Antihepatotoxic Effect and Metabolite Profiling of *Panicum turgidum* Extract via UPLC-qTOF-MS

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Submitted: 09-01-2016 Revised: 10-02-2016 Published: 30-09-2016

ABSTRACT

Background: *Panicum turgidum*, desert grass, has not reported any detailed phytochemical or biological study as yet. Objective: To establish *P. turgidum* secondary metabolite profile and to assess its antihepatotoxic effect. Materials and Methods: Ultra-performance liquid chromatography (UPLC) coupled to quadrupole high-resolution time of flight mass spectrometry (qTOF-MS) was used for large-scale secondary metabolites profiling in *P. turgidum* extract, alongside assessing median lethal dose (LD₅₀) and hepatoprotective effect against carbon tetrachloride (CCl₄) intoxication. Results: A total of 39 metabolites were identified with flavonoids as the major class present as O/C-glycosides of luteolin, apigenin, isorhamnetin and naringenin, most of which are first time to be reported in *Panicum* sp. Antihepatotoxic effect of *P. turgidum* crude extract was revealed via improving several biochemical marker levels and mitigation against oxidative stress in the serum and liver tissues, compared with CCl₄ intoxicated group and further confirmed by histopathological examination. Conclusion: This study reveals that *P. turgidum*, enriched in C-flavonoids, presents a novel source of safe antihepatotoxic agents and further demonstrates the efficacy of UPLC-MS metabolomics in the field of natural products drug discovery.

SUMMARY

- UPLC coupled to qTOF-MS was used for large scale secondary metabolites profiling in *P. turgidum*.
- A total of 39 metabolites were identified with flavonoids amounting as the major metabolite class.
- Anti-hepatotoxic effect of *P. turgidum* extract was revealed via several biochemical markers and histopathological examination.
- This study reveals that *P. turgidum*, enriched in C-flavonoids, present a novel source of antihepatotoxic agents.

INTRODUCTION

*Panicum turgidum* Forssk. (fam. Poaceae), known as desert grass by natives in Egypt, is a common perennial bunchgrass from Mauritania and Senegal eastwards through the Sahara and Sahel to Sudan, Ethiopia, Eritrea, and Somalia and through northern Africa and western Asia to Pakistan and India, and very common in the Mediterranean and Red Sea coastal regions of Egypt. Previous studies on *Panicum* genus revealed the presence of steroidal saponins, phenolics including caffeic acid, ferulic acid, p-coumaric acid, p-hydroxybenzoic acid, protocatechuic acid, quercetin-3-O-rhamnose, and rutin flavonoids. With regard to biological effect of its members, investigation of antioxidant activity, diuretic effect, antidiabetic activity, and antiinflammatory and antipyretic properties has been reported. Albeit, no previous detailed study of *P. turgidum* either on its phytochemical composition or its biological effect has been reported.

In addition, complete secondary metabolites profile in plants belonging to *Panicum* genus is still lacking, with most studies based on a targeted type of analysis. An essential purpose of the scientific discipline of metabolomics is the study of the biochemical composition of living organisms in a rather holistic manner. The current progress in plant metabolomics techniques has made it possible to profile plant crude extracts in a rather untargeted manner. Metabolomics makes the use of hyphenated techniques that rely on chromatographic separation of metabolites coupled to mass spectrometry (MS) to analyze complex mixtures. Considering flavonoid known antihepatotoxic effects and in search for other resources of active hepatoprotective agents, *P. turgidum* was assessed herein and results from this study provides a new evidence for its possible use as an adjunct therapy for alleviating acute and chronic...
MATERIALS AND METHODS

Plant material and extraction process

The panic grass (Panicum turgidum) used in this study was grown wild in Wadi-Habes, 18 km Mersa-Matruh, Egypt. The plant was collected on April 2012 and identified by Dr. Attia Mohamed, Desert Research Center, Egypt (herbarium specimen no., 5/3/2015-B, Fac. of Pharmacy, Cairo Univ.). Freeze-dried aerial parts of the plant were ground with a pestle in a mortar using liquid nitrogen. The powder (20 mg) was then homogenized with 4 ml 70% ethanol containing 10 µg/ml umbelliferone (internal standard) using an ultrasonic bath for 30 min. Extract was then vortexed and centrifuged at 10,000 g for 10 min to remove plant debris and filtered through 22 µm Millipore filter and stored at −20°C for high-resolution Ultra-performance liquid chromatography (UPLC–PDA–MS (UPLC/ photodiode array detection/MS) analysis. For biological assays, dried, powdered aerial parts of the plant were extracted with 70% ethanol till exhaustion.

