GC-MS analysis and hepatoprotective activity of the n-hexane extract of Acrocarpus fraxinifolius leaves against paracetamol-induced hepatotoxicity in male albino rats

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**ABSTRACT**

Context: In Egypt, the burden of liver diseases is exceptionally high.

Objective: To investigate the components of the n-hexane extract of Acrocarpus fraxinifolius Arn. (Leguminosae) and its hepatoprotective activity against paracetamol (APAP)-induced hepatotoxicity in rats.

Material and methods: TRACE GC ultra gas chromatographic spectrometry was used for extract analysis. Thirty albino rats were divided into six groups (five rats in each). Group 1 was the healthy control; Groups 2 and 3 were healthy treated groups (250 and 500 mg/kg b.w. of the extract, respectively) for seven days. Group 4 was hepatotoxicity control (APAP intoxicated group). Groups 5 and 6 received APAP + extract 250 and APAP + extract 500, respectively.

Results: Chromatographic analysis revealed the presence of 36 components. Major compounds were \(\alpha\)-tocopherol (18.23\%), labda-8 (20)-13-dien-15-oic acid (13.15\%), lupeol (11.93\%), phytol (10.95\%) and squalene (7.19\%). In the acute oral toxicity study, the mortality rates and behavioural signs of toxicity were zero in all groups (doses from 0 to 5 g/kg b.w. of \textit{A. fraxinifolius}). LD\textsubscript{50} was found to be greater than 5 g/kg of the extract. Only the high dose (500 mg/kg b.w.) of extract significantly alleviated the liver relative weight (4.01 ± 0.06) and biomarkers, as serum aspartate aminotransferase (62.87 ± 1.41), alanine aminotransferase (46.74 ± 1.45), alkaline phosphatase (65.96 ± 0.74), lipid profiles (180.39 ± 3.51), bilirubin profiles (2.30 ± 0.06) and hepatic lipid peroxidation (114.20 ± 2.06), and increased body weight (11.58 ± 0.20), serum protein profile (11.09 ± 0.46) and hepatic total antioxidant capacity (23.78 ± 0.66) in APAP-induced hepatotoxicity in rats.

Conclusion: Our study proves the antihepatotoxic/antioxidant efficacies of \textit{A. fraxinifolius} hexane extract.

**INTRODUCTION**

Liver diseases are considered as a major public health problem around the world due to their potentiality to cause morbidity and mortality (Hasan & Khan 1997). About more than two million people in the world die annually from liver-related disorders (Bukhsh et al. 2014). Hepatitis viral infection, alcohols, drugs, industrial chemicals and pollutants are the major risk factors (Paraskevi & Ronald 2006).

Among drug-induced liver injury, paracetamol (acetaminophen, \(N\)-acetyl-\(p\)-aminophenol; APAP), is one of the most widely used hepatotoxic agents. It is safe at therapeutic doses, but causes liver failure in overdoses (Lewerenz et al. 2003). Paracetamol is used as an antipyretic drug (Ahmed & Khater 2001). It is safe at therapeutic doses, but at high doses, it produces acute liver failure and hepatic necrosis (Abraham 2005) and so, the American Association of Poison Control Center (AAPCC) announced that paracetamol toxicity leads to 401 deaths due to acute hepatic failure (Bronstein et al., 2009).

Oxidative stress plays a basic role in the pathogenesis of paracetamol-induced liver damage (Srinivasan et al. 2001). In overdose, paracetamol is metabolized by liver cytochrome-450 enzymes into highly reactive metabolite \(N\)-acetyl-p-benzoquinoneimine (NAPQI) which causes depletion of 70% glutathione and subsequently oxidative stress (Bajt et al. 2001; Jaeschke et al. 2011; Gelotte et al. 2007). Also, NAPQI binds covalently to cellular proteins causing mitochondrial dysfunction, ATP depletion, lipid peroxidation, DNA damage and necrosis of parenchymal cells as well as hepatic necrosis (Jones et al. 2010). At the same time, damaged hepatocytes activate the liver’s innate immune response like Kupffer cells, natural killer cells, natural killer T cells. This results in producing pro-inflammatory mediators such as tumour necrosis factor-\(\alpha\), interferon-\(\gamma\) and interleukin-\(\beta\) causing liver injury (Kaplowitz et al. 2008).

