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**New acyclic secondary metabolites from the biologically active fraction of *Albizia lebbeck* Flowers**

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**Abstract** The total extract of *Albizia lebbeck* flowers was examined *in vivo* for its possible hepatoprotective activity in comparison with the standard drug silymarin at two doses. The higher dose expressed promising activity especially in reducing the levels of AST, ALT and bilirubin. Fractionation via liquid- liquid partition and reexamination of the fractions revealed that the *n*-butanol fraction was the best in improving liver biochemical parameters followed by the *n*-hexane fraction. However, serum lipid parameters were best improved with $CHCl_3$ fraction. The promising biological activity results initiated an intensive chromatographic purification of *Albizia lebbeck* flowers fractions. Two compounds, identified from natural source for the first time, the acyclic farnesyl sesquiterpene glycoside1-O-[6-O-$\alpha$-L- arabinopyranosyl-$\beta$-D-glucopyranoside]– (2*E*,6*E*-)-farnesol (**6**) and the squalene derivative2,3-dihydroxy-2,3-dihydrosqualene (**9**), in addition to eight compounds reported here for the first time from the genus *Albizia*; two benzyl glycosides, benzyl 1-O-$\beta$-D- glucopyranoside (**1**) and benzyl 6-O-$\alpha$-L-arabinopyranosyl $\beta$-D-glucopyranoside (**2**); three acyclic monoterpene glycosides, linalyl $\beta$- D- glucopyranoside (**3**) and linalyl 6-O-$\alpha$-L-arabinopyranosyl -$\beta$-D-glucopyranoside (**4**); 2*E*)-3,7-dimethylocta-2,6-dienoate-6-O-$\alpha$-L arabinopyranosyl-$\beta$-D-glucopyranoside

(**5**), two oligoglycosides, *n*- hexyl-α-L arabinopyranosyl-(1→6)-β-D-glucopyranoside (creoside) (**7**) and *n*-octyl α-L- arabinopyranosyl-(1→6)-β-D-glucopyranoside (rhodiooctanoside) (**8**); and ethyl fructofuranoside (**10**). The structures of the isolated compounds were elucidated based on extensive examination of their spectroscopic 1D and 2D-NMR, MS, UV, and IR data. It is worth mentioning that, some of the isolated linalol glycoside derivatives were reported as aroma precursors.

**KEYWORDS**

*Albizia lebbeck* flowers; Hepatoprotective; Aroma precursors; Farnesol derivatives; Dihydrosqualene derivatives.

### 1. Introduction

The genus *Albizia* (Fabaceae), embraces around 150 species, mostly trees and shrubs native to warm areas of Asia and Africa (Migahid, 1989). It is widely cultivated in tropical and subtropical regions as an ornamental plant. A perusal of the literature revealed that plants belonging to genus *Albizia* have great medicinal values. *A. anthelmintica* A. Brogne is used for the treatment of malaria and febrile convulsions (Carpani et al., 1989; Johns et al., 1994), while the leaves, boiled with water, are given by traditional healers in Dar es Salaam, Tanzania for the treatment of epilepsy (Moshi et al., 2005). On the other hand, the bark of *A. odoratissima* is used to treat cough, bronchitis, rheumatism and diabetes (Kumar et al., 2011). Seeds of *A. amara* are used in the treatment of piles, diarrhea and gonorrhea (Gundamaraju et al., 2014).

Since the fifties of the last century, genus *Albizia* has been a rich source of several classes of bioactive secondary metabolites including saponins, tannins, alkaloids, flavonoids and

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phenolic glycosids (El-Mousallamy, 1998; Kang et al., 2007; Sanjay, 2003; Varshney and Farooq, 1952). Recently, an antitumor triterpene saponin julibroside $J_{28,}$ isolated from the stem bark of *A. julibrissin* has displayed significant, *in vitro*, antitumor activity against PC-3M-1E8, Bel-7402 and HeLa cancer cell lines at 10μM assayed by SRB method (Liang et al., 2005).

*A. lebbeck* L. is one of the most common species of *Albizia* worldwide, known by various names like Indian siris, flea tree, frywood, koko and Laback in Arabic. The tree was imported to Saudi Arabia from India, years ago, as an ornamental tree, well adapted to the hot environmental conditions of Najd state in the central region of Saudi Arabia (El Gamal et al., 2015).

It is usually flowering, between April and September, with cream-colored fragrant hermaphroditic flowers (Migahid, 1989). The plant is well-known, in traditional folk medicine, for the treatment of various ailments in several areas around the world. In Ayurveda, all parts of the tree including roots, leaves, bark and flowers are used to cure asthma and other inflammatory conditions such as, arthritis and burns (Ayurvedic Pharmacopoeia of India, 2001). In traditional Chinese medicine the flowers are commonly used to treat anxiety, depression and insomnia (Kang et al., 2007). The decoction of the flower in a dose of 50 mg/kg induces muscle relaxation and can protect the guinea pig against histamine-induced bronchospasm (Tripathi and Das, 1977).

In our previous research on *A. lebbeck*, we found that different fractions from the flowers of *A. lebbeck* possessed antipyretic, analgesic, estrogenic and anti-inflammatory activities (Farag et al., 2013). Our earlier phytochemical study of the alcoholic extract of the flowers led to the isolation of traxerol triterpenes, ceramide derivatives and two

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flavonoids, in addition to a novel β-lactam derivative, albactam, which was also evaluated for platelet anti-agreggatory effect (El Gamal et al., 2015).

In view of the versatile biological activities of this plant and as a part of our continuing interest in identifying biologically active drug leads from natural sources, we conducted this research with the aim of discovering new compounds with hepatoprotective potential.

## 2. Materials and methods

### 2.1. General experimental procedures

$^{1}$H and $^{13}$C NMR spectra were recorded at NMR Unit at the College of Pharmacy, Sattam Bin Abdulaziz University on a UltraShield Plus 500MHz (Bruker) spectrometer operating at 500 MHz for proton and 125 MHz for carbon, respectively. The chemical shift values are reported in δ (ppm) relative to the internal standard TMS or residual solvent peak, the coupling constants (*J*) are reported in Hertz (Hz). 2D-NMR experiments (COSY, HSQC, HMBC and NOESY) were obtained using standard Bruker program. Jeol JMS-700 High Resolution Mass Spectrophotometer was used for accurate mass determination. Electron Impact mode with ionization energy of 70ev was accustomed. Direct probe, temperature ramp setting was used with initial temperature 50 $^{o}$C; increasing 32 $^{o}$C per minute and final temperature 350 $^{o}$C set up, resolution was adjusted to 10k. Thin layer chromatography (TLC) was performed on pre-coated silica gel F$_{254}$ plates (E. Merck, Darmstadt, Germany); detection was done at 254 nm and by spraying with p-anisaldehyde/H$_2$SO$_4$.reagent followed by heating at 110 $^{o}$C for 1-2 min. Centrifugal preparative thin layer chromatography (CPTLC) was performed on chromatotron (Harrison Research, Palo Alto, California, CA, USA). Plates coated with 2 mm of silica

gel 60, 0.04-0.06 mm were used. All solvents used were of analytical grade. Silymarin was purchased from Sigma Aldrich (St. Louis, USA).

