



Acacia pennata L. leaves: chemical profiling and impact on DNA damage, alteration of genotoxicity—related genes expression and ROS generation in hepatic tissues of acetaminophen treated male rats

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Abstract

From the phenolic profile of *Acacia pennata* L. leaves hydromethanolic extract, nine flavonoids were tentatively characterized (liquid chromatography-electrospray ionization mass spectrometry), of which Kaempferol 3,7-di-*O*-hexoside, Apigenin 6,8-di-*C*-hexoside, Luteolin-6-*C*-pentoside-8-*C*-hexoside, Apigenin-6-*C*-hexoside-8-*C*-pentoside and Apigenin-6-*C*-pentoside-8-*C*-hexoside were detected for the first time from the plant. Three doses of the plant extract (12.5, 25 and 50 mg/kg bw/d, four weeks treatment) were used to assess its protective effect against acetaminophen induced genotoxicity in hepatic cells of male rats (2 g/kg bw twice per week). *A. pennata* extract and Acetaminophen treated animal groups attenuated DNA damage rates (Comet assay) by 5.6, 6.2 and 6.4% for the three doses, respectively, compared with the acetaminophen treated and untreated groups by 21.2% and 5.8%, respectively. It also decreased DNA fragmentation, significantly down-regulate the expression of p53 tumour suppressor gene and mdr1b multidrug resistance gene [real-time PCR (qPCR)] and ROS generation rates induced by Acetaminophen by 715.4, 453.8 and 376.9% in rats in a dose dependant effect. The results highlighted the DNA protection potential of *A. pennata* phytoconstituents from drug abuse harmful effects.

Keywords *Acacia* · LC–ESI-MS · Comet assay · DNA fragmentation · Gene expression analysis

Introduction

Acacia is the second largest genus of family Leguminosae, comprising more than 1200 species (Sulaiman and Gopalakrishnan 2013). *Acacia pennata* L., is a thorny woody climber distributed in South and Southeast Asia including India, China and Thailand (Lalchhandama 2013). Traditionally, it has been used in rheumatism, headaches and fever

treatment (Nguyen et al. 2018). Several biological reports have been performed on different plant parts, leaves possessed anti-nociceptive, anti-inflammatory (Dongmo et al. 2005) and antifungal effects particularly towards *C. albicans* and *K. polysporus* (Malabadi and Vijay Kumar 2007) as well as anti-transcription activities (Rifai et al. 2010). The twigs were beneficial in the Alzheimer's disease prevention, from which tetracosane and 2-(heptyloxy)-octadecane were isolated and reported to be potent inhibitors of β -amyloid aggregation (Lomarar et al. 2015), whereas the bark showed antioxidant effect (Sowndhararajan et al. 2013).

Previous phytochemical reports on *A. pennata* declared the isolation of flavonoids as apigenin-, quercetin- and kaempferol diglycoside, isorhamnetin mono-glycoside, isovitexin and flavanol glycosides (Dongmo et al. 2007; Kim et al. 2015). Several studies investigated the antioxidant potential of phenolic phytoconstituents and showed their protective effects on cells against reactive oxygen species-induced DNA damage (Lin et al. 2002). ROS are

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often originated as biological reactions by-product or from exogenous factors (Pietta 2000). It can attack lipids in cell membranes and DNA resulting in genotoxicity and several pathological conditions including cancer, arthritis and liver diseases (Kannan and Jain 2000).

Nowadays, in the market over-the-counter analgesic and antipyretic drugs like Acetaminophen are widely consumed and considered safe upon normal drug use (Scaffidi et al. 2002). Acetaminophen (paracetamol), possessing the chemical name *N*-acetyl-*p*-aminophenol (APAP), is the most commonly administered analgesic worldwide (Ennis et al. 2016), with the brand names of Tylenol in US and Panadol in UK (Coulthard et al. 2014). It is also the recommended antipyretic for fever aligned with COVID-19 (Bose et al. 2020). However, long-term exposure to elevated therapeutic dose of Acetaminophen leads to formation of a reactive intermediate *N*-acetyl-*p*-benzoquinone imine (NAPQI) which causes P-450 dependent centrilobular hepatotoxicity (Lee 2003; Park et al. 2005). Furthermore, it is correlated with Acetaminophen-induced tumours in liver tissues of mice (Bessemers and Vermeulen 2001). Since no previous work has been performed on the preventive effect of *A. pennata* leaves against apoptotic DNA fragmentation and damage, thus this study goal was to carry on further phytochemical investigation on *A. pennata* L. leaves extract studying its chemical profile using liquid chromatography–electrospray mass spectrometry (LC–ESI–MS) and perceiving its role in modulation of DNA damage (comet assay and DNA fragmentation), gene expression analysis of tumour suppressor gene (p53) and multidrug resistance gene (*mdr1b*) as stress related genes and ROS generation to counteract Acetaminophen-induced hepatotoxicity in male rats.