Conventional drugs that are currently used in the treatment of liver diseases have poor efficacy with long-term use and potential toxic effects. Subsequently, scientists have been paying serious attention to alternative herbal therapies that have antioxidant properties with minimum side effects in the treatment of many diseases like liver diseases, to replace currently used drugs of doubtful efficacy and safety (Muthulingam et al. 2010). Medicinal plants play important roles in treating liver diseases. Lots of medicinal plants were used in the protection and treatment of various liver diseases (Saleem & Naseer 2014). Leaves of \textit{Sapium sebiferum} (L.) Roxb (Euphorbiaceae) (Hussain et al. 2015) and \textit{Malva sylvestris} L. (Malvaceae) (Hussain et al. 2014)
showed strong hepatoprotective effects against paracetamol-induced induced liver injury. Leguminosae embraces several genera of reported hepatoprotective species (Huo et al. 2011; Rajendran et al. 2009; Arulkumaran et al. 2009; Rehman et al. 2015; Gamal El-Din et al. 2014; Azah et al. 2013).

*A. fraxinifolius* Arn. (Leguminosae) is a member of the tribe Caesalpinieae (El-nashar et al. 2015). Mundani, shingle and pink cedar are its common names. It is a deciduous tree (heights of 30–60 m) and is considered as a part of tropical evergreen and sub-evergreen forests. This species is distributed naturally in countries such as India, Burma and China and is widely cultivated in Egypt. Traditionally, in Yaneshas, boiled *A. fraxinifolius* bark is prepared as a drink and used for internal bruises (Valadeau et al. 2009). Recently, bioactive phenolics such as gallic acid, catechin, epicatechin and catechin gallate were identified from the bark extract by HPLC-DAD, and may be responsible for its radical scavenging activity (Rosales-castro et al. 2015). Total ethanol and aqueous ethanol extracts of the leaves exhibited high potencies as compared with metformin in decreasing the glucose level after four weeks. In addition, aforementioned extracts showed an in vivo antioxidant activity when compared to vitamin E (Abou Zed et al. 2012).

This study was designed to investigate the hexane extract of *A. fraxinifolius* phytochemically for the first time as well as its hepatoprotective activity of the extract against APAP-induced hepatotoxicity in albino rats. At the same time, it investigated any deleterious effects caused by the consumption of *A. fraxinifolius* extract by healthy albino rats.

**Materials and methods**

**Plant material**

Leaves of *A. fraxinifolius* were collected from Giza Zoo Botanical Garden, in January 2013. They were kindly authenticated by Mrs. Tereize Labib, the taxonomy specialist in El-Orman Botanical Garden, Giza, Egypt. A voucher specimen (PHG-P-AF130) has been deposited at Pharmacognosy Department, Faculty of Pharmacy, Ain Shams University.

**Preparation of n-hexane extract**

The intact air-dried plant material (2 kg) was comminuted to fine powder then soaked in 80% methanol for 4 days and filtered. The filtrate was completely evaporated in vacuo at \( \approx 55^\circ \text{C} \) to achieve complete dryness. The dried residue was further successively fractionated with *n*-hexane. The combined *n*-hexane extracts were evaporated in vacuo until dryness to give 50 g of a sticky dark greenish material.

**Instruments and chromatographic conditions**

Gas chromatography mass spectrography (GC-MS) analysis of *n*-hexane extract was carried out using GC-MS spectrometry instrument at Department of Medicinal and Aromatic Plants Research, National Research Centre. A TRACE GC ultra gas chromatograph (THERMO Scientific Corp., Waltham, MA, USA) is coupled with a thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-5MS column (30 m x 0.25 mm i.d., 0.25 μm film thickness). Helium is used as carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10. Temperature programming is applied (50 °C for 3 min; rising at 5.0 °C/min to 300 °C and held for 20 min). The injector and detector were held at 280°C. Diluted samples (1:10 hexane, v/v) of 0.2 μL were injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of *m/z* 40–450.

**Chemicals and kits**

Paracetamol or acetaminophen (*N*-acetyl-*p*-aminophenol; APAP) was purchased from Sanofi-aventisegypts.a.e., El Sawah, El Amiriya, Cairo, Egypt. The kits used for biochemical measurements were all purchased from Bio-diagnostic Company, Dokki, Giza, Egypt. Other chemicals were of the highest purity commercially available.