*2.2. Plant Materials*

The flowers of *Albizia lebbeck* Linn. were collected in April, 2011 from Riyadh, kingdom of Saudi Arabia. The plant was identified by Dr. Mohammed Yusuf, taxonomist of the Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC), College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. A voucher specimen (# 16182) has been deposited at the Pharmacognosy Department, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

*2.3. Extraction, fractionation and isolation*

The air-dried powdered flowers of *A. lebbeck* (1 kg) were extracted with 80% ethanol (3 × 4 L) at room temperature. The pooled extracts were concentrated under reduced pressure using rotatory evaporator to get a dark brownish residue (250 g), part of which (100 gm) was suspended in methanol (400 mL) and filtered to remove the precipitated insoluble part. The dried methanol-soluble fraction (55 gm) was dissolved in 40% ethanol and successively partitioned with *n*-hexane (300 mL x 4), chloroform (300 mL x 4), ethyl acetate (300 mL x 4) and *n*-butanol (300 mL x 4) to provide the corresponding fractions. The *n*-hexane fraction (12 g) was subjected to column chromatography on pre-packed silica gel column (35 mm i.d. × 350 mm) using *n*-hexane-ethyl acetate gradient; collected fractions were examined with TLC and similar fractions were pooled together. The fraction eluted with *n*-hexane/EtOAc (20/80) was further purified by subjecting to RP-18 column (120 g x 60 cm x 3 cm) using MeOH/CH$_3$CN, 10/90 to give an almost pure

compound which was further purified on the chromatotron using solvent system 1% acetone–CHCl$_3$ to give compound **9** (25 mg).

The chloroform fraction (13 g) was applied on a silica gel column using CHCl$_3$/MeOH gradient and collected fractions were grouped to obtain four main subfractions: A (3.7 g), B (3.4 g), C (2.6 g) and D (3.3 g). Fraction A was rechromatographed on a RP-18 column (MeOH–H$_2$O, gradient) to yield compound **10** (15 mg). Fraction B was subjected to purification by chromatotron (8% MeOH/CHCl$_3$), followed by RP-18 column chromatography (MeOH–H$_2$O, 65:35) to yield compound **4** (23 mg). Fraction C was purified on a RP-18 column (MeOH–H$_2$O, 65:35) to give compound **5** (8 mg), while compound **6** (19 mg) was obtained by RP-18 column (10% MeOH/H$_2$O) of fraction D.

The *n*-butanol fraction (10 gm) was chromatographed on Diaion HP-20 column, using MeOH/H$_2$O gradient to afford two main subfractions (i and ii). Sub fraction i, eluted with 40% MeOH/H$_2$O was further subjected to column chromatography (CHCl$_3$/MeOH, gradient) to afford compounds **1- 3** (using 9- 15 % MeOH/CHCl$_3$ as eluents respectively). Sub fraction ii, eluted with 60% MeOH/H$_2$O was further subjected to RP-18 column chromatography (100 g x 50 cm x 3 cm, MeOH/H$_2$O gradient) to afford compounds **7** and **8** (eluted with 50 and 65% MeOH/H$_2$O respectively).

*2.4. Animals*

Wistar male albino rats weighing 150–200 g, almost the same age (8–10 weeks), obtained from the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh were used. The animals were kept under constant temperature (22± 2 $^{\circ}$C), humidity (55%) and light/dark conditions (12/12 h). They had *ad libitum* access to a dry

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commercial diet and free access to drinking water (Abdel-Kader et al., 2010). The procedures and experiments used in this research were approved by the Ethics Committee of the College of Pharmacy, King Saud University.

## 2.5. Hepatoprotective activity

The male albino rats were randomly assigned into four groups (5 animals each). Group IV was divided into eight sub groups of five animals each. Group I received only normal saline and serve as control. Groups I– IV received single dose of $CCl_4$ (1.25 ml/kg body weight). Group II received $CCl_4$ treatment only. Group III was administered silymarin at a dose of 10 mg/kg p.o. The sub groups of were treated with 200, 400 mg/kg of the total extract, 50 and 100 mg/kg of the different fractions. Treatment started 6 days prior to $CCl_4$ and continued till day seven. After 24 h, following $CCl_4$ administration in day 7, the animals were sacrificed using ether anesthesia. Blood samples were collected by heart puncture and the serum was separated to evaluate the biochemical parameters (Abdel-kader and Alqasoumi, 2008).

## 2.6. Determination of AST, ALT, GGT, ALP, bilirubin, Cholesterol, Triglycerides, HDL, LDL and VLDL levels

Five biochemical parameters, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltranspeptidase (GGT), alkaline phosphatase (ALP) and total bilirubin were estimated, as reflection of liver function, by reported method (Edwards and Bouchier, 1991). The enzyme activities were measured using diagnostic strips (Reflotron®, ROCHE) and were read on a Reflotron®Plus instrument (ROCHE). TG (Foster and Dunn, 1973), TC (Zlatkis et al., 1953), HDL-C (Burstein et al., 1970), LDL-C and VLDL (Friedwald et al., 1972) were assessed using Roche Diagnostics Kits.

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*2.7. Determination of non-protein sulfhydryl groups (NP-SH), malonaldehyde (MDA)*

*and total protein (TP)*

The livers were separately cooled in a beaker immersed in an ice bath. The tissues were homogenized in 0.02 M ethylenediaminetetraacetic acid (EDTA) in a Potter– Elvehjem type C homogenizer.

Homogenate equivalent of 100 mg tissues were used for the measurements. Non-protein sulfhydryl groups (NP-SH) and MDA were quantified spectrophotometrically as previously described (Sedlak and Lindsay, 1968); Utley et al., 1967).

Total protein was determined according to the method of Lowry et al. (1951) Homogenates were treated with 0.7ml of Lowry's solution, mixed and incubated for 20 min in dark at room temperature. Diluted Folin's reagent (1 ml) was then added and samples were incubated at room temperature in dark for 30min. The absorbance of the resulted solutions was then measured at 750 nm (Lowry et al., 1951).

*2.9. Statistical analyses*

Results are expressed as Mean±Standard Error (SE) of mean. Statistical analysis was achieved, using a one-way analysis of variance (ANOVA). When, the F-value was found statistically significant ($p < 0.05$), further comparisons among groups were made using Dunnett's multiple comparisons test. All statistical analyses were performed using SPSS software 17.0 (Released Aug. 23, 2008), Chicago, USA.

**3. Results**

Phytochemical study of *A. lebbeck* flowers resulted in the isolation of ten compounds, benzyl 1-O-$\beta$-D- glucopyranoside (**1**), benzyl 6-O-$\alpha$-L-arabinopyranosyl $\beta$-D-glucopyranoside (**2**), linalyl $\beta$- D- glucopyranoside (**3**), linalyl 6-O-$\alpha$-L-arabinopyranosyl

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-$\beta$-D-glucopyranoside (**4**), 2*E*)-3,7-dimethylocta-2,6-dienoate-6-O-$\alpha$-L arabinopyranosyl-$\beta$-D-glucopyranoside (**5**), 1-O-[6-O-$\alpha$-L- arabinopyranosyl-$\beta$-D-glucopyranoside]–(2*E*,6*E*) -farnesol (**6**) *n*- hexyl-$\alpha$-L arabinopyranosyl-(1→6)-$\beta$-D-glucopyranoside (creoside) (**7**), *n*-octyl $\alpha$-L- arabinopyranosyl-(1→6)-$\beta$-D-glucopyranoside (Rhodiooctanoside) (**8**), 2,3-dihydroxy-2,3-dihydrosqualene (**9**) and ethyl fructofuranoside (**10**). The structures of the compounds are presented in Fig. 1. The total alcoholic extract, *n*-hexane, chloroform (CHCl$_3$) and *n*-butanol fractions were tested for hepatoprotective effect against CCl$_4$ induced toxicity. The results are shown in Tables 4-5.

