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Hepatoprotective Activity of Ethanol Extract of *Conyza bonariensis* against Paracetamol Induced Hepatotoxicity in Swiss Albino Mice

Mohammad Saleem[1,*], Faiza Naseer[1], Sohail Ahmad[2], Attiya Nazish[1], Fiza Raza Bukhari[1], Atta Ur Rehman[1], Imran Mahmood Khan[3], Samra Sadiq[1], Fatima Javed[1]

[1]College of Pharmacy, GC University, Faisalabad, Pakistan
[2]Department of Biochemistry, Hazara University, Mansehra, KPK, Pakistan
[3]Department of Pharmacology, Independent University Hospital, Faisalabad, Pakistan
*Corresponding author: saleem2978@hotmail.com

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Abstract The hepatoprotective activity of Conyza bonariensis ethanol extract was studied against paracetamol induced hepatotoxicity in mice. The results showed that an extract of C.B (250 mg/kg and 500 mg/kg) produced ($p<0.05$) decline in paracetamol induced liver marker enzymes and total bilirubin, but its 750 mg/kg showed remarkable decline in ALT, AST, ALP and TB levels, compared to the reference levels of silymarin and results were supported by histopathology of liver section. So, it is concluded that 750 mg/kg is highly potent dose of ethanol extract of Conyza bonariensis and this potential may be due to the presence of the active constituent: qurecetin and chromatogram by HPLC confirmed its presence.

Keywords: *Conyza bonariensis, hepatoprotective, Paracetamol*

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1. Introduction

Phytotherapy is grabbing interest in the conventional therapy day by day because medicines originate from natural sources help to maintain the hormonal balance and stimulate the body's immune system without causing serious adverse effects. They provide essential phytochemicals and nutrients for optimizing the therapy and boosting its effectiveness (Saleem et al, 2010 Bag and Mumtaz, 2013). Liver, a most important organ plays a vital role in pharmacokinetics. Hepatic cell injury caused by various toxicants like chemotherapeutic agents, anti tuberculosis drugs, carbon tetrachloride, paracetamol, chronic alcohol consumption and pathogenic microbes are well reported (Hegde and Joshi, 2010). They catabolize the free radicals, cause lipid peroxidation, damage the liver cell membranes and cause inflammation and necrosis of hepatocytes and leads to the liberation of cytosolic enzymes into the systemic transmission (Saleem and Naseer, 2014). The medicinal herbs which have ability to protect the liver from various hepatotoxins have phyto constituents like poly phenols, flavonoids, steroids, quinones, coumarins, essential oils, terpenoids, anthocyanidines, saponins, alkaloids and nitrogenous compounds (Valan et al., 2010). *Conyza bonariensisis* a cosmopolitan herb, family Asteraceae (Compositeae), commonly known as mrich booti or gulava is one of the plants having all these constituents (Zahoor et al., 2012) and traditionally used for fungal and bacterial infections (Chaudhry et al., 2001), anticoagulation (Favila and Antonio, 2006), hepatic and gastro enteritis toxicities, diarrhea, leucorrhoea, menorrhagia, anticancer in breast carcinoma (MCF7), colorectal carcinoma (HCT116), cervical carcinoma (HELA) cell lines (Zalabani et al., 2012) and antioxidant (Shahwar et al., 2012). This herb is traditionally used as homeostatic, tonic, astringent (Ahmad, 2007), cholinergic (Khan et al., 2006), anti-inflammatory and antimitotic (Santana et al., 2011). The current study was conducted to determine hepatoprotective activity of ethanol extract of *Conyza bonariensisis* against paracetamol hepatotoxicity in swiss albino mice.

Collection of plant: *Conyza bonariensisis* was collected from local fields of Faisalabad and its surroundings. It was identified by a plant taxonomist Dr. Mansoor Hameed, Head of Botany department, Agriculture University, Faisalabad before starting any experimental work. The plant was kept in the University herbarium for future reference.