Animals

Adult male Sprague-Dawley rats weighing 200–250 g, aged from 10 to 16 weeks, were purchased from the Lab. animal colony, Ministry of Health and Population, Helwan, Cairo, Egypt and maintained under standard laboratory conditions at the animal center, College of Pharmacy, Al-Azhar Univ., Cairo, Egypt. Rats were fed with basal diet pellets, supplied with water ad libitum, and kept in a temperature-controlled environment (20–22°C) and 40–60% relative humidity with an alternating 12-h light–dark cycle. The experiments were conducted in accordance with the ethical guidelines for investigations in laboratory animals and comply with the Guidelines for the Care and Use of Laboratory Animals.

Chemicals and drugs

Acetonitrile and formic acid (LCMS grade) were obtained from J.T. Baker (Deventer, Netherlands), milliQ water was used for LC analysis. Umbelliferone (99.8%) (St. Louis, MO, USA), Silymarin, Ellman’s reagent, thiobarbituric acid (TBA), reduced glutathione reductase (GSH), catalase (CAT), 1,1,3,3-tetraethoxypropane, pyrogallol, trichloroacetic acid (TCA) and EDTA-Na<sub>2</sub> were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA), while (CCl<sub>4</sub>) was obtained from Loba-chemie (India). Neutral formalin, HCl, n-butanol, α-phosphoric acid, potassium chloride, sodium chloride, sodium diabasic phosphate were obtained from El-Nasr Pharm. Chem. Co. (Cairo, Egypt). anhydrous potassium hydrogen phosphate and potassium dihydrogen phosphate were purchased from Fluka Lab. Chem. and Anal. Reagents Co. (Steinheim, Germany), and H<sub>2</sub>O<sub>2</sub> was provided from Fisher scientific (Fair lawn, NJ, USA.).

Analysis of plant extracts using UPLC/PDA/MS

UPLC/PDA/high resolution TOF-MS

Chromatographic separations were performed using an Acquity UPLC system (Waters) equipped with a HSS T3 column (100–1.0 mm, particle size 1.8 µm; Waters). The following elution binary gradient was used at a flow rate of 150 µL min<sup>−1</sup>: 0 min, 100% A (water/formic acid, 99:0.1 v/v), 5% B (acetonitrile/formic acid, 99:0.1 v/v), 1–16 min, linear from 5–95% B; 16–18 min, isocratic 95% B; 18–20 min, isocratic 5% B. Eluted compounds were detected from m/z 100 to 1000 using a MicroTOF-Q hybrid quadrupole time-of-flight mass spectrometer (BrukerDaltonics) equipped with an Apollo-II electrospray ion source in negative ion modes using the following instrument settings: nebulizer gas, nitrogen, 1.6 bar; dry gas, nitrogen, 6 l min<sup>−1</sup>; capillary, 5500 V (+4000 V); end plate offset, 500 V; funnel 1 RF, 200 Vpp.

Identification of metabolites

UPLC–MS files were converted to netcdf file format using the File Converter tool in BrukerDaltoniks software and further processed using AMDIS software. Metabolites were characterized by their UV–vis spectra (220–600 nm), retention times, mass spectra, and searching the phytochemical dictionary of natural products database and reference literature.

Biological assays

Acute and subchronic toxicity

Lethality test (LD<sub>50</sub>) was estimated in mice orally, according to OECD guidelines No. 420. In a preliminary test, four groups of each 5 animals received the following different doses of the tested extract suspended in the vehicle (3% v/v Tween 80): 5, 50, 300 and 2000 mg/kg b.wt. Animals were observed for 24 h for signs of toxicity and number of deaths. From these results, extra doses of 5, 7.5, and 15 g/kg b.wt. of the tested extract were administered. Control animals only received the vehicle and were kept under the same conditions without any treatments. Signs of toxicity and number of deaths per dose in 24 h were recorded and the LD<sub>50</sub> was calculated as the geometric mean of the dose that results in 100% mortality, which causes no lethality. Further, 10 mature rats of both sex (200–250 g) were divided into two groups, for a chronic type toxicity study, each made up of 5 animals. In the first group, control rats received the vehicle, whereas second group was orally administered P. turgidum ethanol extract (500 mg/kg b.wt.). Extracts were administered to animals orally using gastric intubation for a period of 4 weeks.