### 3.1. The physical and spectral data of compounds *1*–*10*

#### 3.1.1. Benzyl 1-O-$\beta$-D- glucopyranoside (*1*)
C$_{13}$H$_{18}$O$_6$; white solid; ^{1}H NMR (500 MHz, MeOD): $\delta_H$ 7.43 (br d, H-2/6), 7.33 (br t H-3/5), 7.28 (m, H-4), 4.93 (d, *J* = 11.9 Hz, H-7a), 4.70 (d, *J* = 11.9 Hz, H-7b), 4.34 (d, *J* = 7.5 Hz, H-1′), 3.20 (m, H-2′), 3.35 (m, H-3′), 3.36 (m, H-4′), 3.17 (m, H-5′), 3.80 (dd, *J* = 11.8, 2.5 Hz, H-6′-a), 3.65 (dd, *J* = 11.8, 5.6 Hz, H-6′b); ^{13}C NMR (125 MHz, MeOD): $\delta_C$ 139.1 (C-1), 129.4 (C-2/6), 129.4 (C-3/5), 128.8 (C-4), 71.8 (C-7), 103.3 (C-1′), 75.1 (C-2′), 78.0 (C-3′), 71.8 (C-4′), 78.1 (C-5′), 62.8 (C-6′); HREIMS *m/z* 270.2780 [M$^+$].

#### 3.1.2. Benzyl-6-O-$\alpha$-L-arabinopyranosyl $\beta$-D-glucopyranoside (*2*)

C$_{18}$H$_{26}$O$_{10}$; white solid; 1H NMR (500 MHz, MeOD): $\delta_H$ 7.43 (br d, H-2/6), 7.33 (br t H-3/5), 7.28 (m, H-4), 4.93 (d, *J* = 11.9 Hz, H-7a), 4.70 (d, *J* = 11.9 Hz, H-7b), 4.35 (d, *J* = 8.0 Hz, H-1′), 3.22 (t, *J* = 8.1 Hz, H-2′), 3.42 (d, *J* = 2.3 Hz, H-3′), 3.43 (m, H-4′), 3.44 (m, H-5′), 4.33 (dd, *J* = 11.0, 5.7 Hz, H-6′a), 3.75 (dd, *J* = 11.0, 5.6 Hz, H-6′b), 4.34 (d, *J* = 7.0 Hz, H-1″), 3.59 (dd, *J* = 9.0, 7.1 Hz, H-2″), 3.64 (dd, *J* = 9.1, 3.0 Hz, H-3″), 3.95 (m, H-4″), 3.89 (dd, *J*= 13.0, 2.2 Hz, H-5″a), 3.65 (br d, *J* = 12.4 Hz, H-5″b); ^{13}C NMR (125 MHz, MeOD): $\delta_C$ 139.1 (C-1), 129.4 (C-2/6), 129.3 (C-3/5), 128.9 (C-4), 72.0 (C-7), 103.4 (C-1′), 75.1 (C-2′), 77.9 (C-3′), 71.6 (C-4′), 76.9 (C-5′), 69.6 (C-6′), 105.3 (C-1″), 72.4 (C-2″), 74.2 (C-3″), 69.6 (C-4″), 66.9 (C-5″); HREIMS *m/z* 402.1526 [M$^+$].

#### 3.1.3. Linalyl-$\beta$- D- glucopyranoside (*3*)
C$_{16}$H$_{28}$O$_6$; white viscous liquid; ^{1}H NMR (500 MHz, MeOD): $\delta_H$ 5.17 (d, *J*= 11.3 Hz, H-1-a), 5.22 (d, *J* =18.0 Hz, H-1-b), 6.07 (dd, *J* =17.7, 11.0 Hz, H-2), 1.64 (m, H-4), 2.04 (m, H-5), 5.11 (br t, *J* = 7.0 Hz, H-6), 1.68 (s, H-8), 1.61 (s, H-9), 1.35 (s, H-10), 4.33 (d, *J* = 7.4 Hz, H-1′), 3.18 (m, H-2′), 3.34 (m, H-3′), 3.36 (m, H-4′), 3.16 (m, H-5′), 3.80 (dd,

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$J$ = 11.9, 2.3 Hz, H-6′-a), 3.65 (dd, $J$ = 11.9, 5.5 Hz, H-6′-b), ^{13}C NMR (125 MHz, MeOD): $\delta_C$ 115.1 (C-1), 144.5 (C-2), 81.5 (C-3), 41.6 (C-4), 23.5 (C-5), 125.8 (C-6), 32.2 (C-7), 23.7 (C-8), 26.0 (C-9), 17.9 (C-10), 99.2 (C-1′), 75. 1 (C-2′), 78.2 (C-3′), 71.7 C-4′), 77.6 (C-5′), 62. 9 (C-6′); HREIMS *m/z* 316.1886 M[+]].

### 3.1.4. Linalyl-6-O-α-L-arabinopyranosyl -β-D-glucopyranoside (*4*)

$C_{21}H_{36}O_{10}$; viscous liquid; ^{1}H NMR (500 MHz, MeOD): $\delta_H$ 5.22 (d, $J$ = 11.3 Hz, H-1a,), 5.17 (d, $J$ = 11.3 Hz, H-1b), 6.09 (dd, $J$ = 17.7, 11.0 Hz, H-2), 1.56 (m, H-4a), 1.62 (m, H-4b,), 2.07 (m, H-5a), 2.04 (m, H-5b), 5.11 (br t, $J$ = 7.0 Hz, H-6), 1.68 (s, H-8), 1.61 (s, H-9), 1.35 (s, H-10) 4.35 (d, $J$ = 7.9 Hz, H-1′), 3.20 (t, $J$ = 8.0, H-2′), 3.40 (m, H-3′), 3.43 (m, H-4′), 3.43 (m, H-5′), 4.33 (dd, $J$ = 11.0, 5.7 Hz, H-6′a), 3.75 (dd, $J$ = 11.0, 5.5 Hz, H-6′b), 4.33 (d, $J$ = 7.0 Hz, H-1″), 3.60 (dd, $J$ = 9.0, 7.0 Hz, H-2″), 3.63 (dd, $J$ = 9.1, 3.0 Hz, H-3″), 3.93 (m, H-4″), 3.88 (dd, $J$ = 13.0, 2.2 Hz, H-5″a), 3.66 (br d, $J$ = 12.4 Hz, H-5″b); ^{13}C NMR (125 MHz, MeOD): $\delta_C$ Table 2; HREIMS *m/z* 448.2308.