Materials and methods

Plant material

A. pennata L. was collected and identified from the Orman Botanic Garden, Giza, in September 2016. A voucher specimen (M126) was deposited in the herbarium of the National Research Centre (NRC).

Extraction

The air-dried powdered leaves of *A. pennata* L. (0.5 kg) were extracted three times with 70% MeOH (3 × 1litre). The solvent was evaporated under reduced pressure at 50 °C to yield APE (25 g).

LC–ESI–MS analysis of *A. pennata*

LC–ESI–MS analysis was performed on a XEVO TQD triple quadrupole instrument and mass spectrometer (Waters Corporation, Milford, MA01757 USA). 10 µL as injection volumes were applied to UPLC instrument with C-18 reverse phase column (ACQUITY UPLC - BEH C18 1.7 µm particlesize – 2.1 × 50 mm column). Handling, processing of the sample for LC–MS analysis and the parameters for analysis were accomplished using negative ion mode as described by Ibrahim et al. (2016). The Mass spectra detection range was between *m/z* 100–1000, using the Maslynx 4.1 software for data processing. Pure flavonoids obtained from Phytochemical and Plant Systematics Department, NRC, Giza, Egypt were used as authentics in this study. Correlation between their fragmentation pattern and mass spectra with the known peaks were performed.

Biological investigation

Experimental animals

Eighty adult albino male rats weighting 100–120 g were obtained from the Animal House Colony of the National Research Centre, Giza, Egypt. They were sustained on standard laboratory diet and water *ad libitum* and kept individually in a filter-top polycarbonate and thermoregulated (23 ± 1 °C) cages, under 12 h dark-light cycle room without chemical contamination. The adaptation period was 1 week prior to commencing experiment, then divided into eight groups (10 rats/ group). Human care was fulfilled to all animals in accordance to the Animal Care and Use Committee guidelines of National Research Centre, Egypt and followed the recommendations of Health Guide for Laboratory Animals care and usage No. MP (1899).

Experimental design

The animals were classified as the following for 4-weeks period treatments: Group (1) untreated group (Control); Groups (2–4) were treated orally with low, medium and high doses of *A. pennata* hydroalcoholic extract (APE-L, APE-M and APE-H) (12.5, 25 and 50 mg/kg bw/day) which were equivalent to 0.1, 0.2 and 0.4 of its LD₅₀, respectively (Khare 2016); Group (5) was treated orally with Acetaminophen (2 g/kg bw twice per week), with a dosing interval every 84 hours (Kannan et al. 2013); Groups (6–8) were treated with APE (similar to those in groups 2–4) combined with acetaminophen (2 g/kg bw twice per week).

Sample preparation

At the twenty-fourth hour after 4 weeks treatment, all experimental animals were anesthetized and then sacrificed by decapitation. The liver tissue samples were then isolated and stored at $-80\text{ }^{\circ}\text{C}$ for determining the reactive oxygen species, gene expression analysis of p53 tumour suppressor and *mdr1b* multidrug resistance genes, DNA fragmentation and comet assay as described by Linjawi et al. (2017).

Statistical analysis

General Linear Models (GLM) method was applied for data analysis, then Scheffé-test for the significant differences assessment between groups. The software was SAS, Version 9.1 (Statsoft Inc., Tulsa, USA). The values were expressed as mean \pm SEM with significance established on probability of $P < 0.05$.

Comet assay

Isolated liver tissues of all groups of rats were exposed to the modified single-cell gel electrophoresis or comet assay (Olive and Banáth 2006), the liver tissues were subjected to several washings, lysis, denaturation of the DNA, electrophoresis, staining and examination under fluorescence microscope (Lu et al. 2002). About 100 cells were examined for each animal which appeared like comets for determining the percentage of cells with DNA damage.

Effect of APE on ROS generation

Intracellular ROS generation was measured in liver tissues as described by El-Hagrassy (2017), after single-cell suspensions were made (Cetica et al. 2001).