Hepatoprotective activity

A total of 40 rats were divided into four groups, ten animals each, and the schedule of treatment was adopted as follows: Group 1: rats were given corn oil (2 ml/kg b.wt., s.c) twice a week for two consecutive weeks and served as negative control group. Group 2: rats were injected CCl<sub>4</sub> (2 ml/kg b.wt., s.c) diluted in corn oil (50% v/v) twice a week for two consecutive weeks. Group 3 and 4: rats were treated with silymarin orally (50 mg/kg b.wt.) and alcohol extract of P. turgidum orally (500 mg/kg b.wt.), respectively, daily for three consecutive weeks and injected CCl<sub>4</sub> (2 ml/kg b.wt., s.c) diluted in corn oil (50% v/v) twice a week for two consecutive weeks (starting from the 2<sup>nd</sup> week of study). Plant extract and silymarin drug were each administered orally to the animals by...
gastrointestinal intubation for 3 weeks following the module described by Dutta et al., 2013.[15]

Serum and tissue preparation
Blood samples were collected from retro-orbital venous plexus under light ether anesthesia in nonheparinized tubes and the serum was separated for measuring different biochemical parameters. Animals were later killed and the liver sample was divided into two portions: first was formalin fixed for histopathological examination and the second part was homogenized for the determination of liver malondialdehyde (MDA) and glutathione reductase (GSR) levels as well as catalase (CAT), and superoxide dismutase (SOD) enzymatic activities. Kidney samples for the chronic toxicity module were also maintained for histopathological examination.

Biochemical analysis
Serum was carefully separated into clean dry Eppendorf tubes using a Pasteur pipette and used for the determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea and creatinine.[16] total bilirubin and direct bilirubin, total proteins, albumin, HDL-cholesterol, total cholesterol, and triglycerides.[17] using colorimetric Spectrum Diagnostics and Diamond kits (Cairo, Egypt). The liver homogenate was centrifuged at 5000 rpm for 10 min and the resulting supernatant was used for estimation of lipid peroxides MDA determination, GSH, CAT, and SOD activities.[18]

Histopathological examination
Autopsy samples were taken from the liver and kidney of rats in different groups of both models and fixed in 10% neutral buffered formalin for 24 h and decalcification was done in formic acid. Washing was performed with water, and then serial dilution of alcohol (methyl, ethyl, and absolute ethyl) was used for tissue dehydration. Specimens were cleared in xylene and embedded in paraffin at 56°C in a hot air oven for 24 h. Paraaffin bees wax tissue blocks of 4-μm thickness were prepared for sectioning by sledge microtone. The obtained tissue sections were collected on glass slides, de-paraffinized, and stained by hematoxylin and eosin stain for examination under light electric microscope.[19]

Statistical analysis of data
All data are presented as mean ± SE. Statistical analysis was performed using GraphPad prism version 5 (GraphPad, San Diego, CA). Group differences were analyzed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer for multiple comparison tests. The difference was considered significant when P ≤ 0.05.

RESULTS

Chemical analysis
Chemical constituents of Panicum turgidum Forssk were analyzed by reversed phase UPLC/PDA/ESI-qTOF-MS, using a gradient mobile phase consisting of acetoniitile and aqueous formic acid that allowed for a comprehensive elution of plant analytes, that is, flavonoids and fatty acids within 17 min (ca. 1000 s). MS base peak intensity chromatogram of P. turgidum is presented in Figure 1. The identities, retention times, Ultraviolet (UV) characteristics, and observed molecular and fragment ions for 39 identified peaks are presented in Table 1.