Preparation of plant extract: The whole plants were washed, chopped and dried under shade at room temperature for many days until fully dried, ground by electric grinder, powdered and sieved. This material was macerated in ethanol for 7 days with frequent shaking

every day, filtered out by using Whatman filter paper, separating out solvent from solid material by using a rotary evaporator at 40-50°C and residues obtained were stored in small amber jars at 4°C (Saleem et al., 2014).

Experimental animals: 6-8 weeks old Swiss albino mice of either sex weighing between 20-30 g were obtained from National Institute of Health Islamabad and kept at animal house in College of Pharmacy, Government College, University Faisalabad, Pakistan, under controlled conditions of Temperature (25±1°C) and humidity (50±5°C). These were feed with standardized pellet diet and water *ad libitum* (Ali et al., 2013). Mice were acclimatized to environment for one week prior to commencement of the experiment.

Chemicals: Ethanol (Analytical grade), Distill Water, Silymarin, Paracetamol

Study design for hepatotoxicity in mice: In 7 days study, 60 healthy mice were divided in 6 groups and 10 mice in each group (Ali et al., 2013). **Group-I** received distill water for week (p.o); **Group-II** received paracetamol at 250 mg/kg/day for a week; **Group-III** received silymarin 50 mg/kg/day and after 3 hours paracetamol 250 mg/kg/day for a week; **Group-IV** received *Conyza bonariensis* ethanol extract at 250 mg/kg/day and after 3 hours paracetamol 250 mg/kg/day for a week; **Group-V** received *Conyza bonariensis* ethanol extract at 500 mg/kg/day and after 3 hours paracetamol 250 mg/kg/day for a week; **Group-VI** received *Conyza bonariensis* ethanol extract at 750 mg/kg/day and after 3 hours paracetamol 250 mg/kg/day for a week.

Biochemical investigations: Mice were sacrificed after the last treatment and blood was collected by 3 ml syringes, transferred to test tubes and allowed to stand for an hour at room temperature. Test tubes were centrifuged at 4,000 RPM for 20 mints. Serum was separated from blood, stored in eppendhrof tubes at -4°C and labeled. Serum samples of control and treated mice were analyzed for biochemical investigations including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin (TB).

Histopathological studies: A portion of liver approximately of 6 mm^3 size was cut, put in phosphate buffered 10% formaldehyde solution and embed in paraffin wax. A thin portion of 5 µm thickness was again incised and stained by acid-base dye i.e. haematoxylin–eosin and examined under high resolution microscope.

Chromatogram by HPLC for identification of active constituent: The *Conyza bonariensis* contains an important flavonoid i.e. quercetin which has been proven to exhibit the hepatoprotective activity. High performance liquid chromatography (HPLC) was performed to confirm the presence of quercetin in *Conyza bonariensis* ethanol extract. The sample was dissolved in 5 ml distilled water and 12 ml methanol, kept for 5 mints, again added 6 ml distilled water, stayed for 5 mints and added 10 ml 5 M HCl in this solution. Placed in oven for 2 hrs and filtered the solution by syringe filter. Isocratic: dichloromethane: methanol (60:20:20) was used as the mobile phase with the flow rate of 1 mL/min. The column was ODS 250 mm x 4.6 mm and UV detector was used to obtain chromatogram at 280 nm at room temperature (Saleem et al., 2014).

Statistical analysis: One way ANOVA (analysis of variance) was used for statistical analysis. The results were represented by Mean ± STD.