### 3.1.5. (2E)-3,7-dimethylocta-2,6-dienoate-6-O-α-L-arabinopyranosyl-β-D glucopyranoside (*5*)

$C_{21}H_{34}O_{11}$; colorless amorphous powder; ^{1}H NMR Table 1; ^{13}C NMR Table 2; HREIMS *m/z* 462.2101 [M[+]].

### 3.1.6. 1-O-[6-O-α-L- arabinopyranosyl β-D-glucopyranoside] – (2E,6E-)-farnesol (*6*)

$C_{26}H_{44}O_{10}$; viscous liquid; ^{1}H NMR Table 1; ^{13}C NMR Table 2; HREIMS *m/z* 516.2934 [M[+]].

### 3.1.7. Hexyl-α-L arabinopyranosyl-(1→6)-β-D-glucopyranoside (*Creoside IV*) (*7*)

$C_{17}H_{32}O_{10}$; colorless amorphous powder; ^{1}H NMR Table 1; ^{13}C NMR Table 2; HREIMS *m/z* 396.1995 [M[+]].

### 3.1.8. Octyl-α-L arabinopyranosyl-(1→6)-β-D-glucopyranoside (rhodiooctanoside) (*8*)

$C_{19}H_{36}O_{10}$; white amorphous powder; ^{1}H NMR Table 1; ^{13}C NMR Table 2; HREIMS *m/z* 424.2308 [M[+]].

### 3.1.9. 2,3-dihydroxy-2,3-dihydrosqualene (*9*)

$C_{30}H_{52}O_2$; oily; ^{1}H NMR (500 MHz, MeOD): $\delta_H$ 1.16 (s, H-1), 1.22 (s, H-25), 1.60-1.70 (18 H, s, CH$_3$-24,26-30), 1.98-2.22 (m, CH$_2$-4,5,8,9,12,13,16,17,20,21), 5.22 (H-7), 3.37 (d, $J$ = 7.5 Hz, H-3), 5.12-5.22 (5H, t, $J$ = 6.1 Hz, C-H-7,11,14,18,22); ^{13}C NMR (125 MHz, MeOD): $\delta_C$ 23.2 (C-1), 73.1 (C-2), 78.3 (C-3), 29.7 (C-4), 36.8 (C-5), 135.2 (C-6), 124.3 (C-7), 26.8 (C-8), 39.8 (C-9), 134.9 (C-10), 124.4 (C-11), 28.3 (C-12), 26.4 (C-13), 124.4 (C-14), 135.0 (C-15), 39.8 (C-16), 26.7 (C-17), 125.1 (C-18), 134.9 (C-19), 39.8 (C-20), 26.7 (C-21), 124.2 (C-22), 131.3 (C-23), 23.3 (C-24), 25.7 (C-25), 15.9 (C-26), 16.0 (C-27), 16.0 (C-28), 16.1 (C-29), 17.7 (C-30); HREIMS *m/z* 444.3967 [M[+]].

### 3.1.10. 4-Ethyl fructofuranoside (*10*)

$C_8H_{16}O_6$; viscous liquid; ^{1}H NMR (500 MHz, MeOD): $\delta_H$ 3.90 (m, H-1), 3.70 (dd, $J$ = 2.8, 3.0 Hz, H-2), 4.04 (d, $J$ = 2.8 Hz, H-3), 3.66, 3.90 (dd 2.5, 12.00 CH$_2$-5), 3.56, 3.77 (m, CH$_2$-6), 3.57 (m, $J$ = 7.4 Hz CH$_2$-1′), 1.20 (t, $J$ = 7.5 Hz CH$_3$-2′), 5.15 ^{13}C NMR 125

MHz, MeOD): δ$_C$ 84.1 (C-1), 78.6 (C-2), 83.0 (C-3), 109.0 (C-4), 61.5 (C-5), 62.7 (C-6), 57.8 (C-1′), 16.1 (C-2′); HREIMS *m/z* 208.0947 [M$^+$].

## 4. Discussion

The administration of CCl$_4$ causes damage of the hepatocytes which is reflected by increase in the biochemical parameter levels such as aspartateaminotransferase (AST), alanine minotransferase (ALT), gamma glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), and total bilirubin (Table 3). Investigation of the curative potentials of the total alcoholic extract at two doses revealed that pretreatment of rats with 400mg/kg provided protection comparable to that of silymarin. The levels of AST, ALT, GGT and bilirubin were reduced by 52.01, 33.67, 29.67 and 48.05 % respectively. The least improvement was observed in the level of ALP 918.89 %). All results were highly significant (p<0.001) (Table 3). Liquid- Liquid fractionation of the total extracts with *n*-Hexane, CHCl$_3$ and *n*-butanol and evaluation of the hepatoprotective potential of the fractions at 50 and 100 mg/kg revealed that the *n*-butanol fraction is the most active in reduction of the levels of AST (36.54%), ALT (48.56%), MDA (1.82±0.20) and restoring the levels of NP-SH (4.50±0.30) and total protein (82.19±0.19) (p<0.001and p<0.01) (Tables 3 and 5). In the second place in improving these parameters comes the *n*-hexane fraction (Tables 3 and 5). However, the CHCl$_3$ fraction was superior in the improvement of blood lipid picture (Table 4). Cholesterol, triglycerides, HDL, LDL and VLDL were improved significantly (p<0.001) following the treatment of the rats with 100 mg/kg.

Phytochemical analysis of the *n*-hexane, chloroform and *n*-butanol fractions obtained from the flowers of *A. lebbeck* led to the isolation of two benzyl glycosides, benzyl 1-O-*β*-D- glucopyranoside (**1**) (De Rosa et al., 1996; Wen et al., 2012) and benzyl

6-O-$\beta$-L-arabinopyranosyl β-D-glucopyranoside (**2**) (Chassagne et al., 1996); three acyclic monoterpene glycosides, linalyl $\beta$- D- glucopyranoside (**3**) (Moon et al., 1994), linalyl 6-O-$\alpha$-L-arabinopyranosyl -$\beta$-D-glucopyranoside (**4**) (Chassagne et al., 1996; Pabst et al., 1991) and (2*E*)-3,7-dimethylocta-2,6-dienoate-6-O-$\alpha$-L-arabinopyranosyl-$\beta$-D-glucopyranoside (**5**) (Yang et al., 2013), an acyclic sesquiterpene glycoside 1-O-[6-O-$\alpha$-L-arabinopyranosyl-$\beta$-D-glucopyranoside]–(2*E*,6*E*-)-farnesol (**6**) (Magid et al., 2005; Magid et al., 2008) in addition to creoside IV (**7**) (Nakamura et al., 2008), octyl $\alpha$-L arabinopyranosyl-(1--->6)-$\beta$-D-glucopyranoside (rhodiooctanoside) (**8**) (Yoshikawa et al., 1996), the squalene derivative, 2,3-dihydroxy-2,3-dihydrosqualene (**9**) (Brown and Martens, 1977) and ethyl fructofuranoside (**10**) (Liu et al., 2012; Lu et al., 2013) which were identified using 1D and 2D- NMR and HREIMS and correlation with known compounds. The compounds **6** and **9** are reported here for the first time from natural sources. On the other hand and from the chemotaxonomic point of view, the sugar part of the isolated bioside glycosides consist of *α-L arabinopyranosyl-(1--->6)-β-D-glucopyranoside* which might be considered as chemotaxonomic marker for *A. lebbeck*.