Gene expression analysis

Isolation of total RNA

Total RNA was extracted from male rats liver tissues by using TRIzol® Reagent (Invitrogen, Germany) as claimed

by the manufacturer’s instructions with minor modifications and in accordance with the method described by Linjawi et al. (2017).

Reverse transcription (RT) reaction

Reverse transcription of the isolated RNA was applied to synthesize the complementary DNA (cDNA). The reaction volume of 20 μL was prepared in accordance to the instructions of the RevertAid™ First Strand cDNA Synthesis Kit (MBI Fermentas, Germany). An amount of total RNA (5 μg) was used with a master mix as described by Linjawi et al. (2017). Then, it was followed by centrifugation for 30 sec. and transferred to the thermocycler. The RT reaction was carried out in 3 consecutive steps. The reaction tubes were then placed in an ice chamber to be used for cDNA amplification via quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) (El-Baz et al. 2016).

QIAGEN’s real-time polymerase chain reaction (Qrt-PCR)

Qrt-PCR cyler (Rotor-Gene Q, USA) was applied for determination of the number of liver cDNA copy. PCR were configured in 25 μL reaction mixtures containing 12.5 μL 1 \times SYBR® Premix Ex Taq™ (TaKaRa, Biotech. Co. Ltd.), 6.5 μL distilled water, 0.5 μL 0.2 μM antisense primer, 0.5 μL 0.2 μM sense primer and 5 μL of cDNA template. The reaction strategy was processed in 3 steps (Linjawi et al. 2017) with distilled water control in each inspection. A melting curve analysis was applied at 95 $^{\circ}\text{C}$ after each qrt-PCR to inspect the used primers quality. The sequences of specific primer of the genes used are listed in Table 1.

Calculation of gene expression

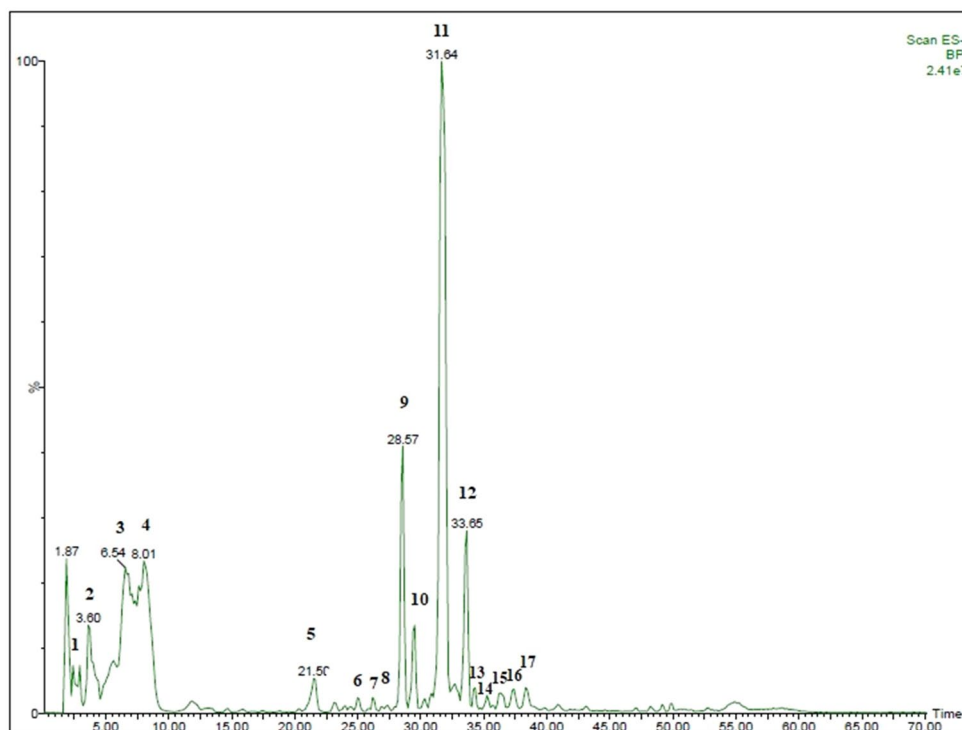
The relative quantification of the target to the reference was determined by using the $2^{-\Delta\Delta\text{CT}}$ method (Kassem et al. 2016).