2. Results

Table 1. Effect of ethanolic extract of *Conyza bonariensis* on liver markers and total bilirubin (mean ± STD) Significant [a]p<0.05; [b]p<0.01; [c]p<0.001

Group	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	TB g/dl
Control	217.33+7.788	62.5+5.612	72.16+4.400	0.72+0.044
Paracetamol 250 mg/kg	427.66+5.125	159.8+3.488	194.83+2.857	2.323+0.382
Silymarin + Paracetamol 250 mg/kg	221.16+6.177[c]	63.33+3.614[c]	74.83+2.639[c]	0.748+0.099[c]
C.B 250mg/kg + Paracetamol 250 mg/kg	348.66+3.829[c]	90.66+6.439[c]	104.66+2.943[c]	1.05+0.035[c]
C.B 500mg/kg + Paracetamol 250 mg/kg	311.33+7.421[c]	84.166+3.430[c]	95.83+2.786[c]	0.95+0.027[c]
C.B 750mg/kg + Paracetamol 250 mg/kg	229.33+8.57[c]	68.166+3.311[c]	76.66+3.502[c]	0.766+0.035[c]

The normal mean value of ALP was 217.33+7.788 IU/L. After administering paracetamol it raised up to 427.66+5.125 IU/L. The administration of 250 and 500 mg/kg Conyza extract decreased the level up to 348.66+3.829 IU/L and 311.33+7.421 IU/L, respectively but 750 mg/kg extract cause significant decrease in the ALP level up to 229.33+8.57 IU/L which was comparable ($p<0.001$) to the standard silymarin level 221.16+6.177 IU/L (Table 1).

The normal mean value of ALT was 62.5+5.612 IU/L and paracetamol administration raised up to 159.8+3.488 IU/L level. The Conyza extract at 250, 500 and 750 mg/kg decreased ALT at 90.66+6.439 IU/L, 84.166+3.430 IU/L and 68.166+3.311 IU/L level (Table 1). So, 750 mg dose result was very close to the standard silymarin 63.33+3.614 IU/L ($p<0.001$).

The normal mean value of AST was 72.16+4.400 IU/L. After administering paracetamol it raised up to 194.83+2.857 IU/L and 750 mg/kg Conyza extract decreased its level up to 76.66+3.502 IU/L which was comparable ($p<0.001$) to the standard silymarin level 74.83+2.639 IU/L (Table 1).

The normal mean value of TB was 0.72+0.044 g/dl and paracetamol doubled its level of 2.323+0.382 g/dl extent. 250, 500 and 750 mg/kg extract treated group showed 1.05+0.035 g/dl, 0.95+0.027 g/dl and 0.766+0.035 g/dl respectively. The maximum dose result was comparable ($p<0.001$) to the reference level, i.e. 0.748+0.099 g/dl (Table 1).

Histopathology of normal, paracetamol and extracts treated mice indicated that healthy cells were observed with normal morphology, round nucleus with centrally plus homogenous cytoplasm, flat endothelial cells around central vein and sinusoid shown in figure 1(a). However, paracetamol-treated mice liver showed replacement of hepatocytes by inflammatory cells comprising neutrophils and rare lymphocytes, cell necrosis with intervening hemorrhage and some ballooning of cells figure 1(b) but the reference silymarin treated group showed mild portal inflammation with no ballooning and necrosis, less injury

of endothelial cells around central vein and less fat vacuoles in hepatocytes figure 1(c). The treated group with 250 mg/kg extract showed moderate degree of inflammation mild hepatitis and ballooning with mild dilatation of sinusoid figure 1(d) while 500 mg/kg extract treated group showed less alteration than lesser dose figure 1(e) and 750 mg/kg extract showed less damage to the liver parenchyma with no necrosis and ballooning as compared to paracetamol-treated groups figure 1(f).