It is noteworthy that linalyl $\beta$- D- glucopyranoside (**3**) and linalyl 6-O-$\alpha$-L-arabinopyranosyl -β-D-glucopyranoside (**4**) were reported as aroma precursors from *Jasminum sumbac* and passion fruit (*Passiflora edulis*), respectively (Chassagne et al., 1996; Moon et al., 1994). On the other hand, ethyl fructofuranoside (**10**) is formed as a byproduct by the *Wickerhamomyces anomala*, a yeast strain capable of selective utilization of fructooligosaccharides (FOSs) syrup (Lu et al., 2013).

Compound **6** was obtained as a viscous liquid. HREIMS of compound **6** showed a molecular ion peak at *m/z* 516.2934 [M$^+$] suggesting the molecular formula $C_{26}H_{44}O_{10}$

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and 5 degrees of unsaturation. Careful examination of NMR spectra for compound **6** revealed the presence of a farnesyl type sesquiterpene glycoside. Its ^{13}C NMR and DEPT spectrum (Table 1) exhibited 26 carbons including four olefinic methyls, seven methylenes, twelve methines nine of which are oxygenated and three quaternary carbons. The aglycone part was proved to be a farnesyl type sesquiterpene by four olefinic methyls observed in the NMR spectra, at $\delta_H$ 1.62, 1.63, 1.69 and 1.72, connected, in the HSQC, to carbons at $\delta_C$ 17.9, 16.3, 26.1 and 16.8 ppm; assigned for Me-13, Me-14, Me-12 and Me-15 respectively. Long range coupling was observed between the olefenic protons at $\delta_H$ 5.11 ppm (H-10, m) and Me-12 and Me-13; and 5.14 (H-6, m) and Me 14; and 5.41 (H-2, t) with Me-15. The presence of oxymethylene at C-1 was clarified by one pair of two coupled oxygenated protons at $\delta_H$ 4.21 ppm (H-1a, dd, $J$= 11.8, 7.6 Hz), 4.36 ppm (H-1b, dd, $J$= 9.3, 6.5 Hz), both showing cross peak correlations with the olefenic proton at $\delta_H$ 5.41 (H-2, t, $J$= 6.45 Hz).

Moreover, NMR showed the presence of four upfield methylene protons at $\delta_H$ 2.08 (H$_2$-4, m), 2.16 (H$_2$-5, m), 1.99 (H$_2$-8, m) and 2.10 ppm (H$_2$-9, m). The above mentioned spectral data together with 15 carbon atoms ascribed by ^{13}C NMR and DEPT experiments as 4 methyls, 5 methylenes, three olefinic methines at $\delta_C$ 121.6, 125.2 and 125.5 ppm assigned for C-2, C-6 and C-10 respectively and three quaternary carbons at $\delta_C$ 132.2, 136.3 and 142.5 assigned for carbons 11, 7 and 3 respectively confirmed the presence of acyclic farnesyl type sesquiterpene. The ^{13}C NMR chemical shift values for the olefenic methyls, Me-14 and Me-15 observed at $\delta_C$ 16.3 and 16.8 ppm and those for the two methylene at C-4 and C-8 appeared at $\delta_C$ 40.8 and 40.9 ppm, respectively are closely similar for those reported for (2*E*, 6*E*)- farnesol (Kasai et al., 1986) while the C-

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12 resonated at $\delta_C$ 20.1 ppm. The chemical structure of the sugar part of the molecule was confirmed to be a bioside of $\beta$-D glucose and $\alpha$-L-arabinose based on the data obtained from 1D ($^1$H, $^{13}$C, and DEPT) experiments and 2D (COSY, HSQC and HMBC). The presence of two anomeric protons at $\delta_H$ 4.32 and 4.33 ppm with large coupling constants $J$= 7.9 and 6.8 correlate directly through one bond length in HSQC to their carbons at $\delta_C$ 103.1 and 105.1 ppm indicating the presence of $\beta$-D- glucose and $\alpha$-L-arabinose respectively. Additionally two oxymethylene protons appeared at $\delta_H$ 3.76 (dd, $J$ = 11.1, 5.8 Hz), 4.11 (dd, $J$ = 11.5, 1.9 Hz) and $\delta_H$ 3.90 (dd, $J$ = 12.6, 2.1 Hz), 3.66 (brd, $J$= 12.4 Hz) assigned for Glc-6 and Ara-5. The remaining protons and carbons data for $\beta$-D-glucose and $\alpha$-L-arabinose was matched with those reported (Nakamura et al., 2007) and with the glycosides isolated from the currently studied plant (compounds 5-8) (Nakamura et al., 2008; Yang et al., 2013; Yoshikawa et al., 1996). The linkage between the two sugar moieties was found to be (1→6) by clear downfield shift of C-6 of $\beta$- D-glucose ($\delta_C$ 69.5 ppm) and significant three bond correlations, observed in HMBC spectrum, from the protons at $\square_H$ 3.76, 4.11 ppm (H-6′a and H-6′b) and the anomeric carbon of arabinose resonating at $\delta_C$ 105.1 ppm. On the other hand, he linkage, between the farnesyl and the sugar moiety, was proved to be at C-1 of the aglycone by a significant downfield shift of C-1 of the farnesol ($\delta_C$ 66.7) as well as the three bond correlation, observed in HMBC experiment, between H-1 ($\delta_H$ 4.21, 4.36) and the $\beta$-D-glucose anomeric carbon at $\delta_C$ 103.0 ppm the above aforementioned data prove compound **6** to be 1-O-[6-O-$\alpha$-L-arabinopyranosyl $\beta$ -D-glucopyranoside] –(2*E*,6*E*-)-

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farnesol. It is worth to note that crenulatoside derivatives (A-G) have been isolated before from *Guioa crenulata* (Magid et al., 2005).

Based on collective data, compound **9** (2,3-dihydroxy-2,3-dihydrosqualene), is identified here from natural source for the first time. However, it was previously synthesized (Brown and Martens, 1977) and also used as precursor in the synthesis of squalene oxides (D'Accolti et al., 2005)

This study proved that *Albizia lebbeck* is a rich source of terpenoid compounds, where we isolate mono, sesqui and triterpenes derivatives. Moreover the sugar part of the isolated biosides (compounds 4-8) consisting of α-L-arabinopyranosyl *(1→6) β*-D-glucopyranoside and this finding is considered as a chemotaxonomic marker for genus *Albizia*.



