


## Research Article

# Uroprotective and Hepatoprotective Potential of *Anagallis arvensis* against the Experimental Animal Model

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*Anagallis arvensis* (*A. arvensis*) belonging to the family *Primulaceae* is traditionally used for liver and kidney diseases. The aim of the study was to evaluate the uroprotective and hepatoprotective potentials of *A. arvensis* in cyclophosphamide-induced interstitial cystitis and paracetamol-induced hepatotoxicity rat model, respectively. Nociception, bladder weight, vesical vascular permeability, Gray's criteria for edema and hemorrhage, and levels of nitric oxide, catalase, and glutathione were estimated and studied in the cystitis model. Liver function test, lipid profile, and histopathological evaluation were carried out in the hepatoprotective activity. Oral administration of methanol extract of *A. arvensis* significantly reduced bladder weight, vesical vascular permeability, edema, hemorrhage, nitric oxide, IL-6, and TNF- $\alpha$ , while the level of catalase and glutathione peroxide was increased. In hepatoprotective activity, pretreatment with *A. arvensis* significantly decreased the level of liver markers (Bilirubin, ALT, AST, and ALP) and lipid profile (cholesterol, TG, LDL, and VLDL). Histopathological studies confirmed the biochemical findings of both studies. GC-MS analysis presented the presence of antioxidant phytoconstituents. Thus, it was concluded that *A. arvensis* might act as uroprotective and hepatoprotective due to the presence of antioxidant phytochemicals in the rodent model. Isolation and identification of phytochemicals present in the methanol extract of *A. arvensis* and evaluation of their exact mechanism of action become mandatory in future studies.

## 1. Introduction

Interstitial cystitis (IC) is a painful condition of the urinary bladder. Manifestations may incorporate urinary incontinence, direness, and pain. This can incorporate an unexpected desire to urine and increase urine recurrence and urinate at night [1–3]. Reactive oxygen species (ROS) assume a significant part in cyclophosphamide-induced IC [4, 5]. It was accounted that an increase in reactive oxygen species (ROS) prompts bladder edema, irritation, and extravasation,

demonstrating that ROS might assume a basic part in a rodent model of cyclophosphamide (CYP)-induced cystitis [6, 7].

Free radicals, pollutants, xenobiotic, food additives, and use of alcohol in excess amounts leads to liver damage, hepatitis, and cirrhosis. The liver is a significant organ in the body, playing a significant job in digestion, biotransformation, and detoxification of toxins [8]. The use of some medications may elevate liver function and cause damage to the liver. One such medication is acetaminophen (paracetamol). Paracetamol overdose causes hepatotoxicity and elevates liver function and

thus causes liver damage, hepatic necrosis, hepatic lesions, and even death in humans [9].

Medicinal plants are very important for human health. Traditional medicines are being used by 80% of the world population [10]. The plant, *Anagallis arvensis* (*A. arvensis*), from the family *Primulaceae* is utilized for liver diseases, skin rashes, moles, urinary contamination, irritation, wounds, and kidney problems [11]. *A. arvensis* is a low-growing plant present in Asia, Europe, and North America [12]. This plant has reported antimicrobial, antiviral, antifungal, antioxidant, anti-inflammatory, antidiuretic, expectorant, and anticancer activities and can be used for liver cirrhosis, kidney stone, urinary infection, and dermatological purposes. Various studies have shown the presence of saponin, glycosides, kaempferol, rutin, stigmasterol, alkaloids, anagalligenin, carbohydrates, and arvenin-I and II in different parts of *A. arvensis* [13]. The aim of the present study was to investigate the protective effect of methanol extract of the whole plant of *A. arvensis* against cyclophosphamide-induced cystitis and paracetamol-induced hepatotoxic rodent models.

## 2. Materials and Methods

**2.1. Plant Collection.** The plant *Anagallis arvensis* was collected from a village near Lahore, Pakistan. The plant was dried under shade for 14 days. Identification of the specimen was done by the Department of Botany, GC University, Lahore (GC.Herb.Bot.3747).

**2.2. Animals Used.** Wistar rats (both male and female) of weight 200–250 g were used. Animals were kept in a controlled laboratory environment (by monitoring temperature, humidity, and light/dark cycle). Animals were permitted for seven days, for adaptation prior to any investigational techniques and fed with standard food and water.