![Figure 1: Representative UPLC-MS trace of P. turgidum methanol extract characterized by two main regions: (220–420 s) with peaks principally due to flavonoid conjugates and a region (420–940) for fatty acids. Peak numbers follow those listed in Table 1 for metabolite identification using UPLC–UV–MS](image)

Table 1: Peak assignments of metabolites in P. turgidum 70% ethanol extract using UPLC–PDA–MS in negative ionization mode

| Peak | Rt (s) | UV | (M-H)- (m/z) | Formula | Error (ppm) | MSn product ions | Identification |
|------|--------|----|-------------|---------|-------------|----------------|---------------|
| 1    | 222.2  | 269.4, 347.3 | 609.1441 | C_{27}H_{29}O_{16} | 3.7 | 591, 519, 489, 399, 369 | Luteolin 6,8 di-C-glucoside |
| 2    | 225.8  | 268.9, 343.8 | 431.1899 | C_{20}H_{14}O_{10} | 4.7 | 311,341 | Vitexin (apigenin-8-C-hexoside) |
| 3    | 228.2  | 270.9, 323.3 | 593.1493 | C_{22}H_{19}O_{15} | 3.8 | 575, 503, 473, 383, 353, 311 | Apigenin-6, 8-di-C-glucoside |
| 4    | 243    | 269.9, 330.8 | 593.1499 | C_{27}H_{28}O_{15} | 3.9 | 575, 503, 473, 383, 353, 311,369 | Apigenin 8-C-glucoside, O-hexoside |
| 5    | 248    | 269.9, 321.8 | 563.1394 | C_{26}H_{27}O_{14} | 2.2 | 503, 473, 443, 383, 369, 353, 311 | Apigenin-C-glucoside, C-pentoside |
| 6    | 250.4  | 269.9, 339.3 | 447.0921 | C_{16}H_{16}O_{11} | 2.8 | 357, 327, 285 | Orientin (luteolin-8-C-hexoside) |
| 7    | 251.7  | 269.9, 339.3 | 623.1597 | C_{26}H_{26}O_{16} | 4.4 | 477, 316, 315 | Isorhamnetin-3-O-rutinoside |
| 8    | 260.5  | 269.9 | 537.1951 | C_{20}H_{21}O_{12} | 2.3 | 507, 447, 401, 375, 345 | Unknown glycoside |
| 9    | 272.3  | 269.9, 321.8 | 609.1434 | C_{27}H_{29}O_{16} | 3.3 | 447,357, 327 | Luteolin-8-C-hexoside, O-hexoside |
| 10   | 274.6  | 268.9, 326.8 | 755.2026 | C_{33}H_{38}O_{20} | 2.1 | 623, 593, 476, 461, 315 | Isorhamnetin-6-pentosyl-O-rhamnoglucoside |

Contd...
Flavonoids

*P. turgidum* chromatogram showed different subclasses of flavonoids (structures of the main nucleus are shown in Figure 2). UV spectra revealed the presence of flavone, flavonol, and flavanone structures.\(^{[20]}\) In MS analysis, the nature of the sugars could be revealed by elimination of the sugar residue, that is, 176 amu (hexuronic acid), 162 amu (hexose: glucose or galactose), 132 amu (pentose), and 146 amu (rhamnose). Interpretation of the UV and MS spectra led to the identification of 15 flavonoid conjugates of C- and O-glycosides. Luteolin conjugates were detected in peaks (1, 6, and 9), all of which showed the neutral loss of 120 amu (0,2 cross-ring cleavage), 90 amu (0,3 cross-ring cleavage), and 18 amu (loss of H₂O), which are characteristic of C-hexosides.\(^{[21]}\) In detail, peak 1 (609.1441, M-H) exhibited fragment ions at m/z 591 (M⁺ -18), 519 (M⁺ -90), 489 (M⁺ -120), 399 (M⁺ aglycone + 113), and 369 (M⁺ +83)

Table 1: Contd...

