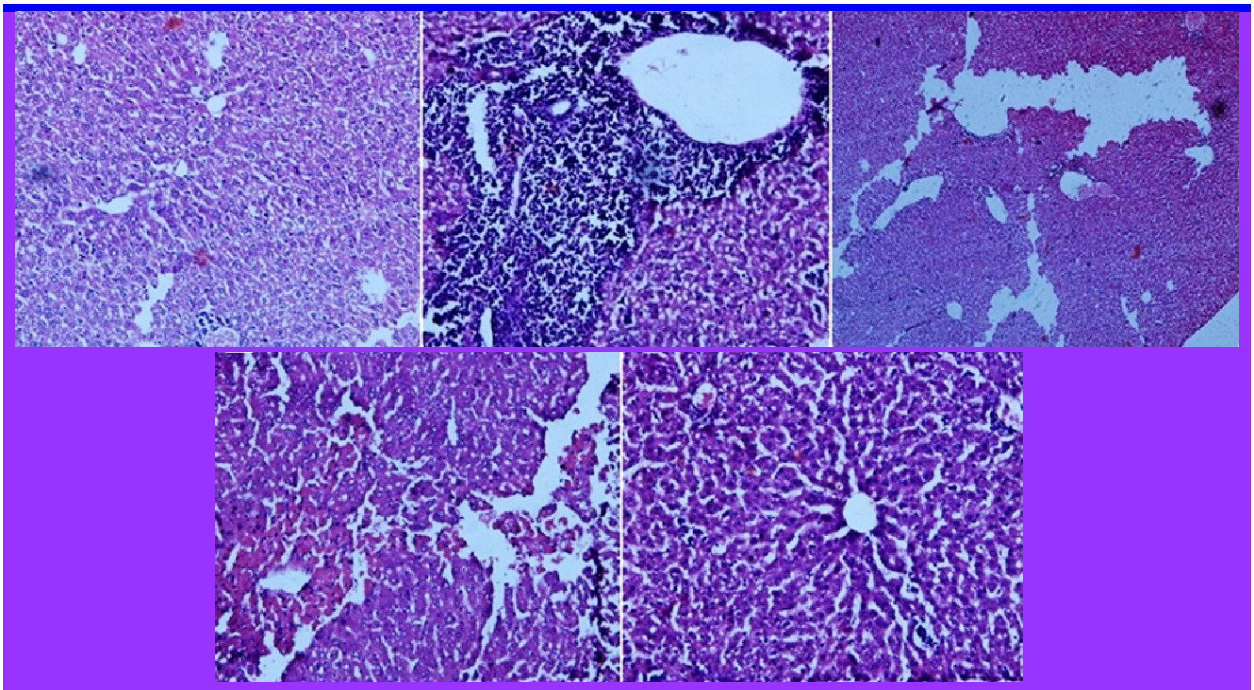

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Hepatoprotective effect of *Chenopodium murale* in mice

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Abstract

Discovery of drugs has its roots in medicinal plants that appeal researchers to identify new therapeutical entities from plants. The current study was conducted to determine its hepatoprotective activity. The results showed that aqueous methanolic extract of *Chenopodium murale* (200 and 500 mg/kg) produced significant ($p<0.001$) decrease in paracetamol induced increased levels of liver enzymes (alanin transaminase, aspartate transaminase, alkaline phosphatase) and total bilirubin. These findings were further supported by histopathological investigations by microscope and detection of phyto-constituents having hepatoprotective potential e.g. qurecetin, kaempferol and gallic acid by HPLC. Conclusively aqueous methanolic extract of *C. murale* possess hepatoprotective activity against paracetamol induced liver damage in mice.

Introduction

Liver is a crucial organ of our body having vital role in metabolism and elimination of various agents. Chemical agents pass through liver before entry to blood. So liver is at high risk of damage then other body organ (Samuel et al., 2012). Liver detoxify all toxins absorbed through gut but these toxins may harm to liver. That's why liver damage found one of the major disease in the world (Ravikumar et al., 2012). Drugs, xenobiotics, viral infections, alcohol and other chemical agents may damage the liver. The injury to liver is characterized by hepatic necrosis and lipid per-oxidation resulting in elevated levels of liver enzymes and bilirubin (Ramachandra et al., 2007).