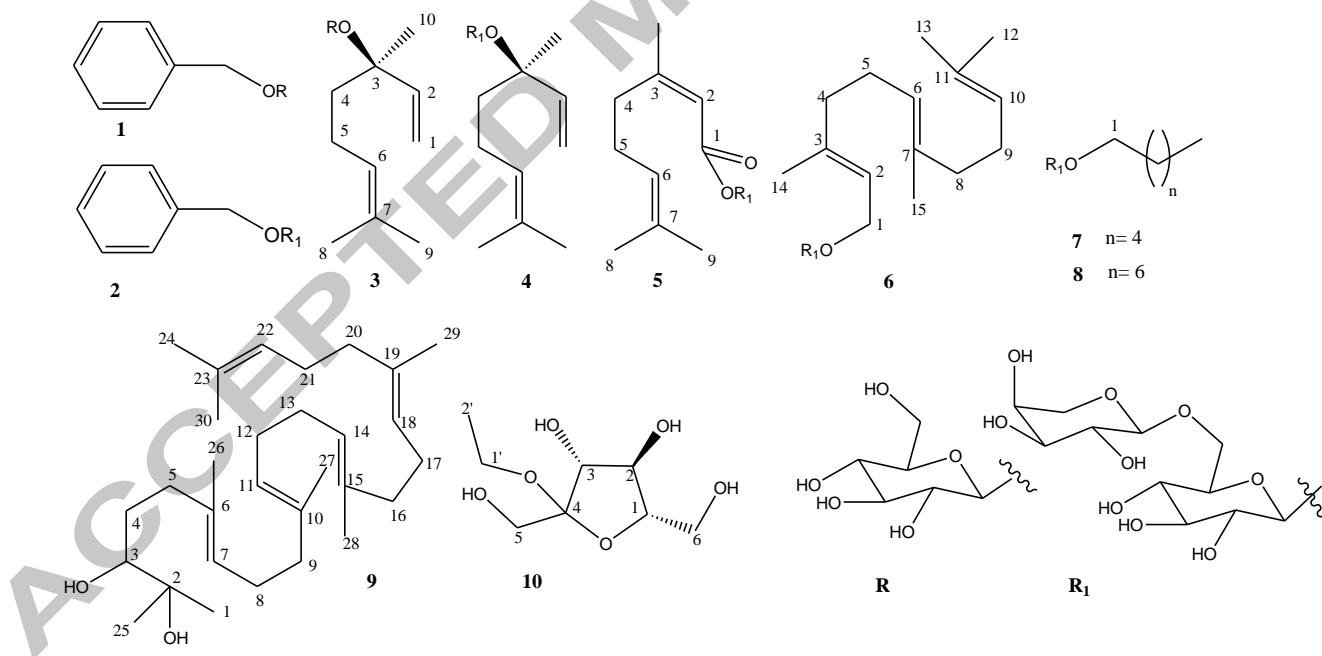Fig. 1 Chemical structures of the isolated compounds from *A. lebbeck* flowers

**Table 1** ^{1}H NMR data of compounds **5–8** (500 MHz, MeOD)

| | 5 | 6 | 7 | 8 |
|---|---|---|---|---|
| **1** | - | 4.21 (dd, $J$ = 11.8, 7.5 Hz); 4.36 (dd, $J$ = 9.3, 6.5 Ha) | 4.20 (dd, $J$ = 12.4, 7.6 Hz) 4.35 (dd, $J$ = 12.4, 6.9 Hz) | 3.83 (m) |
| **2** | 5.76 (s) | 5.41 (t, $J$ = 6.5 Hz) | 1.61 (m) | 1.61 (m) |
| **3** | - | - | 1.37 (m) | |
| **4** | 2.23 (m) | 2.08 (m) | 1.34 (m) | |
| **5** | 2.23 (m) | 2.16 (m) | 1.34 (m) | 1.22-1.47 (m) |
| **6** | 5.20 (dd, J = 6.6, 6.9 Hz) | 5.14 (m) | 0.91 (t, $J$ = 6.8 Hz) | |
| **7** | - | - | - | |
| **8** | 1.70 (s) | 1.99 (m) | - | 0.90 (t, $J$ = 6.9 Hz) |
| **9** | 1.64 | 2.10 (m) | - | - |
| **10** | 2.20 (s) | 5.11 (m) | - | - |
| **11** | - | - | - | - |
| **12** | - | 1.69 (s) | - | - |
| **13** | - | 1.62 (s) | - | - |
| **14** | - | 1.63 (s) | - | - |
| **15** | - | 1.72 (s) | - | - |
| **1′** | 5.49 (d, $J$ = 8.2 Hz) | 4.32 (d, $J$ = 7.9 Hz) | 4.26 (d, $J$ = 8.0 Hz) | 4.22 ((d, $J$ = 8.0 Hz) |
| **2′** | 3.55 (m) | 3.23 (t, $J$ = 8.2 Hz) | 3.18 (dd, $J$ = 8.2, 8.9 Hz), | 3.19 (m) |
| **3′** | 3.46 (dd, $J$ = 8.9, 8.9 Hz) | 3.40 (d, $J$ = 2.3 Hz) | 3.34 (m) | 3.32 (m) |
| **4′** | 3.53 (m) | 3.42 (m) | 3.34 (m) | 3.32 (m) |
| **5′** | 3.39 (m) | 3.43 (m) | 3.42 (m) | 3.40 (m) |
| **6′** | 4.12 (dd, $J$ = 11.2, 2.1 Hz) 3.75 (dd, $J$ = 11.2, 5.3 Hz) | 4.11 (dd, $J$ = 11.5, 1.9 Hz) 3.76 (dd, $J$ = 11.1, 5.8 Hz) | 4.09 (brd, $J$ = 10.0 Hz) 3.73 (m) | 4.09 (brd, $J$ = 11.4 Hz) 3.77 (d, $J$ = 6.6 Hz) |
| **1′′** | 4.30 (d, $J$ = 6.8 Hz) | 4.33 (d, $J$ = 6.8 Hz) | 4.32 (d, $J$ = 6.5 Hz) | 4.31 (d, $J$ = 6.7 Hz) |
| **2′′** | 3.57 (dd, $J$ = 6.4, 8.5 Hz) | 3.60 (dd, $J$ = 9.3, 7.0 Hz) | 3.59 (m) | 3.62 (m) |
| **3′′** | 3.37 (dd, $J$ = 8.2, 8.7 Hz) | 3.65 (dd, $J$ = 9.3, 3.1) | 3.53 (m) | 3.53 (m) |
| **4′′** | 3.81 (m) | 3.97 (m) | 3.80 (m) | 3.82 (m) |

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| 5'' | 3.87 (dd, $J$ = 3.2, 12.5 Hz)<br>3.53 (dd, $J$ = 5.3, 11.2 Hz) | 3.90 (dd, $J$ = 12.6, 2.1 Hz)<br>3.66 ( brd, $J$ = 12.4 Hz) | 3.85 (m)<br>3.52 (m) | 3.83 (m) |
|---|---|---|---|---|