Table 1 Primers sequences used

Target cDNA	Primer sequence (R)	Primer sequence (F)
p53	5'-GGG CTC GAG TCT AGA CTT TTG AGA AGC -3'	5'-GCG GTA CCC CAG GTC GGC GAG AAT CC-3'
<i>mdr1b</i>	5'-GGT TTC ATG GTC GTC GTC TCT TGA-3'	5'-GAA ATA ATG CTT ATG AAT CCC AAA-3'
β -actin	5'-CTC TTT GAT GTC ACG CAC GAT TT-3'	5'-GTG GGC CGC TCT AGG CAC CAA-3'

F forward primer, R reverse primer

Fig. 1 LC–ESI-MS chromatogram of APE



DNA fragmentation

The laddering pattern of nuclear DNA was perceived to qualitatively analyse the apoptotic DNA fragmentation. According to conditions and methods described by Lu et al. (2002) which included extraction of the DNA, lysis, precipitation, centrifugation and then electrophoresis by using a molecular size marker (100-bp DNA ladder, Invitrogen, USA). Finally, the agarose gel was exposed to ultraviolet transillumination for visualization and photographing the DNA fragments.

Results

LC-mass of APE analysis

The LC–ESI-MS chromatogram of APE is presented in Fig. 1. The identity, retention time, observed fragment ions and deprotonated molecular ion for each compound were presented in Table 2. The chromatogram showed the presence of 17 peaks from which 9 compounds were tentatively identified through comparison of their retention times and fragmentation pattern with standard and with the available literature data.

Assessment of DNA damage

DNA damages of treated male rats with acetaminophen (2 g/kg bw), APE (12.5, 25 and 50 mg/kg bw/d, four weeks

treatment) and APE with acetaminophen (2 g/kg bw) were detected by comet assay shown in Table 3 which provides a highly sensitive indicator of DNA strand breaks in individual mammalian cells (Wong et al. 2005). The DNA damage increased by 21.2% in rats treated with acetaminophen. APE-L, APE-M and APE-H decreased the rates of DNA damages to 18.6, 15.2 and 11.8%, respectively when combined with acetaminophen, compared with that of untreated control group 5.8%. Moreover, the DNA damage rates in rats treated with APE-L, APE-M and APE-H were 5.6, 6.2 and 6.4%, respectively which were relatively similar to that of untreated control group. The results revealed that APE at the three different doses (12.5, 25 and 50 mg/kg bw/d) significantly lowers these damages induced by acetaminophen by $P < 0.05$, < 0.05 and < 0.01 for the low, medium and high doses, respectively (Table 3).

DNA fragmentation

DNA fragmentations in rats treated with different doses of APE (12.5, 25 and 50 mg/kg bw/d, for 4 weeks treatment) were relatively equivalent to that in the control group where low DNA bands were observed (Fig. 2). Acetaminophen treatment (2 g/kg bw) increased DNA fragmentation after observation of several DNA bands as compared to the control group.

Table 2 LC–ESI-MS analysis of identified phenolic compounds of *Acacia pennata*

Peak no.	R _t (min)	M	[M-H] ⁻	Fragment signals (m/z)	Tentative identification
1	2.00	274	273	159, 131	Unknown
2	3.63	318	317	79	Unknown
3	6.54	195	194	97, 80	Unknown
4	8.01	195	194	97, 80	Unknown
5	21.496	594	593	285, 152, 108	Kaempferol 3- <i>O</i> -rhamnopyranosyl-(1→4)-glucopyranosyl ^a
6	24.96	440	439	255, 167, 153	Unknown
7	26.169	610	609	285, 447	Kaempferol 3,7-di- <i>O</i> -hexoside ^b
8	27.371	610	609	301, 447	Quercetin 4'- <i>O</i> -α-L-rhamnopyranosyl-3- <i>O</i> -β-D-allopyranoside ^a
9	28.573	594	593	503, 473, 383, 353	Apigenin 6,8-di- <i>C</i> -hexoside ^{b,c}
10	29.374	580	579	519, 489, 459, 399, 369	Luteolin-6- <i>C</i> -pentoside-8- <i>C</i> -hexoside ^b
11	31.777	564	563	503, 473, 443, 383, 353	Apigenin-6- <i>C</i> -hexoside-8- <i>C</i> -pentoside (vicenin isomer) ^{b,c}
12	33.646	564	563	503, 473, 443, 383, 353	Apigenin-6- <i>C</i> -pentoside-8- <i>C</i> -hexoside ^{b,c}
13	34.314	564	563	503, 473, 443, 383, 353	Apigenin 6- <i>C</i> -hexoside-8- <i>C</i> -pentoside (vicenin isomer) ^{b,c}
14	35.248	564	563	503, 473, 443, 383	Apigenin 6- <i>C</i> -pentoside-8- <i>C</i> -hexoside ^{b,c}
15	36.31	712	711	563, 411, 217	Unknown
16	37.38	364	363	199, 155, 111	Unknown
17	38.45	416	415	393, 199, 155	Unknown