**2.3. Chemicals and Reagents.** Methanol of analytical grade (Sigma Aldrich) was used to prepare the extract of the plant. All the chemicals used like buffered formalin, Evans blue dye, formamide, sodium hydroxide, paracetamol, silymarin, cyclophosphamide, and mesna were of high quality and pure analytical grade.

**2.4. Plant Extract Preparation.** The extract of the plant was prepared by soaking 988 g grinded dried plant in ethanol (3, l) in a manner that a layer of solvent was maintained 24 hours on the top. After 3 days, the filtration of the extract was carried out with a cotton cloth and the plant material was again subjected to be soaked in a further 1.5 L of 70% methanol for further three days. Finally, the second part of the extract was filtered in the same manner and both extracts were mixed. The extract obtained was further concentrated using a rotary evaporator for 12 hours at a temperature not more than 40°C at 60 revolutions per minute (RPM) and 0.09 megapascal (Mpa) pressure. The concentrated extract was placed in an oven at a temperature of 40°C to get a semisolid form. The percentage yield of the plant extract was 16.1%.

### 2.5. Cyclophosphamide-Induced Cystitis

**2.5.1. Study Design.** Animals were divided in 5 groups and each group contained 4 animals. Group I (Control): Normal saline was administered to rats. Group II (diseased control): cyclophosphamide (i.p.) 150 mg/kg was administered to rats on the 1<sup>st</sup>, 4<sup>th</sup>, and 7<sup>th</sup> day. Group III: (standard drug treated): rats received mesna (i.v.) (40 mg/kg) and cyclophosphamide was also used on the 1<sup>st</sup>, 4<sup>th</sup>, and 7<sup>th</sup> day 3 hours post mesna administration. Group IV and V: (methanol extract of treated *Anagallis arvensis*): Rats received methanol extract of *Anagallis arvensis* (MEOAA) 250 and 500 mg/kg dissolved in normal saline orally and cyclophosphamide (150 mg/kg) on the 1<sup>st</sup>, 4<sup>th</sup>, and 7<sup>th</sup> day 3 hours post extract administration, respectively [14].

**2.5.2. Sample Collection.** Animals were sacrificed on the 8<sup>th</sup> day and blood was collected, clotted, and centrifuged at 4000 rpm for 20 minutes to separate serum from blood. The urinary bladders were collected and weighed. The macroscopic evaluation was done according to Gray's criteria. The urinary bladders were preserved in 10% formalin for histological assessment.

**2.5.3. Assessment of Nociception.** Before the dissection on day 8, rats were put exclusively in a case isolated in 9 squares for 5 minutes. A number of squares crossed with 4 paws were counted and noted [14].

**2.5.4. Vesical Vascular Permeability.** Evans blue dye (25 mg/kg) was used to measure vesical vascular permeability. It was administered through I.V route 1 hour before the animal was dissected. After dissection, the bladder was placed in 1 ml formamide and incubated over night at room temperature. Then, the optical density of the extracted dye was evaluated spectroscopically at 600 nm [14].

**2.5.5. Assessment of Weight.** Bladder wet weight was measured to quantify the vesical edema immediately after the dissection. The bladder wet weight was measured in mg [14].

**2.5.6. Macroscopic Evaluation.** Bladders were examined for edema and hemorrhage according to Gray's criteria. Edema was considered extreme (3+) when fluid was seen outside and inside in the bladder wall, moderate (2+) when seen distinctly in inner mucosa, mild (1+) among typical and moderate, and none (0) as normal. Hemorrhage was scored as (3+) intravesical clusters, (2+) mucosal hematomas, (1+) widening of bladder vessels, and (0) as normal [14].

**2.5.7. Determination of Bladder Nitric Oxide, Oxidative Stress, IL-6, and TNF- $\alpha$ .** Nitric oxide, glutathione, catalase, IL-6, and TNF- $\alpha$  were measured calorimetrically in blood samples of different groups by commercially available kits [14].