| Position | Retention Time (min) | Peak Area (peak) | Formula | Intensity | Mass (m/z) | Identification |
|----------|----------------------|------------------|---------|-----------|------------|----------------|
| 11       | 276.7                | 268.9, 339.3     | C₂₉H₃₆O₁₁ | 1.7       | 287, 431   | Unknown glycoside |
| 12       | 299.3                | nd               | C₂₈H₃₄O₁₄ | 4         | 533, 503, 413, 383 | Diosmetin-6, 8-di-C-hexoside |
| 13       | 308.3                | 281.9            | C₂₈H₃₂O₁₄ | 4.5       | 271, 316   | Naringenin-O-hexoside |
| 14       | 311.3                | 268.4, 336.3     | C₂₉H₃₄O₁₅ | 2         | 285, 299, 445 | Kampheride-O- rhamnogluicoside |
| 15       | 320.4                | 269.4, 331.3     | C₂₉H₃₂O₁₅ | 3.5       | 315, 461   | Isorhamnetin -3-O-glucuronide |
| 16       | 328.9                | 279.9            | C₂₉H₃₄O₁₅ | 1.8       | 476,448, 329, 314, 271 | Naringenin-O- acyl-hexoside |
| 17       | 349.7                | 280.9            | C₂₉H₃₂O₁₄ | 3.9       | 525, 524, 329, 314 | Unknown glycoside |
| 18       | 358.2                | 285.4            | C₂₉H₃₂N₂O₃ | 2         | 237, 343   | Unknown |
| 19       | 363.2                | 285.9            | C₂₉H₃₂O₄ | 4.4       | 151,174, 237 | Eriodictyol |
| 20       | 380.7                | 283.4            | C₂₈H₃₀O₁₄ | 3.3       | 437, 315   | Isorhamnetin-O-hexoside |
| 21       | 384.8                | 280.4            | C₂₉H₃₂O₁₅ | 6.5       | 358, 325, 287, 272, 259 | Unknown |
| 22       | 410                  | 284.4            | C₂₉H₃₂O₁₄ | 4.4       | 437, 395, 271 | Acylatednaringeninconjugate |
| 23       | 422.5                | 280.9            | C₂₉H₃₂O₃ | 0.7       | 267        | Hydroxycotadecenedioic acid |
| 24       | 425.9                | 270.4, 328.8     | C₂₉H₃₂O₁₄ | 3.2       | 315        | Methylatedisorhamnetin |
| 25       | 441                  | 316.8            | C₂₉H₃₂O₃ | 3.5       | 267        | Unknown glycoside |
| 26       | 447.4                | 280.4            | C₂₉H₃₂O₃ | 0.2       |          | Hydroxycotadecenedioic acid |
| 27       | 531.9                | nd               | C₂₉H₃₂O₁₅ | 1.5       | 793, 721, 664 | Unknown |
| 28       | 556.5                | nd               | C₂₉H₃₂O₄ | 5.3       | 293, 291, 201 | Octadecenedioic acid |
| 29       | 644.1                | nd               | C₂₉H₃₂O₃ | 4.9       | 275, 211   | Hydroxycotadecatrienoic acid |
| 30       | 651.2                | nd               | C₂₉H₃₂O₃ | 4.4       | 275        | Hydroxycotadecatrienoic acid isomer |
| 31       | 688.9                | nd               | C₂₉H₃₂O₃ | 1.1       | 277, 171   | Hydroxycotadecadienoic acid |
| 32       | 709.4                | nd               | C₂₉H₃₂O₃ | 2.5       | 275        | Hydroxycotadecatrienoic acid isomer |
| 33       | 717.8                | nd               | C₂₉H₃₂O₃ | 3.1       | 275, 211   | Hydroxycotadecatrienoic acid isomer |
| 34       | 823.9                | nd               | C₂₉H₃₂O₃ | 0.2       | 180        | Hydroxypalmitic acid |
| 35       | 868.7                | nd               | C₂₉H₃₂O₃ | 3.1       | 481, 480   | Unknown |
| 36       | 831.7                | nd               | C₂₉H₃₂O₃ | 2.2       | 211        | Linolenic acid |
| 37       | 882.5                | nd               | C₂₉H₃₂O₃ | 1.5       | 211        | Linoleic acid |
| 38       | 924.3                | nd               | C₂₉H₃₂O₃ | 1.3       | 113        | Palmitic acid |
| 39       | 940.4                | nd               | C₂₉H₃₂O₃ | 4.6       | 183        | Oleic acid |
that is typical of di-C-glycosyl flavonoids and identified as luteolin-6, 8-di-C-hexoside. Peak 6 with an m/z 447.0921, C_{18}H_{26}O_{14}, showed a neutral loss of 120, 90, and 18 amu indicative of a mono-C-hexose; the high intensity of m/z 357 (M⁺ -90) and absence of (M⁺ -4H₂O) fragment suggested that sugar attachment occurs at the 8-position and was assigned as luteolin-8-C-hexose. Peak 9, m/z269.1434 with molecular formula C_{18}H_{28}O_{13}, showed neutral loss of 162 (characteristic for O-hexose); presence of m/z 357 (M⁺ -90) and absence of (M⁺-4 H₂O) suggested similarly that sugar substitution occurs at the 8-position and was identified as luteolin-8-C-hexosyl-O-hexose.