^aPreviously isolated from *A. pennata*

^bWere detected for the first time in *A. pennata*

^cThe retention times and fragmentation pattern were compared to authentics

In contrast, the DNA fragmentation was decreased clearly in groups treated with low, medium and high doses of APE combined with acetaminophen compared with acetaminophen treatment alone. Moreover, the lowest DNA fragmentation induced by acetaminophen was observed in rats treated with high dose of APE (50 mg/ kg bw/d for 4 weeks) combined with acetaminophen (2 g/kg bw).

Gene expression analysis of p53 and mdrlb genes

The qRT-PCR analysis results of p53 (tumor protein gene) and mdrlb (a multidrug resistance gene) isolated from liver tissues of male rats treated with acetaminophen alone (2 g/kg bw) or combined with APE (12.5, 25 and 50 mg/ kg bw/d) are presented in Fig. 3. Acetaminophen increased significantly the mRNA expression values of p53 and mdrlb

by 372.7 and 293.4%, respectively compared with control group. Treatment with APE exhibited relatively similar expression for the gene expression values as compared to control group, there were significant down regulation in the mRNA expressions of p53 and mdrlb genes after treatment of rats groups with low, medium and high doses of APE (APE-L, APE-M and APE-H) combined with acetaminophen, respectively. Moreover, the lowest expressions of p53 and mdrlb genes were observed after treatment with high doses of APE combined with acetaminophen (Fig. 3).

ROS generation

Protective effect of APE against ROS generation in acetaminophen treated rats is presented in Fig. 4. Treatment

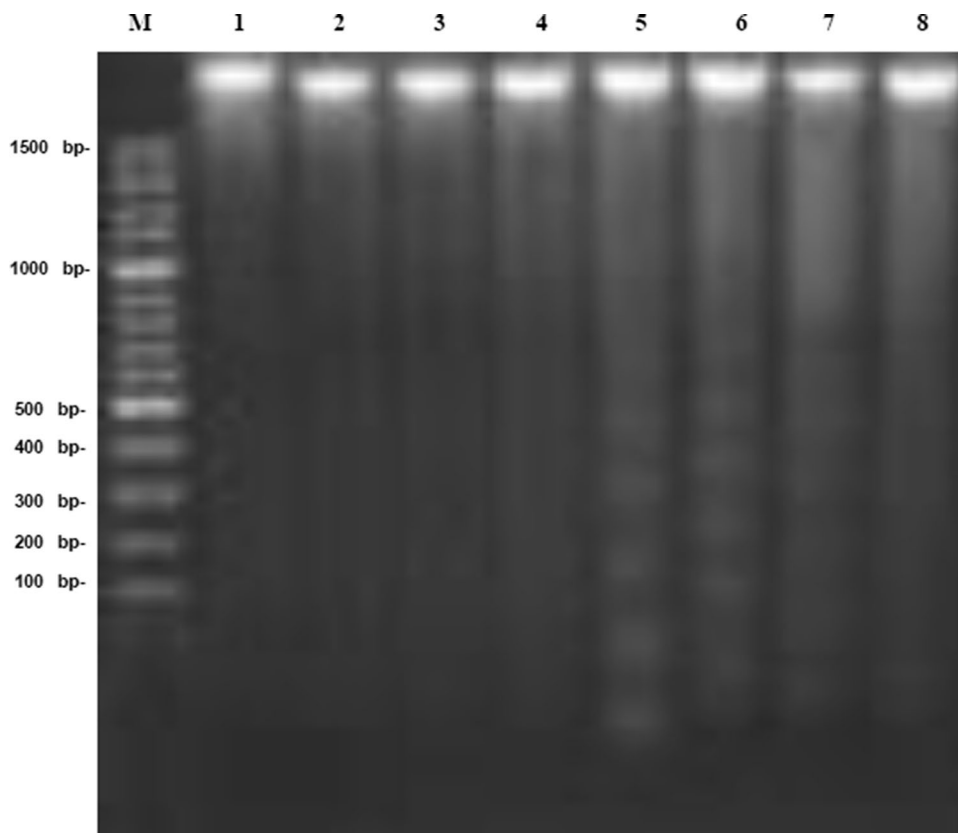
Table 3 Rate of DNA damage in liver tissues of male rats exposed to Acetaminophen alone or combined with APE using comet assay