**Experimental animals**

This study was carried out at Zoology Department, Faculty of Science, Ain Shams University, using clinically healthy mature adult male Wistar albino rats. The animals were obtained from the Animal Breeding House of the National Research Centre (NRC), Dokki, Cairo, Egypt, and maintained in clean plastic cages in the laboratory animal house. Their weights ranged from 130 to 140 g. The rats were acclimatized for 1 week prior to the start of experiments on standard pellet diet (Agricultural-Industrial Integration Company, Giza, Egypt), tap water ad libitum, and daily 12 h dark/light cycle. All animals were humanely treated in accordance with World Health Organization (WHO, 1998) guideline for animal care and the study design was approved by the Ain Shams University Ethics Committee. The animals were accommodated in the laboratory conditions for one week before beginning the experiment.

**Acute toxicity study of the extract**

The acute toxicity test was conducted to determine the median lethal dose (LD\(_{50}\)) of test materials and used to choose the safe dose of the experiment for the *A. fraxinifolius* extract. The doses were selected based on acute toxic class method for Organization for Economic Cooperation and Development guidelines (OECD 423). The rats were divided into six groups. Group I received distilled water only as a vehicle while Groups II, III, IV, V and VI acted as treatment groups receiving single increasing dose of *A. fraxinifolius* extract at 1, 2, 3, 4 and 5 g/kg b.w of rats, respectively. All the doses and vehicle were administered by the oral route. Throughout the study period, all animals were observed for behavioural signs of toxicity, morbidity and mortality. All signs of these were noted after 1, 4 and 24 h of administration of the extract for 14 consecutive days. Finally, 1/20 (250 mg/kg b.w) and 1/10 (500 mg/kg b.w) of the maximum safe dose of the *A. fraxinifolius* extract tested for acute toxicity were selected as two doses for the experimental models.

**Experimental design**

Experimental animals were randomly divided into six groups of five rats each: three healthy groups and three APAP groups. In healthy groups, Group I (healthy control group) animals received orally and daily 1.0 mL distilled water as vehicle by stomach tube. In Group 2 (control treated with *A. fraxinifolius* 250), each animal received *A. fraxinifolius* in a single daily oral dose of 250 mg/kg b.w alone for seven days. In Group 3 (control treated with *A. fraxinifolius* 500), each animal received *A. fraxinifolius* in
a single daily oral dose of 500 mg/kg b.w alone for seven days. In APAP groups, in Group 4 (APAP only), each animal received a single daily oral dose of 400 mg/kg b.w APAP for seven days. Hepatotoxicity was induced in rats according to the procedure described by Kanchana and Mohamed (2011). In Group 5 (APAP + A. fraxinifolius 250), each animal received a single daily low dose of A. fraxinifolius orally starting 1 h before administration APAP and given for seven days. In Group 6 (APAP + A. fraxinifolius 500), each animal was given a single daily high dose of A. fraxinifolius orally starting 1 h before administration APAP and given for seven days.

After 24 h from the last treatment, the rats were humanely sacrificed by light diethyl ether anesthesia. The blood was collected into clean test-tubes without anticoagulant. Then, blood was centrifuged in a cooling centrifuge (IEC centra-4R; International Equipment Co., Needham Heights, MA) for 30 min at 3000 rpm and 4 °C. The serum was separated and divided into samples and preserved at −80 °C for further analysis. Immediately after killing the animals, livers of each animal were separately weighed and homogenized in 5 mL cold buffer (0.5 g of Na2HPO4 and 0.7 g of NaH2PO4 per 500 mL deionized water, pH 7.4) per gram tissue. Then, the homogenates were centrifuged for 15 min at 4000 rpm and 4 °C; and the obtained supernatants were divided into samples and preserved at −80 °C until used for evaluating the lipid peroxidation and total antioxidant parameters.

After centrifugation, the obtained supernatants were divided into samples and preserved at −80 °C until used for evaluating the lipid peroxidation and total antioxidant parameters.