Another flavone structure was detected in apigenin conjugates (peaks 2, 3, 4, and 5). Peak 2 (431.1903, M-H) showed typical loss of 120 and 90 amu of C-hexoses, with high intense m/z 341 (M⁺ -90) compared to m/z 413 (M⁺ -18) fragment and absence of (M⁺ -4H₂O) fragment denoting 8-C-hexose. The presence of minor fragment at m/z 269 of apigenin aglycone in peak 2 led to its identification as apigenin-8-C-hexose. Peaks 3 and 4 (593.1493, M-H) showed the same molecular formula, C_{21}H_{26}O_{13} and typical fragmentation pattern of C-hexose, (M⁺ -18), (M⁺ -90), (M⁺ -120). In contrast, peak 3 showed additional fragments of 383 (M⁺ +113 amu) and 353 (M⁺ +83, Table 1) typical fragmentation pattern of flavone di-C-hexose, whereas peak 4 showed loss of 162 amu characteristic of O-hexose and identifying peaks 3 and 4 as apigenin-6,8-di-C-hexose and apigenin-8-C-hexosyl-O-hexose, respectively. Peak 5 with exact mass m/z 563.1394, C_{21}H_{27}O_{14} was tentatively identified as apigenin-C-hexosyl-C-pentoside showing neutral losses of 90 and 60 amu, characteristic of a C-pentoside in addition to the loss of 120 and 90 amu due to C-hexose. Another flavone structure was detected in peak 12, (623.1593, M-H) showing fragmentation pattern of di-C-hexose [Table 1] and identified as diasminetin-6,8-di-C-hexose. This is the first report of C-flavonoids in this genus.

The second most abundant subclass of flavonoids, flavonol conjugates, was detected in peaks (7, 10, 14, 15, 20, and 24; Table 1), all of which shared the pattern of 3-O-glycosylation as revealed from its band 1 λ_max values. Isorhamnetin, m/z 315.05, was detected as aglycone in peaks 7, 10, 15, 20, and 24 following the neutral elimination of attached O-sugar residues and identified as isorhamnetin-3-O-rutinoside (7), isorhamnetin-6-O-pentosyl-O-rhamnoglucoside (10), isorhamnetin-3-O-glucuronic acid (15) and isorhamnetin-3-O-hexoside (20), peak 24 (m/z 329.0657) showed the loss of 15 amu (C₇H₅) and was tentatively assigned as isorhamnetin bearing an extra methyl group. Formation of flavonol aglycone radical ions in MS/MS with high-collision energies (isorhamnetin-3-O-rhamnoglucoside) UV detection also confirmed the presence of flavone in peaks (13, 16, 19, and 22; Table 1) with a UV maximum at 282 nm. Naringenin, a flavanone with m/z 271.06 was found as aglycone in peaks 13, 16, and 22. Peak 13 (433.1122, M-H) and 16 (491.2129, M-H) both showed neutral losses of 162 amu for O-hexose, peak 16 exhibited fragment ions m/z at 448 (-42 amu, acetyl gp.) assigned as naringenin-O-acetyl hexose, whereas 13 was identified as naringenin-O-hexose. Peak 22 exhibited an (M-H) of 481.1372, C_{18}H_{26}O_{12}, and with fragment ion at m/z 271 of naringenin, whereas the other detected flavanone “eriodictyol” was identified in peak 19 from its (M-H) at m/z 287.0546 and fragment ion at m/z 151.002.

**Oxygenated fatty acids**

In the second half of the chromatographic run (350–900 s), MS spectra of several unsaturated fatty acids, that is, linolenic acid (36), linoleic (37), oleic (39), and also palmitic acid (38), were identified, as evident from high-resolution mass at 277.2162, 279.2321, 281.24714, and 255.2327 with predicted molecular formulae of C_{18}H_{32}O_{5}, C_{18}H_{30}O_{5}, C_{18}H_{28}O_{5}, and C_{18}H_{26}O_{5}, respectively. MS also revealed other hydroxy fatty acids with predicted molecular formulas of C_{18}H_{32}O_{5} and C_{18}H_{30}O_{5} (and (M-H) of 293.2111 and 295.2276, respectively, in peaks (29–33). A mass difference of 2 amu between peaks is indicative of an extra double bond and was tentatively identified as hydroxyoctadecatrienoic and hydroxyoctadecadienoic acid isomers. Similarly, peaks 23 and 26 MS spectra showed a mass weight of 327.2169 and 329.2326 with predicted molecular formula C_{18}H_{32}O_{5} and C_{18}H_{30}O_{5}, respectively, and were tentatively identified as hydroxyoctadecadienoic acid and hydroxyoctadecadienoic acid, respectively. Hydroxyhexadecanoic acid was identified in peak 34 with (M-H) 271.2277, showing an extra hydroxy group (16 amu) than in palmitic acid.