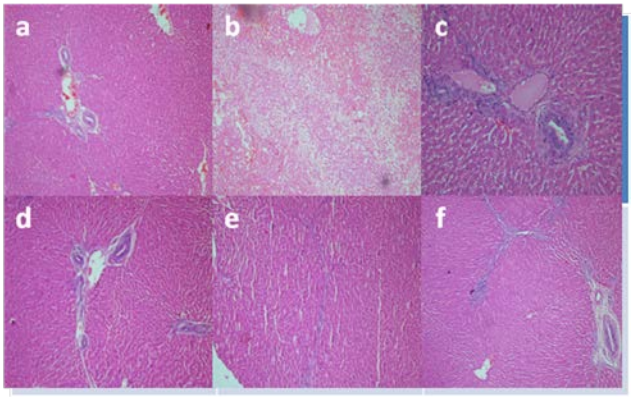

Figure 1. Histological picture of liver parenchyma of normal group (a); paracetamol-treated group (b) showing extensive necrosis, inflammation and ballooning; silymarin-treated group (c) showing mild inflammation; C.B 250 mg/kg (d) showing moderate inflammation and mild ballooning; C.B 500 mg/kg (e) showing mild sinusoid dilatation and inflammation; C.B 750 mg/kg (e) showing less inflammation with no necrosis and ballooning.

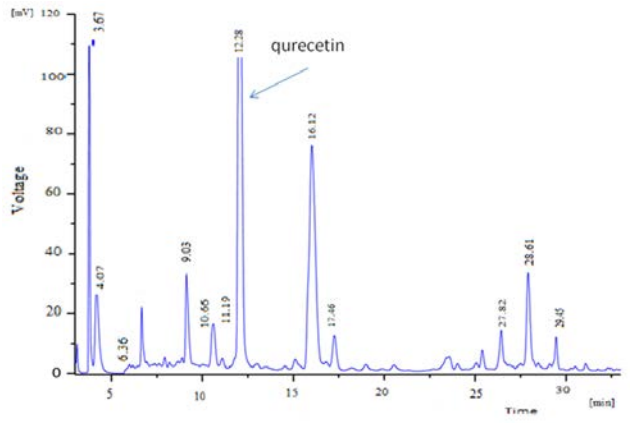

Figure 2. Chromatogram of ethanolic extract of *Conyza bonariensis*

3. Discussion

Paracetamol-induced liver toxicity and cell death is due to generation of toxic metabolites after its metabolism through CYP-450. It is converted into N-acetyl P-benzoquinineimine, produces oxidative stress and cause glycogen and glutathione depletion by irreversible conjugation with sulfhydral groups of glutathione (Saleem and Naseer, 2014; Qadir et al., 2014). In the present study protective effect of ethanol extract of *Conyza bonariensis* against paracetamol induced liver injury in mice has been studied.

After administration of a toxic dose of paracetamol, magnitude of liver marker enzymes boosted up and produced tissue necrosis. When the plant extract in 250, 500 and 750 mg/kg/day for a week was administered p.o. to mice, less increase in enzymes (ALT, AST & ALP) and bilirubin level were observed as compare to paracetamol treated group ($p<0.01$). When these alterations in biochemical parameters were compared with reference (silymarin), a very less difference was observed. The results indicated that extract treated mice showed incredible recovery ($p<0.01$) and supported by histopathology of liver section. Hepatotoxin treated group showed altered morphology and tissue necrosis while extract treated group showed less changes and hepatotoxicity. The main constituents of *Conyza bonariensis* are Quercetin and Kaempferol, confirmed by HPLC shown in figure 2. Both are flavonoid and reported to be hepatoprotective (Ali et al., 2013; Mallhi et al., 2014). It may be concluded that hepatoprotective activity of *Conyza bonariensis* is due to the presence of these important flavanoids.

4. Conclusion

So, it is concluded that 750 mg/kg is highly potent dose of ethanol extract of *Conyza bonariensis* and this potential may be due to the presence of active constituent: qurecetin.

References

[1] Ahmad S. Medicinal wild plants from Lahore-Islamabad motorway (M-2). Pak J Bot. 2007; 39: 355-75.

[2] Ali M, Qadir MI, Saleem M, Janbaz KH, Gul H, Hussain L, Ahmad B. Hepatoprotective potential of *Convolvulus arvensis* against paracetamol-induced hepatotoxicity. Bangladesh J Pharmacol. 2013; 8: 300-04.