Biochemical analysis

Body-weight gain or loss was calculated by using a Sartorius LP2200S balance (Gottingen, Germany) as the following equation: body-weight gain or loss = b.w at the end of the experiment – b.w at the beginning of the experiment. All biochemical analyses were manually done using commercial kits. Determination of amino transaminases enzymes (AST and ALT), ALP activities, total protein, albumin, total and direct bilirubin, total cholesterol, triglycerides and HDL-cholesterol in serum was estimated by the spectrophotometric method described by Ohkawa et al. (1979) and Koracevic et al. (2001), respectively. Serum total globulins and indirect bilirubin concentrations were calculated according to the following equations: total globulins = serum total protein – albumin; indirect bilirubin = total bilirubin – direct bilirubin. In addition, A/G ratio = albumin: globulin. LDL-cholesterol concentration was calculated according to the equation of Friedewald et al. (1972): LDL-cholesterol = total cholesterol – (TAG/5) – HDL-cholesterol. Atherogenic indexes were calculated as follows: atherogenic index (1) = total cholesterol : HDL-cholesterol ratio; atherogenic index (2) = LDL-cholesterol : HDL-cholesterol ratio.

Statistical analysis

Data are presented as mean values with their standard errors. Statistical analysis was performed with analysis of variance (ANOVA), and the differences among groups were determined by Tukey’s multiple comparison test (Turner & Thayer 2001) using Graph Pad Prism version 4.03 for Windows (GraphPad software Inc., San Diego, CA). p values of <0.05, <0.01 and <0.001 were considered statistically significant, highly significant and very highly significant, respectively.

Results

Investigation of the components of n-hexane extract of A. fraxinifolius using GC-MS analysis

The result of the GC-MS analysis of n-hexane extract of A. fraxinifolius leaves is shown in Figure 1 and Table 1. It revealed the presence of 37 compounds from which 29 compounds (95.9%) were identified. Hydrocarbons (acyclic and cyclic) represented 14.10%, steroids; 9.82%, triterpenes; 12.47% and other oxygenated compounds; 59.42%. α-Tocopherol was the major identified compound (18.23%) followed by labda 8 (20)-13-dien-15-oic acid (13.15%), lupeol (11.93%), phytol (10.95%), and squalene (7.19%). Identification of the compounds was carried out by matching their retention times and fragmentation patterns with...
the residue of reference compounds analysed under the same conditions (Selim 2013; Abdelwahab 2009; AOCS Lipid Library 2014).

**Acute toxicity of A. fraxinifolius**

Acute toxicity study in which the animals treated with *A. fraxinifolius* extracts at a higher dose of 5000 mg/kg did not manifest any significant abnormal signs, behaviour changes or macroscopic findings at any time of observation, hence the doses of 250 and 500 mg/kg were taken for the study.

**Modulatory effects of *A. fraxinifolius* on body weight loss and liver relative weight**

Body weight gain and liver relative weight were significantly decreased and increased (*p* < 0.001) by −68.5% and 34.9%, respectively, in APAP-intoxicated group compared with the healthy control rats (Figure 2A, B). Both doses of *A. fraxinifolius* significantly alleviated (*p* < 0.001) the decrease and increase in the body weight gain and liver relative weight, respectively, of APAP-intoxicated rats during the treatment course. The body weight change was reverted to normal levels upon treatment with high dose of *A. fraxinifolius* (*p* > 0.05 compared with the healthy control group). In addition, the improvement of liver relative weight of APAP rats by high dose of *A. fraxinifolius* significantly exceeded (*p* < 0.01) that of low dose of *A. fraxinifolius*. The percentages of changes of the body weight gain and liver relative weight measured in the APAP-only-treated group and groups treated with APAP plus either the low or high dose of *A. fraxinifolius* were −68.5% (*M* = 3.98), −27.8% (*M* = 9.12), −8.4% (*M* = 11.58), 34.9% (*M* = 4.98), 16.3% (*M* = 4.29) and 8.7% (*M* = 4.01), respectively, compared with healthy control group.

**Modulatory effects of *A. fraxinifolius* on hepatocyte integrity markers**

As shown in Figure 3, APAP administration significantly increased (*p* < 0.001) serum ALAT, ASAT and ALP activities by 42.6% (*M* = 58.05), 44.5% (*M* = 80.24) and 27.8% (*M* = 79.51), respectively, compared with the healthy control group. Oral treatment of both doses of *A. fraxinifolius* significantly decreased (*p* < 0.001) serum ALAT, ASAT and ALP activities compared with APAP-intoxicated group. The highest suppressive effect on the decrease on serum hepatocyte integrity markers of APAP rats was induced by the high dose of *A. fraxinifolius* (*14.8%*, 13.2% and 6.0%, respectively, compared with healthy control group). The percentages of changes of the serum ALAT, ASAT and ALP activities measured in the groups treated with APAP plus either