**Acute and subchronic toxicity**

To assess for *P. turgidum* extract safety margin, acute and chronic toxicity effects were monitored in animals including estimation of LD₅₀ and following complete blood picture analysis, liver and kidney serum enzymes measurement and followed by histopathological examination after 4 weeks of administration. Concerning the lethality test, treated mice with different doses of *P. turgidum* extract (up to 15000 mg/ kg b. wt.) were alive during the 24-h observation time, showing no visible signs of acute toxicity. Consequently, according to Hodge and Sterner toxicity scale, the LD₅₀ value of the extract is categorized under the nontoxic category. Similarly, Urea and creatinine, biochemical markers, were used for the diagnosis of possible renal damage, showing no significant difference in (36.4 ± 2.14 and 0.64 ± 0.03 mg/dl, respectively) compared to control animals (41.5 ± 2.16 and 0.70 ± 0.03 mg/dl, respectively) at (P ≤ 0.05) and in agreement with histopathological sections showing normal nephron structure [Figure 4]. Additionally, no significant change was found in kidney weight ratio at a dose of 500 mg/kg b. wt. of *Panicum* extract (0.6 %) when compared with control (0.6%). Moreover, oral administration of *P. turgidum* extract for 4 weeks did not show any significant effect on AST (22.0 ± 1.2 U/L), ALT (24.9 ± 2.3 U/L), ALP (71.2 ± 6.3 U/L), total bilirubin (1.2 ± 0.06 mg/dl), and total protein (8.1 ± 0.46 g/dl) serum levels or on liver weight ratio (3.2 ± 0.10 %) as compared to control group (21.3 ± 1.5, 22.1 ± 0.70, 0.6%, respectively, and following complete blood picture analysis, liver and kidney serum enzymes measurement and followed by histopathological examination after 4 weeks of administration)
Hepatoprotective activity

The ability of alcohol extract analyzed using UPLC-MS to protect against CCl₄ hepatotoxic effects was assessed in a rat animal model along with silymarin as a positive drug control. Treatment of animals with the hepatotoxic agent, CCl₄, resulted in significant (P ≤ 0.05) increase in the liver aminotransferases (AST and ALT) and ALP levels, indicative of hepatocytes damage and decreased serum albumin and total protein. Severe jaundice was also revealed from elevated bilirubin level in addition to elevated serum total cholesterol and triglycerides and accompanied by a decrease in serum HDL-cholesterol and in accordance with,[27,28] Additionally, CCl₄ administration led to significant increase in MDA (lipid peroxidation) and reduction of both GSH content and activity which are endogenous protective antioxidants known to protect from free oxygen radicals in hepatic tissue.[14]

Silymarin, the well characterized antihepatotoxic agent along with P. turgidum extract ameliorated CCl₄-induced alterations [Table 2]. P. turgidum extract induced significant reduction of ALT, AST, ALP, total bilirubin, direct bilirubin, total cholesterol, and Triglycerides (TG) serum elevated levels concurrent with a significant increase in total proteins, serum albumin level, and HDL-cholesterol in comparison to CCl₄-treated group. In addition, Panicum extract significantly mitigated the lipid peroxidation activity in the rat liver induced by CCl₄ as manifested by a decrease in MDA levels and restored GSH content and CAT and SOD activities. It should be noted that animals treated with Panicum extract did not improve CCl₄ effect on body weight, but rather decreased liver-body weight ratio as compared to CCl₄-treated group.

