Evaluation of hepatoprotective activity of Polygonum equisetiforme methanolic extract

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INTRODUCTION

Though the liver is the body’s gateway yet unfortunately; its detoxification systems are recognized in this chemical era with elevated concentrations of toxins that cause hepatic toxicity harm (Jin et al., 2005). CCl₄ hepatotoxicity is caused by its reactive metabolite radical CCI₃,* that attached covalently to the macromolecules and promote to bring lipid peroxidation that liberates lipid peroxides that destroy the liver tissue.

Herbal medicines have been used all over the world for the prevention and management of hepatic illness. Nowadays, clinical investigations have guaranteed the effectiveness of many plants in developing hepatic disorder therapies, so there is a renewed interest of patients with the degenerative hepatic disease to use herbal therapy from the start or adjuvant (Del Prete et al., 2012; Srivastava and Srivastava, 2018; Wu, 2016). These herbal remedies depend on either capturing the free radical or inhibiting enzyme in their mode of action (Al-Snafi, 2016). By inhibiting and/or decreasing lipid peroxidation and growing antioxidant enzymes [glutathione (GSH), superoxide dismutase (SOD)...], the preventive impact of plant polyphenolic extracts reduces this damage. These effects will result in depletion in serum liver enzymes activity, hepatocyte growth factor levels, and cause marked improvement in the histological feature of liver fibrosis (El-Toumy et al., 2011; Salib et al., 2014).

Plants of the genus Polygonum comprise approximately 300 species of 1,500 species of the family Polygonaceae and have been commonly used in folk medicine. Modern studies proved that P. species possess a variety of pharmacological functions including inflammation suppression (Zhang et al., 2007), bacterial suppression (Cecotti et al., 2012), anti-HIV (Lin et al., 2010), used against kidney stones, anti-diabetic, anti-tumor effects (Smolarz et al., 2008), diuretic, and anti-diarrheal agents (Baytop, 1999). Polygonum bistorta seeds are used in the Carpathian Basin to cure diarrhea (Rab, 2001). The methanol extract of P. sachalinensis had antioxidant effects by free radical-scavenging activities (Fan et al., 2010). Polygonum aviculare extracts exhibited anti-obesity
effects by decreasing the activity of the white adipose tissue and increasing antioxidant activity (Sung et al., 2013). Additionally, *P. cuspidatum* extracts also suppressed the multiplication of mouth tumor cells by inducing caspase-dependent apoptosis, suggesting the extracts might be as a good ingredient due to its effective remedy of mouth tumor (Shin et al., 2011). *Polygonum cognatum* has been applied as diuretic agents in Turkish ethnomedicine and for the treatment of urinary tract inflammation (Çakılocu and Türkoglu, 2010). Different parts of some *Polygonum* genus are useful in the treatment of anemia, liver problems, and kidney stones (Ghorbani, 2005). As far as we know, *P. genus* comprises a mixture of different bioactive compounds, including volatile oil, flavonoids, phenylpropanoids, anthraquinones, and terpenoids (Datta et al., 2000; Dong et al., 2014; Wang et al., 2005). In addition, characteristic compounds such as drimane sesquiterpenoids, norsesquiterpenoids, and sulfated flavonoids were isolated from certain species of *Polygonum* (Fukuyama et al., 1982; Haraguchi et al., 1996). Although a significant trial and botanic data were gathered regarding the pharmacological effects of *Polygonum* genus (Nkùeté et al., 2015), no exhaustive studies have yet been published on *Polygonum equisetiforme*. Thus, on our progression of the survey program on the flora of Egyptian plants and compounds with antiproliferative effect; the current research target is to evaluate the hepatoprotective impact of the methanolic extract rich in flavonoids (El-Toumy et al., 2017) of *P. equisetiforme* aerial parts versus CCl₄-induced hepatic illness in male Sprague-Dawley rats.

**MATERIALS AND METHODS**

**Plant material**

*Polygonum equisetiforme* aerial parts were gathered from the Mediterranean region, Egypt during April 2013 (flowering date). The samples were separately air-dried in the shed, powdered, and kept in tightly sealed round flasks and stored for biological studies. Identification of the plant was confirmed by the Botany Department, Faculty of Science, Cairo University, Egypt (Boulos, 1999). A voucher (P75) is installed in the National Research Centre’s herbarium ( Cairo, Egypt).

**Chemicals and biochemicals**

CCl₄ was purchased from Merck, Germany. Silymarin was acquired from Sigma-Aldrich, St Louis, MO. For serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), GSH, glutathione peroxidase (GPx) nitric oxide (NO), malondialdehyde (MDA), SOD, biodiagnostic kits (Biodiagnostic Co., Dokki, Giza, Egypt) were acquired. Solvents and all other used chemicals were of analytical quality.