### Table 1. Identification of the components of *n*-hexane extract of *A. fraxinifolius* leaves.

| No. | Compound | Molecular formula | Molecular weight | Rt (min.) | Relative area (%) | Kf | Method of identification |
|-----|----------|------------------|------------------|-----------|------------------|----|------------------------|
| 1   | Unidentified | – | – | 10.19 | 0.56 | – | – |
| 2   | Undecane | C_{11}H_{24} | 156 | 11.51 | 1.44 | 1099 | Kl, MS |
| 3   | Undecane, 5-methyl- | C_{11}H_{26} | 170 | 13.42 | 0.36 | 1158 | Kl, MS |
| 4   | Undecane, 2methyl- | C_{11}H_{22} | 212 | 13.77 | 0.61 | 1168 | Kl, MS |
| 5   | Undecane, 3-methyl- | C_{11}H_{26} | 170 | 14.00 | 0.46 | 1172 | Kl, MS |
| 6   | Dodecane | C_{12}H_{26} | 198 | 15.07 | 3.43 | 1200 | Kl, MS |
| 7   | Undecane, 2,6-dimethyl- | C_{13}H_{28} | 184 | 15.46 | 0.65 | 1216 | Kl, MS |
| 8   | Phytol | C_{20}H_{40}O | 296 | 41.09 | 10.95 | 1949 | Kl, MS |
| 9   | Methyl stearate | C_{19}H_{38}O | 298 | 41.57 | 0.39 | 2099 | Kl, MS |
| 10  | Oleamide | C_{18}H_{35}O | 281 | 46.79 | 2.70 | 2375 | Kl, MS |
| 11  | Abietic acid | C_{28}H_{36}O | 358 | 53.74 | 5.46 | 2905 | Kl, MS |
| 12  | Tetracosanol-1 | C_{24}H_{50}O | 354 | 56.73 | 5.46 | 2905 | Kl, MS |
| 13  | Tocopherolquinone | C_{29}H_{50}O | 446 | 60.55 | 0.77 | 3113 | Kl, MS |
| 14  | Tocopherol | C_{27}H_{46}O | 410 | 64.35 | 0.58 | 2806 | Kl, MS |
| 15  | Tocopherol | C_{29}H_{48}O | 412 | 62.23 | 3.01 | 2941 | Kl, MS |
| 16  | Tocopherol | C_{29}H_{46}O | 410 | 59.89 | 0.32 | 3113 | Kl, MS |
| 17  | Tocopherol | C_{29}H_{48}O | 412 | 56.91 | 0.31 | 3113 | Kl, MS |
| 18  | Tocopherol | C_{30}H_{50}O | 426 | 61.58 | 0.37 | 3305 | Kl, MS |
| 19  | Tocopherol | C_{29}H_{52}O | 423 | 59.88 | 0.31 | 3305 | Kl, MS |
| 20  | Tocopherol | C_{29}H_{50}O | 426 | 59.89 | 0.32 | 3305 | Kl, MS |
| 21  | Tocopherol | C_{30}H_{50}O | 421 | 61.58 | 0.37 | 3305 | Kl, MS |
| 22  | Tocopherol | C_{30}H_{52}O | 423 | 59.88 | 0.31 | 3305 | Kl, MS |
| 23  | Tocopherol | C_{29}H_{50}O | 426 | 59.89 | 0.32 | 3305 | Kl, MS |
| 24  | Tocopherol | C_{30}H_{50}O | 421 | 61.58 | 0.37 | 3305 | Kl, MS |
| 25  | Tocopherol | C_{29}H_{52}O | 423 | 59.88 | 0.31 | 3305 | Kl, MS |
| 26  | Tocopherol | C_{30}H_{50}O | 421 | 61.58 | 0.37 | 3305 | Kl, MS |
| 27  | Tocopherol | C_{29}H_{50}O | 426 | 59.89 | 0.32 | 3305 | Kl, MS |
| 28  | Tocopherol | C_{30}H_{52}O | 423 | 59.88 | 0.31 | 3305 | Kl, MS |
| 29  | Tocopherol | C_{30}H_{50}O | 421 | 61.58 | 0.37 | 3305 | Kl, MS |
| 30  | Tocopherol | C_{29}H_{50}O | 426 | 59.89 | 0.32 | 3305 | Kl, MS |
| 31  | Tocopherol | C_{30}H_{52}O | 423 | 59.88 | 0.31 | 3305 | Kl, MS |
| 32  | Tocopherol | C_{30}H_{50}O | 421 | 61.58 | 0.37 | 3305 | Kl, MS |
| 33  | Tocopherol | C_{29}H_{50}O | 426 | 59.89 | 0.32 | 3305 | Kl, MS |
| 34  | Tocopherol | C_{30}H_{52}O | 423 | 59.88 | 0.31 | 3305 | Kl, MS |
| 35  | Tocopherol | C_{30}H_{50}O | 421 | 61.58 | 0.37 | 3305 | Kl, MS |
| 36  | Tocopherol | C_{29}H_{50}O | 426 | 59.89 | 0.32 | 3305 | Kl, MS |
| 37  | Tocopherol | C_{30}H_{52}O | 423 | 59.88 | 0.31 | 3305 | Kl, MS |