Liver diseases like hepatitis, liver cirrhosis and fatty liver are very common and problematic. Drugs used for the treatment, like colchicines, interferon, corticosteroids and penicillamine have been found to possess various side effects. So, there is a dire need to develop some effective therapeutic agents from plant source to support liver function and to treat liver diseases having low incidence of side effects. The plants like *Luminetzera racemosa* (Gnanadesigan et al., 2011), *Convolvulus arvensis* (Ali et al., 2013), *Hibiscus vitifolius* (Sameul et al., 2012), *Carica papaya* (Sadeque and Begum, 2010), *Carissa spinarum* (Hegde and Joshi, 2010), *Cocculus hirsutus* (Thakare et al., 2009), *Convolvulus arvensis* (Ali et al., 2013), *Dodonaea viscosa* (Khan et al., 2013), Khamira Gaozaban Ambri Jadwar Ood Saleeb Wala (Akhtar et al., 2013), *Oflpomoea staphylina* (Bag and Mumtaz, 2013), *Suaeda fruticosa* (Rehman et al., 2013), *Trianthema decandra* (Balamurugan and Muthusamy, 2008) and *Trichodesma sedgwickianum* (Saboo et al., 2013) showed hepatoprotective effect.

In the present study *Chenopodium murale* was selected to evaluate its hepatoprotective potential. Previous studies regarding *C. murale* have shown that it possess anti-inflammatory, analgesics (Ibrahim et al., 2007), antifungal (Javaid et al., 2009), antibacterial (Ali et al., 2001) and hypotensive effects (Gohara and Elmazar, 1997). *C. album* the plant of this genus showed hepatoprotective action (Pal et al., 2011; Vijay and Padma, 2011).

C. murale contains flavanoids, saponins and terpenoids (Abbas et al., 2012) and has been proved to be antioxi-

dant (Ali et al., 2001). The presence of flavonoids (Vijay and Padmaa, 2011), saponins, (Kumar et al., 2011) or triterpenoids (Kim et al., 2004) is believed to be hepatoprotective. The detection of flavonoids in aqueous methanolic extract of *Chenopodium* in the current study and its previous findings, give us a clue for its hepatoprotective activity.

Material and Methods

Collection of plants: Fresh plants were collected from district Sialkot, Punjab Pakistan. Plant was identified by Dr. Mansoor Hameed, Department of Botany, University of Agriculture, Faisalabad. Vouchers No 531-2-13 can be used for future reference.

Preparation of plant extracts: Plant was washed, dried under shade and finally grounded to powder. The powdered plant was soaked in aqueous methanolic (30:70) solvent for 7 days with occasional shaking at regular intervals. The extract was filtered and evaporated by using rotary evaporator at 60°C. The residue was stored in amber coloured glass bottle at 4°C.

Experimental animals: Adult Swiss albino mice weighing about 22-34 g were used. All the animals were placed in the animal house of College of Pharmacy, GC University Faisalabad. The animals were allowed to acclimate under standard laboratory conditions prior to perform experiment.

Experimental protocol: All animals were divided into 5 groups containing 5 animals each. Group 1 served as control group receiving distilled water only Group II served as paracetamol control group received paracetamol 250 mg/kg (p.o) 7 days. Group III was treated with silymarin 50 mg/kg (p.o) and followed by paracetamol administration 3 hours after silymarin for 7 days. Group IV was treated with aqueous methanolic extract of *C. murale* at dose of 250 mg/kg (p.o) followed by paracetamol administration 250 mg/kg (p.o) 3 hours after the extract dose for 7 days. Group V was treated with aqueous methanolic extract of *C. murale* at dose of 500 mg/kg (p.o) followed by paracetamol administration 250 mg/kg (p.o) 3 hours after the extract dose for 7 days (Ali et al., 2013).

Biochemical and istopathological investigations: On 8th day, animals were sacrificed and blood was collected for serum separation. The change in aspartate aminotransferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP) and total bilirubin (TB) levels were measured for biochemical investigations. Histolopathological studies were also conducted to examine microscopic changes in liver architecture (Ali et al., 2013).

Histopathological examination: Histopathological study was carried out to see the microscopic changes in the hepatocytes. For this purpose the slides of the liver sections were prepared and were examined by microscope to see the changes in liver hepatocytes.

Identification of active constituents by HPLC: Qualitative determination of active constituents having hepatoprotective activity was done by using HPLC. Sample was prepared by adding small amount of extract in 5 mL distilled water, 12 mL methanol and kept for 5 min. Then 6 mL distilled water was again added and kept for 5 min. 10 mL of 15 M HCl was added and placed in oven for 2 hours. Final solution was filtered with syringe filter. Phenolics were separated using a shimpack CLC-ODS (C-18) column, 25 cm × 4.6 mm, 5 µm. Mobile phase used was isocratic: ACN : dichloromethane: methanol-60:20:20 at a flow rate of 1 mL/min. Samples were analysed using UV-visible detector at 280 nm at room temperature while Kaempferol was separated at 248 nm (Sultana et al., 2008).