**Phytochemical investigation**

Aerial parts of *P. equisetiforme* were crushed and extracted by soaking at room temperature with 70% methanol for five folds. To obtain a dry extract, the collaborated methanol extract was concentrated under reduced pressure and lyophilized. Total dry extract was stored at −20°C in the dark until it was used. A sample (100 g) of the dry extract was fractionated by chromatography on polyamide 6S column. The column was eluted with water and with water/methanol step-gradient. The similar fractions were collected together to give six major fractions (I–VI). The fractions were subjected to Sephadex column LH-20 CC using different percentage of EtOH or/and EtOH-H₂O as solvent system to afford the purified samples of three new flavonoids named; 3,5,7,2′,5′pentahydroxyflavone 3-O-β-D-glucopyranoside, 3,5,7,2′,5′pentahydroxyflavone 3-O-β-D-glucopyranoside 8-C-sulphated, quercetin 3-O-β-D-gluconuride 6″-methyl ester 8-sulphated as well as quercetin 3-O-β-D-gluconuride methyl ester, quercetin 3-O-β-D-glucopyranoside, quercetin 7-O-β-D-glucopyranoside, quercetin, myricetin, P-methoxygallic acid methyl ester, and gallic acid (El-Toumy et al., 2017).

**Animals**

Forty-two Male Sprague-Dawley rats (100–150 g) were used in this study. They were purchased from the Animal House, National Research Center, Egypt. All animals were kept in a controlled air environment (57% ± 2% moisture and 12:12 hours light and dark photo-cycle) and 22°C ± 2°C temperature with access to water and *ad libitum* diet. All experimental methods followed the ethical rules of the National Research Centre’s Medical Ethics Committee in Egypt. The animals were observed daily to guarantee that at any point of the investigations the animals did not tolerate.

**Acute toxicity study**

Doses of *P. equisetiforme* extract at 100, 200, and 1,000 mg/kg b.wt. were given orally to the rats. All rats were examined for the mortality in the duration of 24–72 hours. At a dose of 1,000 mg/kg b.wt., only one dead animal was noted after 24 hours. Therefore, only doses of 100 and 200 mg/kg b.wt were selected to guide this study.

**Experimental design**

CCl₄-induced hepatotoxicity study was performed as stated by Suja et al. (2004). Forty-two male Sprague-Dawley rats (six rats/each) were distributed into seven groups as listed:

**Group I:** rats were administrated saline orally (1 ml/ kg b.wt.) for 21 days as normal control.

**Groups II and III:** rats were orally administrated *P. equisetiforme* extract at two different doses (100 and 200 mg/kg b.wt., respectively), alone for 21 days.

**Group IV:** rats were orally administrated saline for 15 days followed by a single dose of CCl₄ (1.5 mg/kg b.wt.) twice weekly until day 21.

**Group V:** the reference drug silymarin (25 mg/kg b.wt.) was administrated orally for 15 days and CCl₄ (1.5 mg/kg b.wt.) was conducted twice a week until day 21.

**Groups VI and VII:** *P. equisetiforme* extract (100 and 200 mg/kg b.wt., respectively) was administrated orally for 15 days, followed by CCl₄ (1.5 mg/kg, b.wt.) twice a week until day 21.

**Blood sample preparation**

All animals were sacrificed by mild ether anesthesia. Sera were separated from the collected blood samples; in
clean and dry test tubes; by leaving to clot for 10 minutes and centrifuging at 1,500 rpm (4°C). The clear supernatant serum was kept at −20°C for further investigations of liver enzymes levels and hepatic oxidative stress markers assessment. Liver tissue was also separated for histopathological examination.

**Biochemical assays**

Serum enzymes (AST and ALT) activities were assessed colorimetrically using a test-reagent kit as maintained by Reitman and Frankel (1957). According to Lawrence and Burk (1976), the glutathione peroxidase activity was determined by the cyanomet-hemoglobin technique. Suttle’s study (1986) reveals the degree of inhibition of pyrogallol auto-oxidation at an alkaline pH, by the activity of SOD in rat liver cytosol. The level of decreased GSH in the liver tissue homogenate was described just as Moron et al. (1979) stated. Hepatic nitric oxide was maintained by Miranda et al. (2001) procedure. The MDA level was described by Uchiyama and Mihara (1978) technique.

**Histopathological studies**

Liver sections were cut out and washed quickly with normal saline, then handled thoroughly for histopathological observation. First, the materials were placed in 10% buffered neutral formalin and paraffin, then layers of 5 μm thickness were sorted out in alcohol-xylene series and stained with alum hematoxylin and eosin. For histopathology transformation, the liver sections were examined microscopically (Bancroft and Gamble, 2001).

**Statistical analysis**

All information in each group was shown as mean ± SD of six rats. Among several other procedures, we implanted the one-way variance assessment analysis of variance (ANOVA) for statistical analysis, followed by the least significant difference at \( p < 0.05 \).