*Compounds are listed in order of their elution

\(^a\) Kovats retention index calculated on DB-5 column

\(^b\) Average of three analyses

MS: identification based on mass spectral data; Rt: identification based on published retention indices in Wiley Registry of Mass Spectral Data, 8th edition, mainlib Library, replib Library, NIST Mass Spectral Library (December 2005) and other published data. The major components are highlighted in bold.
The significant increase in serum total cholesterol, triglycerides, LDL-cholesterol and atherogenic index was reverted to normal levels upon treatment with both doses of A. fraxinifolius (p < 0.05 compared with the healthy control group), but the utmost modulation on the changes in serum lipid profiles were shown in APAP groups that received high doses of A. fraxinifolius. The percentages of changes of the serum total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol and atherogenic indices measured in the groups treated with APAP plus either the low or high dose of A. fraxinifolius were 4.8% (M = 68.81) vs 4.4% (M = 68.54), 39.4% (M = 64.92) vs 16.3% (M = 54.14), −5.3% (M = 48.41) vs 51.29% (M = 0.31), 42.3% (M = 7.42) vs 23.1% (M = 6.42), 10.7% (M = 1.42) vs 4.09% (M = 1.34) and 50.4% (M = 0.15) vs 21.9% (M = 0.13), respectively, compared with healthy control group.

Modulatory effects of A. fraxinifolius on serum protein profiles

Figure 4 revealed that healthy rats consumed high dose of A. fraxinifolius showed a significant decrease in the serum triglycerides, LDL-cholesterol and atherogenic index 1 (p < 0.05 to p < 0.01) by −12.5% (M = 40.73), −61.9% (M = 1.99) and −7.3% (M = 1.19), respectively, compared with the healthy control animals. The significant increase in serum total cholesterol, triglycerides, LDL-cholesterol and atherogenic indices (p < 0.001) by 22.6% (M = 80.46), 160.2% (M = 121.10), 213.2% (M = 16.33), 56.9% (M = 2.02) and 298.4% (M = 0.41), respectively, as shown in APAP-intoxicated group compared with the healthy control animals. On the other hand, serum HDL-cholesterol significantly decreased (p < 0.001) by −21.9% (M = 39.91) in APAP-intoxicated group compared with the healthy control rats. Both doses of A. fraxinifolius significantly alleviated (p < 0.05–0.001) the changes in the lipid profiles of APAP rats during the treatment course. These changes in LDL-cholesterol, HDL-cholesterol and atherogenic indices were reverted to normal levels upon treatment with both doses of A. fraxinifolius (p < 0.05 compared with the healthy control group), but the utmost modulation on the changes in serum lipid profiles were shown in APAP groups that received high doses of A. fraxinifolius. The percentages of changes of the serum total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol and atherogenic indices measured in the groups treated with APAP plus either the low or high dose of A. fraxinifolius were 4.8% (M = 68.81) vs 4.4% (M = 68.54), 39.4% (M = 64.92) vs 16.3% (M = 54.14), −5.3% (M = 48.41) vs 51.29% (M = 0.31), 42.3% (M = 7.42) vs 23.1% (M = 6.42), 10.7% (M = 1.42) vs 4.09% (M = 1.34) and 50.4% (M = 0.15) vs 21.9% (M = 0.13), respectively, compared with healthy control group.

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indicating that the high dose of *A. fraxinifolius* exceeded that of low dose in APAP rat model.