Furthermore, improvement in biochemical parameters following P. turgidum treatment was also confirmed from the liver histopathological examination [Figure 3]. Liver section of control group showed no histolopathological alterations, with a normal histological structure of the central vein and surrounding hepatocytes [Figure 3A]. In contrast, administration of CCl₄ revealed centrolobular fatty change as observed in most of the hepatocytes associated with inflammatory cells infiltration in the portal area and dilatation in the portal vein [Figure 3C]. Pretreatment with silymarin during CCl₄ administration showed mild degenerative changes in some hepatocyte cells [Figure 3D], whereas treatment with

**Table 2:** Effect of pretreatment with silymarin and P. turgidum extract on changes in body weight, relative liver weight ratio, serum enzymes, total and direct bilirubin, total proteins, albumin and lipid profile levels and oxidative stress and activities of antioxidant liver enzymes in carbon tetrachloride-treated animals (n = 10)

| Group Parameter | Control | CCl₄ | Silymarin + CCl₄ | Panicum Extract + CCl₄ |
|-----------------|---------|------|----------------|-----------------------|
|                  | Mean ± S.E | % Deterioration | Mean ± S.E | % Amelioration | Mean ± S.E | % Amelioration |
| Increase in body weight (g) | 75.0 ± 3.0 | 35.3 ± 3.8a | -53% | 36.1 ± 3.5a | - | 48.9 ± 2.9a | - |
| Relative body-weight ratio (100x) | 3.1 ± 0.09 | 4.7 ± 0.22a | +52% | 4.0 ± 0.21ab | -29% | 4.0 ± 0.20ab | -29% |
| ALT (U/L) | 22.1 ± 1.9 | 66.1 ± 2.5a | +199% | 30.6 ± 2.9b | -54% | 26.1 ± 2.5b | -61% |
| AST (U/L) | 21.3 ± 1.5 | 77.8 ± 3.2a | +265% | 36.2 ± 2.6b | -53% | 34.4 ± 1.5b | -56% |
| ALP (U/L) | 72.3 ± 4.8 | 173 ± 13.6a | +139% | 106 ± 7.8b | -39% | 144 ± 7.9b | -17% |
| Total bilirubin (mg/dl) | 1.1 ± 0.07 | 3.0 ± 0.35a | +173% | 1.8 ± 0.07b | -40% | 1.7 ± 0.12b | -43% |
| Direct bilirubin (mg/dl) | 0.98 ± 0.1 | 2.4 ± 0.09a | +145% | 1.6 ± 0.07b | -33% | 1.5 ± 0.08b | -38% |
| Total protein (g/dl) | 8.0 ± 0.09 | 5.7 ± 0.21a | -29% | 6.9 ± 0.29b | 21% | 7.1 ± 0.28b | +25% |
| Albumin (g/dl) | 6.8 ± 0.18 | 4.6 ± 0.32a | -32% | 6.2 ± 0.27b | +35% | 6.3 ± 0.18b | +37% |
| Cholesterol (mg/dl) | 210.0 ± 7.8 | 384.3 ± 11.7a | +83% | 284.2 ± 14.9b | -29% | 279.7 ± 13.8b | -27% |
| Triglycerides (mg/dl) | 451.7 ± 21.9 | 596.7 ± 27.1a | +32% | 482.8 ± 19.7b | -19% | 480.2 ± 19.9b | -20% |
| HDL (mg/dl) | 74.9 ± 6.5 | 41.4 ± 3.5a | -45% | 59.2 ± 4.7 | +43% | 72.0 ± 6.0b | +74% |

Contd...
**P. turgidum** extract showed dilatation in both central and portal veins associated with mild fatty changes in few hepatocytes [Figure 3E], which was much less severe than that observed in CCl4 group.

### CONCLUSIONS

The approach utilized herein allowed for the identification of several O- and C-glycosyl conjugates of luteolin, apigenin, isorhamnetin, and naringenin, unprecedented in that genus. As such, these derivatives are major secondary metabolites in other monocots, that is, maize, wheat, and rice. In these cereals, C-glycosides of the simple flavones apigenin and/or luteolin predominate predominate and function as natural antioxidants in these plants, which explain the mitigation of oxidative stress resulted from **P. turgidum** extract administration to CCl4– intoxicated rats. Enrichment of these antioxidant flavonoids in plant extract is likely to mediate for its potential antihepatotoxic effects.

Standardization of **Panicum** extract based on these flavonoids could assist in quality control analysis. To the best of our knowledge, this study provides the first metabolite profile not only in **P. turgidum** but also in the whole genus. Further isolation and examination of these metabolites including unknown peaks will help provide more evidence for its potential use in the treatment of liver disorders. Additionally, further studies are still needed for the adjustment of the protecting clinical dose and revealing the mechanism of action mediating the observed antihepatotoxic effect.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

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