**RESULTS AND DISCUSSION**

The obtained results revealed that when the animals are pretreated with *P. equisetiforme* extract at 100 and 200 mg/kg for 6 weeks (groups II and III), it gives normal healthy parameters which revealed the potency hepatoprotectivity of *P. equisetiforme* (Table 1), while oral CCl4 administration showed increased ALT and AST serum concentrations of the group (IV) relative to the control group (I). These enzymes increased impacts justified the hepatic cellular damage, leakage, and liver injury. In comparison to the control group, the oxidative stress mediators levels GSH, GPx, and SOD were significantly decreased, while the NO and MDA levels were remarkably increased (Table 2). CCl4-induced hepatoxicity results from its metabolic byproduct CCl3• which alkylates cellular proteins and other macromolecules with frequent attacks on polyunsaturated fatty acids in the presence of oxygen to form lipid peroxides leading to hepatic illness (Bishayee et al., 1995). On the other hand, improvements were seen in rats that had been medicated with silymarin (V) and increased significantly by the administration of *P. equisetiforme* extract at both doses (100 and 200 mg/kg) (VI and VII) in comparison with the CCl4•-treated group (Tables 1 and 2). The elevated value of GPx and GSH level in the rat’s liver tissues when treated with two doses of *P. equisetiforme* extract and Silymarin may result from GSH regeneration or synthesis of de novo GSH (Sanmugapriya and Venkataraman, 2006). Enhancement of lipid peroxidation reported acceleration in MDA levels in the liver, resulting in tissue distortion and deterioration of antioxidant defense mechanisms to scavenge accumulated free radicals (Ashok et al., 2001). Many illnesses were associated with alterations in GSH concentrations and decreased oxidation stress resistance. These changes are significantly reversed on treating the induced rats with both doses of *P. equisetiforme* extract and silymarin. Many scientists have indicated that flavonoids suppress lipid peroxidation (Husain et al., 1987). GSH (sulphydryl compounds), are one of the well-known antioxidant substance in organisms, which perform a crucial effect against CCl4•-induced injury by covalently binding to CCl3•. In the oxidative chain formation, this is believed to be the initial reactant arising in lipid peroxidation and cell membrane disorder (Brattin et al., 1985). By comparing with the control group, the liver GSH quantity was significantly improved with the treatment of *P. equisetiforme* extract at both doses. The concentration of GSH, SOD, and the GPx were used to monitor the emphasis of oxidative stress and chemo-preventive capacity (Hatono et al., 1996). *Polygonum equisetiforme* extract showed a hepatoprotective impact in the current study, which was resisting to CCl4• cell damage. The antioxidant system associated with SOD, GPx, and GSH has also been improved, which is an evidence of hepatic tissue repair and plasma membrane maintenance from the deficiency induced by CCl4•, particularly in the high dose group (VII), all resulting in the rehabilitation of biological standards. However, *P. equisetiforme*’s protective, curative, and antioxidant characteristics could be assigned to the presence of bioactive components in the plant extract, especially flavonoids and other polyphenolic compounds (Al-Qarawi et al., 2004; Gupta et al., 2004). The variation in the enzymatic antioxidant action and the efficacy and dynamics of this plant’s pure complexes need more research to ensure that their antioxidant features are linked to their role in the protection of the liver.

By studying the histopathology of the liver, the hepatoprotective capability of the *P. equisetiforme* extract was additionally verified. The control animals’ liver section (Fig. 1A) exhibited ordinary hepatic cells. The liver section pretreated with *P. equisetiforme* extract (100 mg/kg) showed normal photomicrograph with ordinary liver tissue and dilation of hepatic

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST(U/ml)</th>
<th>ALT(U/ml)</th>
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<tr>
<td>I (Control)</td>
<td>63.86 ± 0.68</td>
<td>44.22 ± 0.95</td>
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<tr>
<td>II (P. equisetiforme 100 mg/kg)</td>
<td>63.43 ±0.58a</td>
<td>45.02 ±0.48a</td>
</tr>
<tr>
<td>III (P. equisetiforme 200 mg/kg)</td>
<td>64.86 ±0.20a</td>
<td>45.16 ±0.87a</td>
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<tr>
<td>IV (CCl4)</td>
<td>85.86 ±0.36a</td>
<td>73.72 ±0.15a</td>
</tr>
<tr>
<td>V (Silymarin)</td>
<td>81.35 ±0.16a</td>
<td>70.78 ±0.40a</td>
</tr>
<tr>
<td>VI (P. equisetiforme 100 mg/kg + CCl4)</td>
<td>80.88 ±0.30a</td>
<td>70.73 ±0.32a</td>
</tr>
<tr>
<td>VII (P. equisetiforme 200 mg/kg + CCl4)</td>
<td>85.01 ±0.23a</td>
<td>70.73 ±0.46a</td>
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</tbody>
</table>

Hepatotoxicity was induced by a single dose of 1.5 ml/kg (1:1 of CCl4 in olive oil) orally. Values represent the mean ± SE of six animals for each group.