Statistical analysis: Statistical analysis was done with one -way ANOVA (analysis of variance). Results were showed by Mean ± SE.

Results

The average value of ALT of normal animals was 50.2 ± 3.0 (Table I). Treatment with paracetamol raised this value to 172.4 ± 6.2. Administration of aqueous methanolic extract of *C. murale* at dose of 250 and 500 mg/kg brought the level of this enzyme to 92.8 ± 13.5 and 72.2 ± 14.4 respectively which is comparable to ($p>0.001$) standard hepatoprotective drug, silymarin 61 ± 6.1. The average value of AST of normal animals was 57.8 ± 3.4. Treatment with paracetamol raised this value to 167.4 ± 6.5. Administration of aqueous methanolic extract of *C. murale* at dose of 250 and 500 mg/kg brought the level of this enzyme to 90.6 ± 10.3 and 73.4 ± 10.6 respectively which is comparable to ($p>0.001$) standard hepatoprotective drug, silymarin 68.8 ± 2.8. The average value of ALP of normal animals was 173.6 ± 16.2. Treatment

Table I

Effect of aqueous methanolic extracts of *Chenopodium murale* at 250 and 500 mg/kg on ALT, AST, ALP and total bilirubin

	ALT (U/L)	AST (U/L)	ALP (U/L)	TB (mg/dL)
Normal	50.2 ± 3.0	57.8 ± 3.4	173.6 ± 16.2	0.7 ± 0.03
Paracetamol control 250 mg/kg	172.4 ± 6.2	167.4 ± 6.5	407.6 ± 36.4	2.0 ± 0.2
Silymarin 50 mg/kg + PCM 250 mg/kg	61.0 ± 6.1[b]	68.8 ± 2.8[b]	190.4 ± 12.4[b]	0.8 ± 0.02[b]
Extract 250 mg/kg + PCM 250 mg/kg	92.8 ± 13.5[b]	90.6 ± 10.3[b]	220.2 ± 9.3[a]	0.9 ± 0.1[b]
Extract 500 mg/kg + PCM 250 mg/kg	72.2 ± 14.4[b]	73.4 ± 10.6[b]	197.2 ± 17.6[b]	0.7 ± 0.04[b]