Treatment analyzed	Number of animals	No. of cells		Class ^a of comet				DNA damaged cells (%)
		Analyzed ^b	Total comets	0	1	2	3	
Control	5	500	29	471	22	7	0	5.8
APE-L	5	500	28	472	20	8	0	5.6
APE-M	5	500	31	469	23	8	0	6.2
APE-H	5	500	32	468	19	7	6	6.4
Acetaminophen	5	500	106	394	31	33	42	21.2
APE-L + Acetaminophen	5	500	93	407	25	31	37	18.6
APE-M + Acetaminophen	5	500	76	424	26	23	27	15.2
APE-H + Acetaminophen	5	500	59	441	21	18	20	11.8

^aClass 0=no tail, 1=tail length < diameter of nucleus, 2=tail length between 1X and 2X the diameter of nucleus, and 3=tail length > 2X the diameter of nucleus

^bNo. of cells analyzed were 100 per an animal

Fig. 2 DNA fragmentation in liver tissues of male rats treated with acetaminophen alone or combined with APE. M: represents DNA ladder. Lane 1: represents control rats. Lanes 2–4: rats were treated orally with low, medium and high doses of APE, respectively. Lane 5: rats were treated with acetaminophen (2 g/kg bw). Lanes 6–8: rats were treated with APE (similar to those in lanes 2–4) and combined with acetaminophen



with acetaminophen increased intracellular ROS generation by 796.27% as compared to the control group. Whereas low rates of ROS generation which were relatively similar to control were observed in rats treated by

different doses of APE. Furthermore, APE significantly reduced the ROS generation rates induced by acetaminophen by 715.4, 453.8 and 376.9%, respectively for the three different doses used.

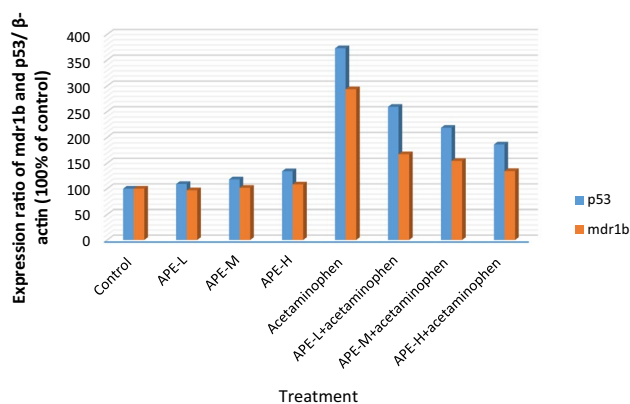


Fig. 3 The alterations of p53 and mdr1b genes in liver tissues collected from male rats treated with acetaminophen alone or combined with APE. Data are presented as mean \pm SEM

Discussion

LC-MS is a valuable and convincing approach to provide a chromatographic fingerprints which elucidate the chemical profile in the plant extract for biological studying. It is an ideal solution to detect and identify constituents in the plant extract that could not be discerned due to their minority. In this study, flavonoids were found to be the major structure types in APE chromatogram where seven compounds were detected for the first time in the leaves of *A. pennata* along with kaempferol 3-*O*-rhamnopyranosyl-(1 \rightarrow 4)-glucopyranosyl and quercetin 4'-*O*- α -L-rhamnopyranosyl-3-*O*- β -D-allopyranoside were previously isolated.

In APE chromatogram (Fig. 1) Peak 5 showed a quasi-molecular ion peak at m/z 593 [M-H]⁻. The fragment ion of kaempferol aglycone at m/z 285 is due to the loss of glucose and rhamnose units attached to the same position of the aglycone [M-H-162-146]⁻. Therefore, it was tentatively identified as kaempferol 3-*O*-rhamnopyranosyl-(1 \rightarrow 4)-glucopyranosyl previously isolated from the leaves of *A. pennata* (Dongmo et al. 2007; Lomarat et al. 2015). Peak 7 showed a deprotonated molecular ion [M-H]⁻ at m/z 609. The main fragment at m/z 447 is assigned to the loss of one hexose unit [M-H-162]⁻ as ether linkage, while that at m/z 285 of kaempferol aglycone indicates the loss of two hexose units [M-H-2 \times 162]⁻. Therefore, it was identified as kaempferol 3,7-di-*O*-hexoside (El-Hagrassy et al. 2017). Peak 8 showed a molecular ion [M-H]⁻ at m/z 609. The fragment at m/z 447 is corresponding to loss of one hexose unit [M-H-162]⁻, while that at m/z 301 of quercetin aglycone indicating further loss of the rhamnosyl moiety [M-H-146-162]⁻. Therefore, it was identified as quercetin 4'-*O*- α -L-rhamnopyranosyl-3-*O*- β -D-allopyranoside

