepG2	100	29.9± 1.45*	100± 0.00
	50	11.9± 0.81*	97.2± 0.57
	25	3.8± 2.10*	60.2± 1.51
	12.5	0.0± 0.00*	32.4± 1.02
MCF7	100	44.8± 1.15*	100± 0.00
	50	18.7±1.73*	84.3± 2.42
	25	6.1± 1.83*	57.6± 1.53
	12.5	0.0± 0.001*	32.7± 0.52

Each value represents the percentage of inhibition growth ± SEM (standard error of mean of percentage of inhibition cells of three replicates).

* Significantly different from Doxorubicin value at $P < 0.005$ according to paired-samples *t*-test.

CONCLUSIONS

Atriplex lindleyi Moq. susp. *Inflate* is an edible plant commonly distributed in the Egyptian deserts used as food for human and animals. A detailed information on phytoconstituent and hepatorenal protection efficacy as well as cytotoxic activity of petroleum ether extract are presented, suggesting its potential medicinal use as hepato-renal protective agent upon further clinical studies.

CONFLICT OF INTEREST

There are no conflicts of interest

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