Mean ± S.E; [a]$p<0.01$; [b]$p<0.001$

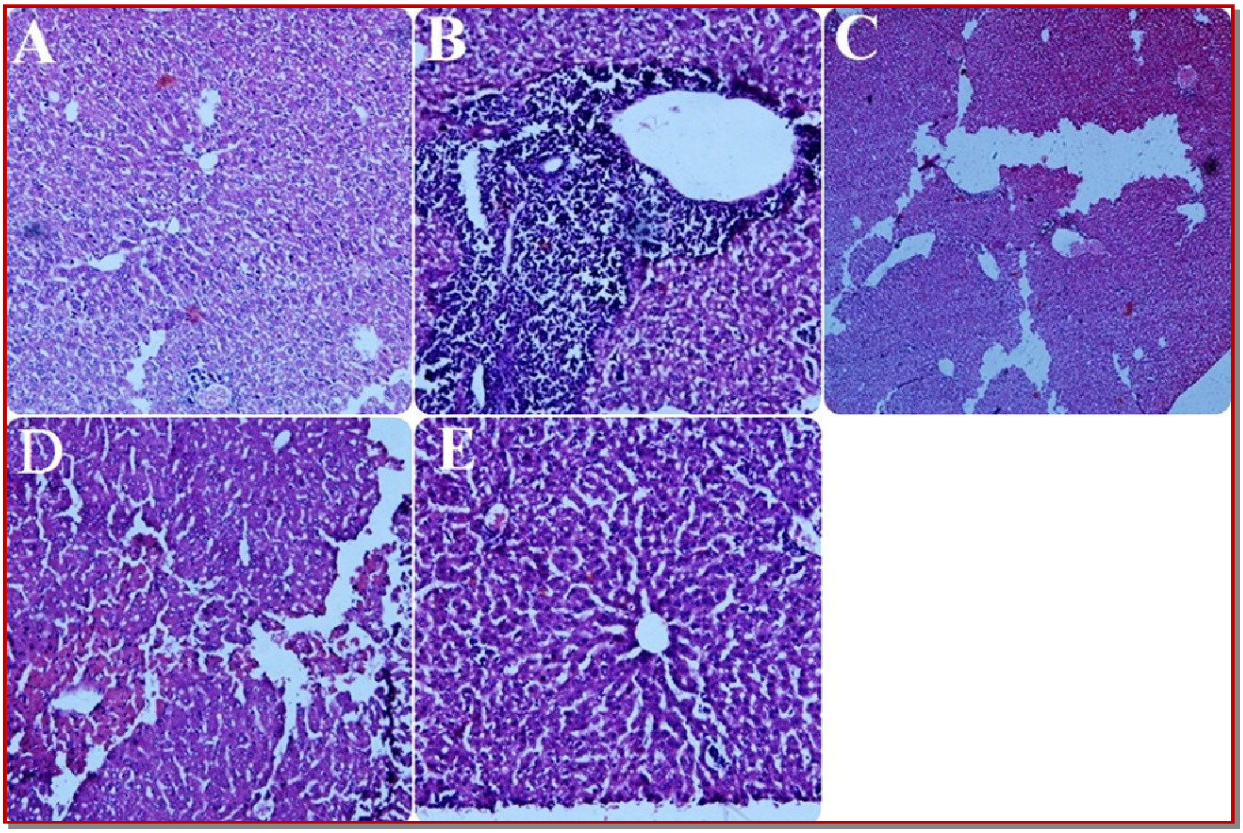

Figure 1: (A) Normal hepatocytes; (B) PCM treated; (C) Silymarin treated; (D) *C. murale* 250 mg/kg treated; (E) *C. murale* 500 mg/kg treated

with paracetamol raised this value to 407.6 ± 36.4. Administration of aqueous methanolic extract of *C. murale* at dose of 250 and 500 mg/kg brought the level of this enzyme to 220.2 ± 9.3 and 197.2 ± 17.6 respectively which is com-parable to ($p>0.001$) silymarin 190.4 ± 12.4. Similarly the average value of total bilirubin of normal animals was 0.72 ± 0.03. Treatment with paracetamol raised this value to 2.0 ± 0.2. Administration of aqueous methanolic extract of *C. murale* at dose of 250 and 500 mg/kg brought the level of this enzyme to 0.9 ± 0.1 and 0.7 ± 0.04 respectively which is comparable to ($p>0.001$) silymarin 0.8 ± 0.02.

Histopathology studies showed that the liver architecture was normal in the control group (Figure 1). While the PCM-treated group showed the sever periportal inflammation, tissue necrosis, ballooning and dilation in sinusoidal spaces. Silymarin-treated group showed only mild inflammation with no ballooning. Animals treated with aqueous methanolic extract of *C. murale* also showed mild inflammation with no ballooning.

HPLC analysis of aqueous methanolic extract of *C. murale* indicated the presence of quercetin, gallic acid, chlorogenic acid, *p*-coumaric acid, sinapic acid and kaempferol (Figure 2). Among which quercetin, gallic acid and kaempferol are hepatoprotective, the chemical structures are shown in Figure 3.

Discussion

In present study aqueous methanolic extracts of *C. murale* were studied at two concentrations. Results were almost similar at both concentrations. Aqueous methanolic extract decreased the elevated level of ALT, AST, ALP and bilirubin as compared to control group. *C. murale* contains flavonoids, saponins and terpenoids (Abbas et al., 2012). Hepatoprotective properties of a plant may be due to the presence of flavonoids (Vijay and Padmaa, 2011), saponins, (Kumar et al., 2011) or triterpenoids (Kim et al., 2004). *C. murale* also contain β-Sitosterol and Stigmasterol (Ahmad et al., 2003) which showed antihepatotoxic action (Patra et al., 2009). Stigmasterol caused decrease in lipid peroxidation in liver and increase in CAT, SOD and GSH activities (Panda et al., 2009). The plant also contained heat stable SOD enzyme which give protection against oxidative stress (Khanna-Chopra et al., 2004).

Previous studies have indicated that quercitin (Janbaz et al., 2004; Rasheed et al., 2013) and kaempferol (Adewusi and Afolayan, 2010) and gallic acid (Jadon et al., 2007) have hepatoprotective activity. In the current study HPLC analysis showed the presence of quercetin, Kaempferol, and gallic acid so it seems that this protection of liver by aqueous methanolic extract of *C. murale* may be due to presence of these hepatopro-