**Modulatory effects of *A. fraxinifolius* on serum bilirubin level**

Figure 6 reveals that the significant increase in serum total, direct and indirect bilirubin levels \((p < 0.001)\) by 27.7\% \((M = 1.33)\), –40.4\% \((M = 0.51)\) and 348.4\% \((M = 0.82)\), respectively, as shown in APAP control toxicity group compared with the healthy control animals. Oral treatment of both doses of *A. fraxinifolius* significantly decreased (from \(p < 0.05\) to \(p < 0.001\)) serum total, direct and indirect bilirubin levels compared with APAP control toxicity group. The modulatory effects of high dose of *A. fraxinifolius* on serum total, direct and indirect bilirubin levels were more efficacious than that of low dose of *A. fraxinifolius*. The percentages of changes of serum total, direct and indirect bilirubin levels, compared with the healthy control group, in APAP groups that received either low or high dose of *A. fraxinifolius* were 14.4\% \((M = 1.19)\) vs 10.6\% \((M = 1.15)\), –30.6\% \((M = 0.59)\) vs –27.3\% \((M = 0.62)\) and 226.4\% \((M = 0.59)\) vs 189.0\% \((M = 0.53)\), respectively, compared with the healthy control animals.

**Modulatory effects of *A. fraxinifolius* on hepatic oxidative stress markers**

Healthy rats that consumed high dose of *A. fraxinifolius* showed a significant decrease/increase \((p < 0.05)\) in the hepatic MDA and total antioxidant levels by –8.5\% \((M = 95.21)\) and 17.4\% \((M = 33.33)\), respectively, compared with the healthy control animals (Figure 7). The hepatic MDA and total antioxidant levels significantly increased/decreased \((p < 0.01)\) by 66.5\% \((M = 173.30)\) and –40.4\% \((M = 16.94)\), respectively, in APAP-intoxicated group compared with the healthy control animals. Both doses of *A. fraxinifolius* significantly alleviated (from \(p < 0.05\) to \(p < 0.001\)) hepatic MDA and total antioxidant levels compared with APAP-intoxicated group. In addition, the improvement of hepatic MDA and total antioxidant levels of APAP rats by high dose of *A. fraxinifolius* significantly exceeded that of low dose of *A. fraxinifolius*. The percentages of changes of hepatic MDA and total antioxidant levels, compared with the
healthy control group, in APAP groups that received either 250 or 500 mg/kg of A. fraxinifolius were 27.3% (M = 132.5) vs 9.70% (M = 114.20), and –22.1% (M = 22.13) vs –16.3% (M = 23.78), respectively, compared with the healthy control animals.

All other parameters measured in this study were not significantly altered (p > 0.05) in healthy rats that received either low or high dose of A. fraxinifolius compared with the healthy control animals. Moreover, the mortality rates were zero in all groups treated with A. fraxinifolius. Therefore, no deleterious effects were detected for the dose of A. fraxinifolius used in this study.

**Discussion**

Liver injury induced by APAP was widely used as a model for the screening and studying of hepatoprotective drugs as well as herbal therapies. Other studies demonstrated that hepatocytes are targeted by APAP (Slattery et al. 1987; Gazzani et al. 1989). This study demonstrated that APAP significantly elevated liver relative weight, the serum hepatic enzymes markers, lipid profiles and bilirubin. These results were mainly due to increase and decrease the hepatic MDA concentration and total antioxidant capacity, respectively. In addition, APAP decreased the body weight and serum protein profile compared with the control animals. Elevated lipid peroxidation causes degradation of cellular macromolecules that was concomitant with a reduction of antioxidant system leading to damage of several tissues (Basu et al. 2012). Also, Basu et al. (2012) reported that elevation serum cholesterol and triglyceride levels in APAP treated rats indicated impaired fat metabolism due to hepatic damage. The serum activity of ALAT and ASAT was used as a biochemical marker for early acute liver necrosis. Approximately 5% of the metabolites of APAP is normally converted into NAPQI (toxic agent responsible for the hepatotoxicity) through the action of CYP P450 system in the liver but during overdosing of APAP, detoxification of NAPQI is limited because of insufficient non-enzymic antioxidant system resulting from their depletion (Kaplowitz 2004; Geiger & Howard 2007; Josephy 2005). Serum protein and bilirubin levels are related to the function of the hepatocytes revealing the functional status of the liver. Hepatotoxicity may directly result either from protein damage or by the accumulation of reactive oxygen and nitrogen species (ROS and NOS) (James et al. 2003). All of the above parameters indicating the presence of oxidative injury were markedly reversed by A. fraxinifolius treatment, suggesting that A. fraxinifolius has a potent anti-oxidant effect on APAP hepatotoxicity. Our data indicate that GC-MS analysis of n-hexane extract of A. fraxinifolius leaves revealed the presence of five major compounds: α-tocopherol (18.23%), labda-8 (20)-13-dien-15-oic acid (13.15%), lupeol (11.93%), phytol (10.95%) and squalene (7.19%). This study found that both doses of A. fraxinifolius significantly alleviated the hepatotoxicity of APAP by alleviating the serum hepatocyte integrity markers (ALAT, ASAT and ALP activities), lipid contents, protein and bilirubin levels through increasing/decreasing the production of hepatic total antioxidant capacity/lipid peroxidation, respectively, and increasing the body weight gain. In addition, this study proves the hepatoprotective activity of 500 mg/kg of A. fraxinifolius over 250 mg/kg of A. fraxinifolius, which has not been established before.