**Table 2** ^{13}C NMR data of compounds **1–8** (125 MHz, MeOD)

| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
|---|---|---|---|---|---|---|---|---|
| **1** | 139.1 | 139.1 | 115.1 | 115.1 | 166.5 | 66.7 | 71.1 | 71.1 |
| **2** | 129.4 | 129.4 | 144.5 | 144.3 | 115.9 | 121.6 | 30.8 | 30.8 |
| **3** | 129.3 | 129.3 | 81.5 | 81.5 | 164.5 | 142.4 | 26.8 | 27.1 |
| **4** | 128.8 | 128.9 | 41.6 | 41.7 | 42.0 | 40.8 | 32.9 | 30.6 |
| **5** | 129.3 | 129.3 | 23.5 | 23.8 | 27.1 | 27.5 | 23.7 | 30.4 |
| **6** | 129.4 | 129.4 | 125.8 | 125.8 | 124.1 | 125.2 | 14.4 | 33.0 |
| **7** | 71.8 | 72.0 | 132.2 | 132.2 | 133.6 | 136.3 | - | 23.7 |
| **8** | - | - | 23.7 | 26.0 | 25.9 | 40.9 | - | 14.5 |
| **9** | - | - | 26.0 | 23.7 | 17.8 | 27.9 | - | - |
| **10** | - | - | 17.9 | 17.9 | 19.3 | 125.5 | - | - |
| **11** | - | - | - | - | - | 132.1 | - | - |
| **12** | - | - | - | - | - | 26.1 | - | - |
| **13** | - | - | - | - | - | 17.9 | - | - |
| **14** | - | - | - | - | - | 16.3 | - | - |
| **15** | - | - | - | - | - | 16.8 | - | - |
| **1'** | 103.3 | 103.4 | 99.2 | 99.3 | 95.2 | 103.0 | 104.4 | 104.4 |
| **2'** | 75.1 | 75.1 | 75.1 | 75.1 | 77.9 | 75.0 | 75.2 | 75.2 |
| **3'** | 78.0 | 77.9 | 78.2 | 78.2 | 77.6 | 77.9 | 78.1 | 78.1 |
| **4'** | 71.8 | 71.6 | 71.7 | 71.7 | 73.9 | 71.5 | 71.7 | 71.7 |
| **5'** | 78.1 | 76.9 | 77.6 | 76.4 | 71.2 | 76.8 | 76.9 | 76.8 |
| **6'** | 62.8 | 69.6 | 62.9 | 69.4 | 69.2 | 69.5 | 69.6 | 69.5 |
| **1''** | - | 105.3 | - | 104.9 | 104.8 | 105.1 | 105.2 | 105.2 |
| **2''** | - | 72.4 | - | 72.5 | 72.4 | 72.4 | 72.5 | 72.5 |
| **3''** | - | 74.2 | - | 74.2 | 74.2 | 74.2 | 74.4 | 74.4 |
| **4''** | - | 69.6 | - | 69.4 | 69.6 | 69.4 | 69.5 | 69.6 |
| **5''** | - | 66.9 | - | 66.4 | 66.8 | 66.8 | 66.8 | 66.8 |

**Table 3** Effect of *A. lebbeck* total extract (Total) and fractions on serum marker enzymes of control and experimental rats

| Treatment (n=5) | Biochemical parameters | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | AST (units/l) | % Change | ALT (units/l) | % Change | GGT (units/l) | % Change | ALP (units/l) | % Change | Bilirubin (mg/dl) | % Change |
| Normal | 106.01±5.44 | | 29.48±1.50 | | 3.55±0.27 | | 283.00±14.36 | | 0.53±0.02 | |
| CCl₄ | 297.66±10.01***[a] | | 192.5±5.70*** [a] | | 10.90±0.41*** [a] | | 503.66±14.84*** [a] | | 2.22±0.16*** [a] | |
| Silymarin | 119.83±5.68***[b] | 59.72 | 100.68±4.27*** [b] | 47.69 | 6.28±0.29*** [b] | 42.36 | 372.00±13.03*** [b] | 36.14 | 1.08±0.04*** [b] | 51.49 |
| Total 200 mg | 163.16±5.05*** [b] | 45.18 | 139.50±3.76*** [b] | 27.53 | 8.31±0.30*** [b] | 23.91 | 452.50±5.97* [b] | 10.15 | 1.33±0.04*** [b] | 40.04 |
| Total 400 mg | 142.83±4.72*** [b] | 52.01 | 127.66±3.48*** [b] | 33.67 | 7.66±0.22*** [b] | 29.67 | 408.50±7.57*** [b] | 18.89 | 1.15±0.04*** [b] | 48.05 |
| Normal | 106.85±4.18 | | 30.95±1.20 | | 4.96±0.19 | | 337.66±6.11 | | 0.54±0.02 | |
| CCl₄ | 331.83±9.78***[a] | | 288.83±11.01*** [a] | | 13.40±0.56*** [a] | | 598.16±8.97*** [a] | | 2.33±0.09*** [a] | |
| Silymarin | 149.66±8.04***[b] | 54.89 | 108.63±7.47*** [b] | 62.38 | 10.40±0.36** [b] | 22.39 | 420.50±6.23*** [b] | 29.70 | 1.03±0.05*** [b] | 55.79 |
| *n*-Hexane fraction 50 mg | 284.66±10.48* [b] | 14.22 | 240.00±8.61** [b] | 16.91 | 11.93±0.29 [b] | 10.97 | 564.33±14.72 [b] | 5.66 | 2.05±0.13 [b] | 12.01 |
| *n*-Hexane fraction 100 mg | 212.50±9.37*** [b] | 35.96 | 183.83±8.04*** [b] | 36.35 | 7.40±0.57 [b] | 44.77 | 531.33±22.83* [b] | 11.17 | 1.95±0.10* [b] | 16.31 |
| Normal | 101.33±3.08 | | 27.36±1.51 | | 4.73±0.35 | | 326.50±10.55 | | 0.53±0.01 | |
| CCl₄ | 319.83±9.11*** [a] | | 248.50±10.00*** [a] | | 12.66±0.58*** [a] | | 606.83±9.29*** [a] | | 2.25±0.10*** [a] | |
| Silymarin | 148.83±6.97*** [b] | 53.46 | 108.51±5.03*** [b] | 56.33 | 6.01±0.18*** [b] | 52.53 | 401.50±9.29*** [b] | 33.84 | 0.98±0.04*** [b] | 56.44 |
| CHCl₃ fraction 50 mg | 300.83±9.18 [b] | 5.94 | 280.50±7.21 [b] | - | 12.31±0.49 [b] | - | 596.66±12.58 [b] | - | 1.98±0.04* [b] | 12 |
| CHCl₃ fraction 100 mg | 273.66±8.89** [b] | 14.43 | 237.16±13.47* [b] | 4.56 | 8.40±0.39*** [b] | 33.65 | 510.33±7.13*** [b] | 15.90 | 1.53±0.07*** [b] | 32 |
| Normal | 109.25±4.25 | | 28.40±1.66 | | 5.21±0.21 | | 312.16±10.36 | | 0.54±0.01 | |
| CCl₄ | 304.66±7.21***[a] | | 282.50±8.98***[a] | | 11.83±0.60*** [a] | | 586.83±10.36*** [a] | | 2.75±0.05*** [a] | |
| Silymarin | 137.83±3.93*** [b] | 54.76 | 107.16±5.16*** [b] | 62.06 | 6.53±0.23*** [b] | 44.80 | 401.33±9.69*** [b] | 31.61 | 1.50±0.05*** [b] | 45.45 |
| *n*-Butanol fraction 50 mg | 223.33±8.94*** [b] | 26.69 | 224.66±16.43* [b] | 20.47 | 9.96±0.29* [b] | 15.80 | 577.50±13.48 [b] | - | 2.52±0.08 [b] | 8.36 |
| *n*-Butanol fraction 100 mg | 193.33±6.43*** [b] | 36.54 | 145.33±4.55*** [b] | 48.56 | 8.86±0.45** [b] | 25.10 | 532.00±11.98** [b] | 9.34 | 2.15±0.09*** [b] | 21.82 |