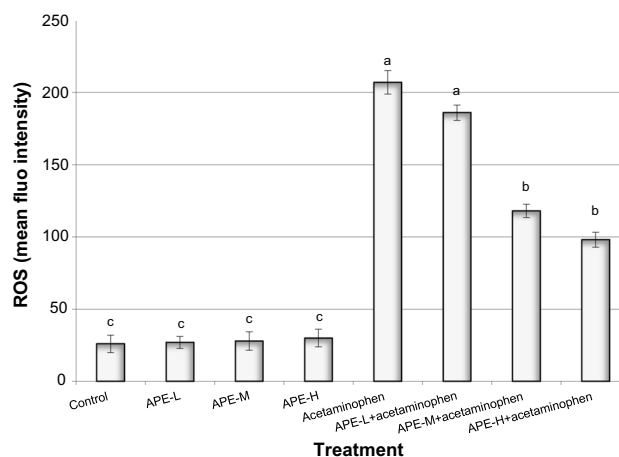


Fig. 4 The intracellular ROS level alterations in liver tissues of male rats treated with acetaminophen alone or combined with APE. Data are presented as mean \pm SEM. ^{a,b,c}Mean values within tissue with unlike superscript letters were significantly different ($P < 0.05$)

previously isolated from the leaves of *A. pennata* (Dongmo et al. 2007). Apigenin-6,8-di-*C*-hexoside was identified in peak (9) at m/z 593 from its (-90 amu) and (-120 amu) losses appearing at m/z 503 and 473, respectively and the characteristic fragment ions at: m/z 383 (Aglycone + 113) and m/z 353 (Aglycone + 83) which were confirmed by comparing with standard. Peak 10 showed a quasi-molecular ion at m/z 579. It showed the characteristic fragments of luteolin di-*C*-hexosides at m/z : 369 [Aglycone + 83]⁻ and 399 [Aglycone + 113]⁻. The fragmentation pathway of flavonoid-*C*-glycoside includes cross-ring cleavages [(O-C1 and C2-C3) or [(O-C1 and C3-C4)] of the sugar units, namely, [M-120/90]⁻ for *C*-hexosides, [M-90/60]⁻ for *C*-pentosides. Therefore, the fragment ions at m/z : 519 [M-60-H]⁻, 489 [M-90-H]⁻, and 459 [M-120-H]⁻ confirmed the identification of peak 10 as luteolin-6-*C*-pentoside-8-*C*- β -D-hexoside isomers (Hussein et al. 2018). Peaks 11–14 showed similar molecular ion at m/z 563 [M-H]⁻. Their fragmentation patterns presented fragment ions at m/z : 503, [M-H-60]⁻, 473 [M-H-90]⁻, 443 [M-H-120]⁻, 383 [M-H-120-60]⁻, 353 [M-H-120-90]⁻. These fragments are characteristics of a hexosyl and pentosyl residues. Therefore, peaks 11, 12, 13 and 14 are isomers of apigenin with hexosyl and pentosyl linked to the 6 and 8 positions and their retention time and fragmentation pattern were compared with standards. The type of substitution of a sugar at the 6 and 8 positions of 6,8-di-*C*-hexosylflavones can be determined using an ion trap and triple quadrupole mass analyzers. These techniques showed the sugar moiety favoured fragmentation at position 6 rather than position 8 (Benayad et al. 2014). In the current study, the two isomers were ascertained by comparing the relative abundance of m/z 473 and 443 fragments. Considering the favoured fragmentation at the *C*-6 position, the fragment ions observed at m/z

503 [M–H-60][−] and 473 [M–H-90][−] assigned to the pentose unit showed a relative lower abundance in the spectra of peaks 11 and 13, suggesting the pentose presence at position 8. The characteristic fragment ions of a hexose moiety at *m/z* 443 [M–H-120][−] and 353 [M–H-90-120][−] were observed with a higher intensity indicating a 6-position substitution. Therefore, peaks 11 and 13 were identified as two isomers of apigenin 6-*C*-hexoside-8-*C*-pentoside. The reverse was the case of peaks 12 and 14, where the [M–H-120][−] peak of hexose moiety was observed at lower intensity than that of pentose moiety [M–H-90][−] and [M–H-60][−]. Therefore peaks 12 and 14 were tentatively identified as isomers of apigenin 6-*C*-pentoside-8-*C*-hexoside (Benayad et al. 2014). Taking into account the previously reported data, peak 11 and 13 were tentatively identified as apigenin-6-*C*-hexoside-8-*C*-pentoside (vicenin isomers) and from the close match of the obtained data and the retention time with that published which was measured under the same conditions (Hussein et al. 2018).