**Figure 6.** Serum total, direct and indirect bilirubin levels of control and intoxicated rats. Values are means, with their standard errors represented by vertical bars. A. fraxinifolius: Acrocarpus fraxinifolius; APAP: N-acetyl-p-aminophenol. **(p < 0.01, ** p < 0.001: compared with the healthy control group; † p < 0.05, † † † p < 0.001: compared with the APAP-intoxicated group that received vehicle; (one-way ANOVA with Tukey’s multiple comparison test).**

**Figure 7.** Hepatic MDA concentration (A) and total antioxidant capacity (B) of control and intoxicated rats. Values are means, with their standard errors represented by vertical bars. A. fraxinifolius: Acrocarpus fraxinifolius; APAP: N-acetyl-p-aminophenol; MDA: malondialdehyde. **p < 0.05, ** p < 0.01, *** p < 0.001: compared with the healthy control group; † p < 0.05, † † † p < 0.001: compared with the APAP-intoxicated group that received vehicle; (one-way ANOVA with Tukey’s multiple comparison test).**
α-Tocopherol is an isoform of vitamin E which has a powerful antioxidant activity in detoxifying free radicals, stabilization of the cell membrane and structure restoration (Sodhi et al. 2003). Also Kothekar et al. (2004) showed that α-tocopherol was effective in reverting the hepatotoxicity induced by isoniazid, rifampicin and pyrazinamide combination.

In addition, α-tocopherol is more potent than β-carotene and ascorbic acid in combating ethanol induced hepatic oxidative stress (Datta et al. 2012). Labdane-type diterpenes isolated from Brazilian propolis extract also exerted a significant hepatoprotective activity (Banskota et al. 2008). Lupeol, a pentacyclic triterpenes, was reported to improve the antioxidant status of the liver in cadmium intoxicated rats (Sunitha et al. 2001). The antioxidant and hepatoprotective activities exerted by Ficus pseudopelasma Blanco (Moraceae) extract against paracetamol-induced oxidative damage was attributed to lupeol (Arimado & Santiago 2015). Lupeol, isolated from the petroleum ether extract of Diospyros cordifolia Roxb (Ebenaceae) stem bark, exhibited significant hepatoprotective activity against carbon tetrachloride induced toxic hepatitis (Mankani et al. 2006). Lupeol, exhibited protective effect against 7,12-dimethylbenz(a)anthracene (DMBA) induced hepatotoxicity (Prasad et al. 2008). Regarding squalene, Sivakrishnan et al. (2009) reported about the presence of mainly α-tocopherol and terpenoidal compounds. In general, A. fraxinifolius is considered to be safe herbal medicine with insignificant adverse/side effects on healthy male rats. The low and high doses of A. fraxinifolius used in this study are equivalent to 2.4 and 4.8 g/day/human and did not induce any deleterious effects in healthy rats.

Conclusion
In conclusion, n-hexane extract of A. fraxinifolius having antihepatotoxic properties may reduce the harmful effects generated by APAP-induced hepatotoxicity by preventing lipid peroxidation, and through its modulation on total antioxidant capacity. The alleviating effects of n-hexane extract of A. fraxinifolius on some parameters measured in this study were partial, but significant, and dose dependent. Subsequently, it may be more useful if the dose or time increased in future studies.

Disclosure statement
The authors report that they have no conflicts of interest.

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