*ab p < 0.05: Statistically significant from the saline control group.*

*ab p < 0.05: Statistically significant from CCl4 group by using ANOVA and LSD.*
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Histopathological changes were observed after CCl4 administration (Fig. 1C) indicating liver damage and were in agreement with preceding results that CCl4 causes cell death (Sun et al., 2001), infiltration of mononuclear cell, steatosis and hepatocytes damage, and accelerate the mitotic process in liver (Teocharis et al., 2001). Figures 1E and F showed that the liver section of P. equisetiforme extract handled in a dose-dependent manner, totally reversed all CCl4-induced deteriorations, with

| Table 2. Effect of oral administration of P. equisetiforme (100 and 200 mg/kg) on NO, MDA, GSH, GPx, and SOD activity in CCl4-induced hepatotoxicity in rats. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | I               | II              | III             | IV              | V               | VI              | VII             |
| NO (µmol/g tissue) | 0.023 ± 0.0004  | 0.024 ± 0.0004  | 0.025 ± 0.0011  | 0.037 ± 0.0011  | 0.027 ± 0.0006  | 0.026 ± 0.0005  | 0.03 ± 0.0006  |
| MDA (nmol/g tissue) | 2.06 ± 0.04  | 2.10 ± 0.04  | 2.10 ± 0.02  | 3.42 ± 0.08  | 2.18 ± 0.04  | 2.16 ± 0.07  | 3.42 ± 0.02  |
| GSH (mg/g tissue) | 4.35 ± 0.06   | 4.18 ± 0.03   | 4.08 ± 0.02   | 3.11 ± 0.08   | 3.84 ± 0.16   | 3.69 ± 0.28   | 3.59 ± 0.15   |
| GPx (µg tissue) | 936.77 ± 71.55 | 882.13 ± 44.96 | 882.13 ± 56.94 | 242.00 ± 11.84 | 761.13 ± 19.90 | 487.91 ± 11.04 | 281.03 ± 27.38 |
| SOD (µ/g tissue) | 133.54 ± 2.18  | 132.58 ± 1.83  | 131.13 ± 2.01  | 38.95 ± 0.40  | 95.56 ± 1.45  | 48.15 ± 2.77  | 41.13 ± 0.97  |

Hepatotoxicity was induced by a single dose of 1.5 ml/kg (1:1 of CCl4 in olive oil) orally. Values represent the mean ± SE of six animals for each group. a p < 0.05: Statistically significant from the saline control group. b p < 0.05: Statistically significant from CCl4 group by using ANOVA and LSD.

Figure 1. Photomicrographs of hepatic histopathology. (A) Negative control, normal histological picture of the hepatic lobule. (B) Liver section from group III treated with the plant extract at dose 200 mg/kg. No noticeable histological changes, except dilated blood sinusoids. (C) Liver section from CCl4-treated rat showing massive fatty degeneration, cytoplasmatic vacuolization, gross necrosis and broad infiltration of lymphocytes and Kupffer’s cells around the central vein. (D) Liver section from the group treated with silymarin and CCl4, the histological was similar to that of the control group. (E) Liver section from the group treated with the plant extract at dose 100 mg/kg and CCl4 showing mild prevention and mild degenerative changes with dilated blood sinusoids. (F) Liver section from the group treated with the plant extract at dose 200 mg/kg and CCl4 revealed partial amelioration of degenerative changes in hepatocytes, but still showed congested central vein and infiltration of inflammatory cells.
mild necrotic hepatocytes and congested central vein Figure 1E at 100 mg/kg dose only. These observations recommended that treatment with greater extract dose (200 mg/kg) improved the histoarchitecture and well-formed the arrangements of the hepatic cord (Fig. 1F).

Although the hepatotoxicity caused by CCl₄ administration was also reserved by Silymarin treatment (Fig. 1D), yet the morphologic picture of the liver was essentially normalized by the administration of P. equestiflorme extract (Group VII) compared to Group IV. However, the liver section in this group (VII) had some diffuse lymphocyte infiltration in the parenchyma along with the normal structure but still the damage of hepatocytes was much less than those treated with Silymarin alone (Fig. 1F and D).

CONCLUSION
This research demonstrates that P. equestiflorme extract (particularly at 200 mg/kg dose) was capable of hindering hepatic damage induced by CCl₄ in rats. Our findings showed that P. equestiflorme extract’s liver-protective impact is triggered by an increase in the antioxidant-defense system effects and lipid peroxidation suppression.

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