The LC–ESI-MS analysis revealed that flavonoids were the major phytoconstituents present in the leaves of *A. pennata* which is in alignment with the previous phytochemical reports (Kim et al. 2015; Lomarat et al. 2015) and this could probably be the main reason of its DNA protection effect as revealed from the current study that APE possess the potential to counteract the genotoxic effect of acetaminophen on liver tissues, while acetaminophen treatment revealed significant upregulation in tumour suppressor gene (p53), multidrug resistance (mdr1b) gene (Fig. 3) and ROS generation (Fig. 4) which were significantly downregulated by APE. Gene p53 is normally present at low level, its activation indicates the occurrence of DNA damage resulting in its elevated cellular level and its binding ability to DNA, activating a set of genes triggering DNA repair (Siddik 2003). Thus it tended to enhance genomic stability by allowing repair of the damaged DNA (Campisi 2005). Whereas multiple drug resistance (mdr) genes are responsible for xenobiotics efflux from cells by encoding P-glycoprotein. The results of this study is in agreement with the previous report that mdr1b mRNA level increased in rat liver after administration of chemotherapeutic drugs (Brady et al. 2002). The possible mechanism by which APE protected the liver tissue from the genotoxic effect of acetaminophen is by lowering ROS generation and initiating DNA repair.

This DNA protection effects of APE were also evidenced by lowering the DNA fragmentation induced by acetaminophen (Fig. 2). The genoprotective effects could be attributed to the flavonoids present in the leaves as they are well known by possessing a broad spectrum antioxidant effect reacting with superoxide anion, alkoxyl, peroxy and hydroxyl radicals (Han and Andrade 2005). Flavonoids also mitigate oxidative-stress DNA damage and arrest cellular lipid peroxidation (Duthie et al. 2000). The presence of the flavonoid analogues, apigenin and kaempferol could have a

role in the DNA protection effect as these analogues prevent the damage resulting from intracellular ROS generation (Lin et al. 2002). Kaempferol decreases oxidative DNA damage in isolated human lymphocytes (Birt et al. 2001), also the presence of vicenin isomers could probably play a crucial role as they are efficient free radical scavengers, in addition to enhancement of DNA repair through its high antioxidant capacity, it significantly scavenge DPPH and ABTS. Vicenin also significantly reduced radiation-induced DNA damage in mice splenocytes and allowed for its faster repair (Satyamitra et al. 2014). Pretreatment with vicenin along with orientin significantly reduced all type of DNA-aberration induced by different factors as radiation, cytotoxic medications and smoking. Thus, protecting mouse bone marrow against different factors inducing chromosomal abrasion and stem cell death (Nayak and Devi 2005).

Conclusions

The study is considered the first report for the chemical LC–ESI-mass profiling of *A. pennata* L. leaves extract which revealed the presence of nine flavonoids seven of which were tentatively identified for the first time. The extract exerted a potential effect on modulation of the apoptotic DNA damage and fragmentation, induced significant down-regulation of p53 and mdr1b-genotoxicity related genes and protected cells from oxidative stress in acetaminophen treated rats. The results revealed that *A. pennata* L. leaves extract could improve acetaminophen therapeutic index by increasing the capability of the liver tissue to withstand or even repair the molecular damage caused by toxic intermediates.

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Compliance with ethical standards

Ethical statement Ethical ideologies and rules for Scientific Experiments on Animals originated by guidelines of the Animal Care and Use Committee of National Research Centre, Egypt and the recommendations of Health Guide for care and use of Laboratory Animals No. MP (1899) were strictly followed.

Conflict of interest Eman M. M. El-Taher has no conflict of interest. Moshera M. El_Sherei has no conflict of interest. Riham Salah El Dine has no conflict of interest. Dina M.Y. ElNaggar has no conflict of interest. Wagdy K. B. Khalil has no conflict of interest. Salwa M. Kassem has no conflict of interest. Ahmed Elkhateeb has no conflict of interest. Mona E. S. Kassem has no conflict of interest.

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