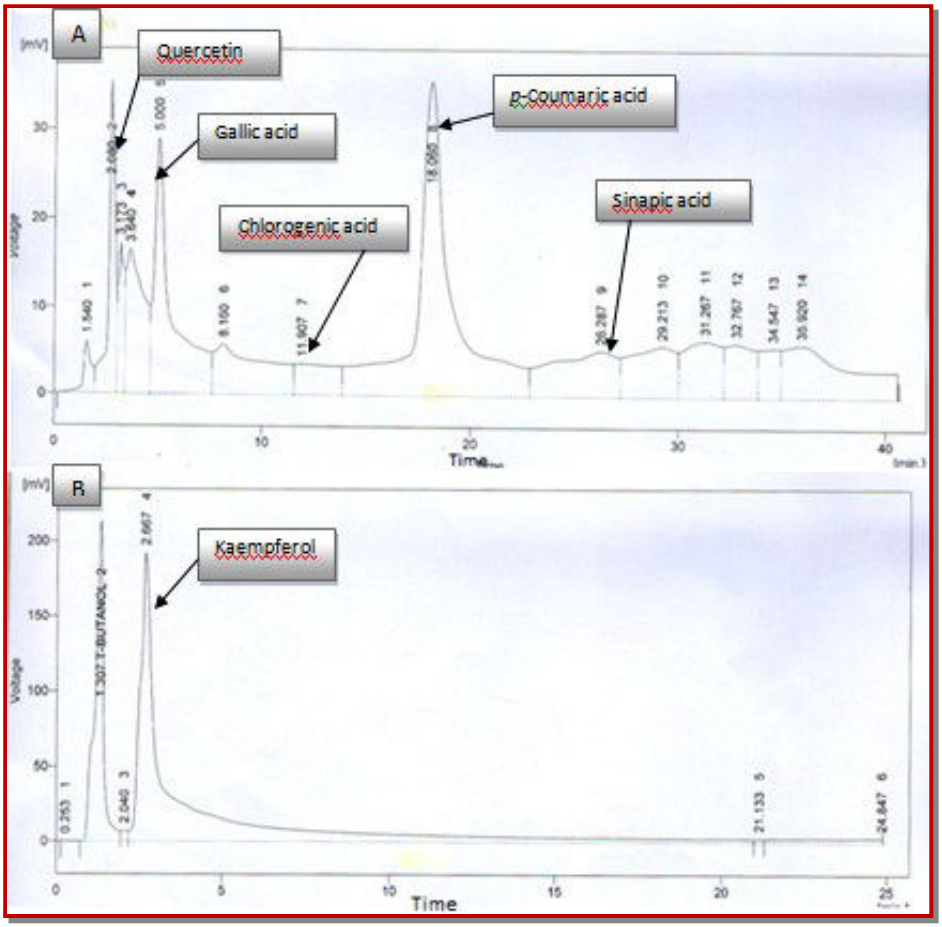

Figure 2: HPLC analysis of aqueous methanolic extract of *C. murale*. A) Detection at 280 nm; B) Detection at 248 nm

tective constituents. Our findings suggest that aqueous methanolic extract of *C. murale* is hepatoprotective against paracetamol induced liver injury in mice.

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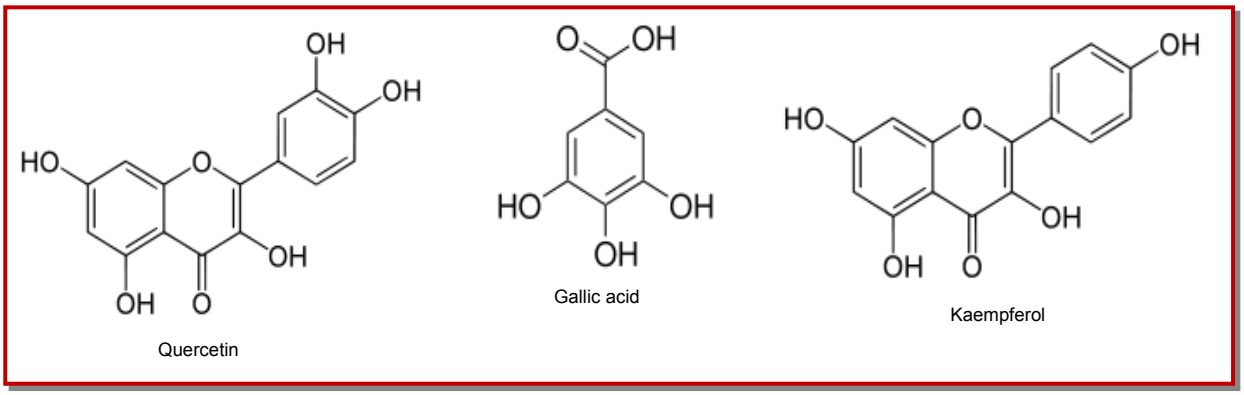

Figure 3: Hepatoprotective constituents of *C. murale*

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