[3] Bag AK, Mumtaz SMF. Hepatoprotective and nephroprotec-tive activity of hydroalcoholic extract *OfIpomoea staphylina* leaves. Bangladesh J Pharmacol. 2013; 8: 263-68.

[4] Chaudhry A, Janbaz KH, Uzair M, Ejaz AS. Biological Studies of Conyza and Euphorbia species. J Res (Sci). 2001; 12 (1): 85-88.

[5] Favila C, Antonio M. Chemical and Biological Study of Conyza bonariensis (L.) Cronquist (Asteraceae), Thesis dissertation. 2006.

[6] Hegde K, Joshi AB. Hepatoprotective and antioxidant effect of *Carissa spinarum* root extract against CCl_4 and paracetamol-induced hepatic damage in rats. Bangladesh J Pharmacol. 2010; 5: 73-76.

[7] Khan RA, Bukhari IA, Nawaz SA, Choudhary MI. Acetylcholinesterase and butyrylcholinesterase inhibitory potential of some Pakistani medicinal plants. J Basic Appl Sci 2006; 2: 07-10.

[8] Mallhi TH, Abbas K, Ali M, Qadir MI, Saleem M, Khan YH. Hepatoprotective activity of methanolic extract of *Malva parviflora* against paracetamol-induced hepatotoxicity in mice. Bangladesh J Pharmacol. 2014; 9: 00-00.

[9] Qadir MI, Murad MSA, Ali M, Saleem M, Farooqi AA. Hepatoprotective effect of leaves of aqueous ethanol extract of *Cestrum nocturnum* against paracetamol-induced hepatotoxicity. Bangladesh J Pharmacol. 2014; 9: 167-70.

[10] Rates SM. Plants as source of drugs. Toxicon. 2001; 39: 603-13.

[11] Saleem M, Ahmed B, Qadir MI, Rafiq M, Ahmad M, Ahmad B. Hepatoprotective effect of Chenopodium murale in mice. Bang J Pharmacol. 2014; 9: 124-28.

[12] Saleem M, Chetty M, Ramkanth S, Rajan V, Kumar MK, Gauthaman K. Hepatoprotective Herbs-A Review. Int. J. Res. Pharm. Sci. 2010; 1: 01-05.

[13] Saleem M, Naseer F. Medicinal plants in the protection and treatment of liver diseases. Bangladesh J Pharmacol. 2014; 10: 511-526.

[14] Santana PM, Miranda M, Gutiérrez Y, García G, Orellana T, Orellana-Manzano A. Anti-inflammatory and antimitotic effect of the alcoholic extract and chemical composition of the oil from Conyza bonariensis (L.) Cronquist (deer shinbone) leaves. Rev Cubana Plant Med. 2011; 16: 13-23.

[15] Shahwar D, Raza MA, Saeed A, Riasat M, Chattha F, Javaid M, Ullah S, Ullah S. Antioxidant potential of the extracts of Putranjiva roxburghii, Conyza bonariensis, Woodfordia fruiticosa and Senecio chrysanthemoids. African Journal of Biotechnology. 2012; 11: 4288-95.

[16] Valan MF, Brittob AJ, Venkataramanc R A Brief Review-Phytoconstituents With Hepatoprotective Activity. Int J Chem Sci 2010; 8: 1421-32.

[17] Zahoor A, Hussain H, Khan A, Ahmed I, Ahmad V, Krohn K. Chemical Constituents from Erigeron bonariensis L. and their Chemotaxonomic Importance. Rec Nat. Prod. 2012; 6: 376-80.

[18] Zalabani S, Hetta M, Ismail A. Genetic Profiling, Chemical Characterization and Biological. Evaluation of Two Conyza Species Growing in Egypt. J App Pharmaceut Sci. 2012; 2: 54-61.