*P<0.05, **P<0.01, ***P<0.001
[a] compared to Normal saline group
[b] compared to Carbon tetrachloride group

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**Table 4** Effect of *A. lebbeck* fractions on serum lipid metabolism and serum lipoproteins of control and experimental rats

| Treatment (n=5) | Cholesterol (mg/dl) | % Change | Triglycerides (mg/dl) | % Change | HDL (mg/dl) | % Change | LDL (mg/dl) | % Change | VLDL (mg/dl) | % Change |
|---|---|---|---|---|---|---|---|---|---|---|
| Normal | 105.16±4.52 | | 60.83±2.70 | | 56.06±2.69 | | 36.92±4.76 | | 12.17±0.54 | |
| CCl₄ | 175.66±3.42***[a] | | 150.33±3.94***[a] | | 25.78±1.78***[a] | | 119.81±4.83*** [a] | | 30.06±0.78*** [a] | |
| Silymarin | 152.33±5.60** [b] | 13.28 | 127.83±3.41**[b] | 14.96 | 38.33±2.40** [b] | 48.68 | 87.83±7.20** [b] | 26.69 | 25.66±0.68** [b] | 14.64 |
| *n*-Hexane fraction 50 mg | 160.50±6.30 [b] | - | 141.00±3.56 [b] | - | 33.38±1.80* [b] | 29.48 | 98.91±6.81* [b] | 17.44 | 28.20±0.71 [b] | - |
| *n*-Hexane fraction 100 mg | 155.00±7.15* [b] | 11.76 | 131.83±4.07* [b] | 12.31 | 35.35±2.75* [b] | 37.12 | 93.38±8.98* [b] | 21.86 | 26.36±0.81** [b] | 12.31 |
| Normal | 103.96±4.93 | | 68.40±2.04 | | 56.21±2.08 | | 34.07±5.48 | | 13.68±0.40 | |
| CCl₄ | 189.66±6.76*** [a] | | 170.66±6.11*** [a] | | 26.66±1.02*** [a] | | 128.96±6.20*** [a] | | 34.03±1.22*** [a] | |
| Silymarin | 148.66±6.72** [b] | 21.61 | 123.66±4.42*** [b] | 27.54 | 42.28±2.05*** [b] | 58.58 | 83.41±6.81*** [b] | 35.32 | 24.73±0.88*** [b] | 27.33 |
| CHCl₃ fraction 50 mg | 165.16±4.47* [b] | 12.91 | 146.16±4.00* [b] | 14.36 | 40.51±2.64*** [b] | 51.95 | 100.00±4.73** [b] | 22.46 | 29.23±0.80* [b] | 14.11 |
| CHCl₃ fraction 100 mg | 144.33±4.12*** [b] | 23.90 | 117.66±4.91*** [b] | 31.05 | 42.28±1.68*** [b] | 58.58 | 78.51±4.58*** [b] | 39.12 | 23.53±0.98*** [b] | 30.86 |
| Normal | 100.63±4.32 | | 70.13±3.61 | | 58.61±2.79 | | 28.00±5.32 | | 14.02±0.72 | |
| CCl₄ | 196.83±7.42*** [a] | | 172.83±4.32*** [a] | | 23.10±1.44*** [a] | | 139.16±6.74*** [a] | | 34.56±0.86*** [a] | |
| Silymarin | 148.66±6.72** [b] | 24.47 | 123.66±4.42*** [b] | 28.45 | 42.28±2.05*** [b] | 83.03 | 83.41±6.81*** [b] | 40.06 | 24.73±0.88*** [b] | 28.44 |
| *n*-Butanol fraction 50 mg | 203.00±10.27 [b] | - | 159.33±3.73 [b] | 7.81 | 34.03±1.69*** [b] | 47.31 | 137.10±10.82 [b] | - | 31.86±0.74* [b] | 7.81 |
| *n*-Butanol fraction 100 mg | 188.50±5.07 [b] | - | 142.50±3.22*** [b] | 17.55 | 41.50±1.80*** [b] | 79.65 | 118.49±4.67* [b] | 14.85 | 28.50±0.64*** [b] | 17.53 |

All values represent mean ± SEM. *p<0.05; **p<0.01; ***p<0.001; ANOVA, followed by Dunnett's multiple comparison test.
[a] -as compared to normal saline group
[b]- as compared to Carbon tetrachloride group

**Table: 5** Effect of *A. lebbeck* fractions on NP-SH, MDA and TP in rat liver

| Treatment (n=5) | MDA (nmol/g) | NP-SH (nmol/g) | Total protein (g/l) |
|---|---|---|---|
| **Normal** | 0.46±0.04 | 6.01±0.37 | 98.61±6.74 |
| **CCl₄** | 6.37±0.46\*\*\*[a] | 3.37±0.28\*\*\* [a] | 25.04±3.41\*\*\* [a] |
| **Silymarin** | 1.89±0.20\*\*\*[b] | 5.84±0.29\*\*\* [b] | 59.16±4.50\*\*\* [b] |
| **_n_-Hexane fraction 50 mg** | 5.31±0.42 [b] | 3.92±0.17 [b] | 34.28±2.95 [b] |
| **_n_-Hexane fraction 100 mg** | 2.98±0.25\*\*\* [b] | 4.61±0.28\* [b] | 51.20±5.56\*\* [b] |
| **Normal** | 0.46±0.03 | 7.19±0.31 | 131.20±9.47 |
| **CCl₄** | 7.64±0.35\*\*\* [a] | 4.35±0.28\*\*\* [a] | 30.20±2.63\*\*\* [a] |
| **Silymarin** | 2.02±0.21\*\*\* [b] | 6.25±0.22\*\*\* [b] | 63.53±4.63\*\*\* [b] |
| **CHCl₃ fraction 50 mg** | 5.09±0.42\*\*\* [b] | 3.26±0.16\* [b] | 47.98±3.32\*\* [b] |
| **CHCl₃ fraction 100 mg** | 2.87±0.200\*\*\* [b] | 3.35±0.21\* [b] | 72.85±5.80\*\*\* [b] |
| **Normal** | 0.52±0.04 | 7.20±0.38 | 128.44±9.21 |
| **CCl₄** | 5.66±0.54\*\*\* [a] | 3.61±0.13\*\*\* [a] | 38.54±2.82\*\*\* [a] |
| **Silymarin** | 1.46±0.19\*\*\* [b] | 4.99±0.25\*\*\* [b] | 94.98±11.06\*\*\* [b] |
| **_n_-Butanol fraction 50 mg** | 4.46±0.29\*\*\* [b] | 4.30±0.32 [b] | 59.24±4.83\*\* [b] |
| **_n_-Butanol fraction 100 mg** | 1.82±0.20\*\*\* [b] | 4.50±0.30\* [b] | 82.19±7.19\*\*\* [b] |

All values represent mean ± SEM. \*p<0.05; \*\*p<0.01; \*\*\*p<0.001; ANOVA, followed by Dunnett's multiple comparison test.
[a] -as compared to Normal saline group
[b] - as compared to Carbon tetrachloride group

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