## 2.6. Paracetamol-Induced Hepatotoxicity

**2.6.1. Study Design.** The animals were divided into 5 groups with five animals in each group. Group-I (control group) received 0.9% normal saline (5 ml/kg). Group-II (diseased control) received paracetamol (250 mg/kg) dissolved in water for 7 days on daily basis. Group-III (standard drug treated) received silymarin (50 mg/kg) for 7 days on a daily basis and paracetamol was also used 3 hours post silymarin administration. Group-IV and V (MEOAA treated): received MEOAA 250 and 500 mg/kg for 7 days on a daily basis and paracetamol was administered 3 hours after silymarin treatment [15].

**2.6.2. Biochemical Parameters.** All the animals were cervical decapitated 24 hours after the last treatment and the blood was collected, clotted, and centrifuged at 4000 rpm for 20 minutes for serum separation. Lipid profile and liver function tests were evaluated by commercially available kits (Germany) with an automated analyzer (Micro lab 300, Merk Germany) [15].

**2.6.3. Histopathological Studies.** For the fixation of the liver and urinary bladder, the formalin (10%) buffered solution was used; then the urinary bladder and liver were cut into thin sections (5-6 micrometers) and put on slides which were stained by Eosin and Hematoxylin (*H* and *E*). These slides were examined under a light microscope for inflammation, ballooning, degeneration, and necrosis of the hepatocytes and bladder tissue.

**2.6.4. Statistical Analysis.** Data were presented as mean  $\pm$  SEM. Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's Test. The significant difference between groups was indicated by  $P < 0.05$ .

**2.6.5. GC-MS Analysis.** For GC-MS analysis, a capillary column (0.25  $\mu$ m, 30 m  $\times$  0.25 mm) was used. As a carrier, helium gas was preferred. The velocity flow of the column was set to 1 ml/min by using split less mode and 0.5  $\mu$ L injection volume. First, the oven temperature was set at 110°C for 2 minutes and then increased at a rate of 10°C per minute until the temperature reached 200°C. After that, the temperature was decreased at a rate of 5°C per minute until the temperature reached to 280°C per minute. The final temperature was maintained and set at 280°C. 70 eV ionizing voltage was used for MS and 200°C was set for the quadrupole analyzer. The mass to charge range was set at 20 to 800 [16].

## 3. Results

### 3.1. Cyclophosphamide-Induced Cystitis

**3.1.1. Macroscopic Evaluation.** It was observed that the edema and hemorrhage levels were markedly increased in the diseased control group when compared to the control

group and decreased significantly in the standard-drug treated and MEOAA (250 mg/kg and 500 mg/kg)-treated groups of rats as shown in Table 1.

**3.1.2. Effect of *A. arvensis* on Bladder Weight.** The weight of the bladder was measured immediately after the dissection for the control, diseased control (cyclophosphamide 150 mg/kg), standard drug (mesna 40 mg/kg), and MEOAA (250 mg/kg and 500 mg/kg) -treated groups of rats. It was seen that the weight of the bladder of the diseased control group was more when contrasted with the control group. Whereas, the weight was less in MEOAA (250 mg/kg and 500 mg/kg)-treated groups (Figure 1).

**3.1.3. Effect of *A. arvensis* on Nociception.** On the 7<sup>th</sup> day of dosing, nociception was assessed by making 9 square boxes and a number of squares crossed with 4 paws were counted in 5 minutes. It was observed that diseased control rats crossed fewer boxes as compared to control group rats and the number of crossed boxes markedly increased in MEOAA (250 mg/kg and 500 mg/kg)-treated groups (Figure 2).

**3.1.4. Effect of *A. arvensis* on Vesical Vascular Permeability.** Evans blue dye was used to determine vesical vascular permeability. The optical density of extracted dye was checked spectroscopically at 600 nm. It was determined that the optical density was increased in the diseased control group (cyclophosphamide 150 mg/kg) as compared to the control group and significantly decreased in standard drug (Mesna 40 mg/kg) -treated and extract of *A. arvensis* (250 mg/kg and 500 mg/kg) treated groups of rats as shown in Table 2.

**3.1.5. Effect of *A. arvensis* on Nitric Oxide, Catalase, Glutathione Peroxide, IL-6, and TNF- $\alpha$ .** Nitric oxide, catalase, glutathione peroxide, IL-6, and TNF- $\alpha$  were measured for control, diseased control (cyclophosphamide 150 mg/kg), standard drug (Mesna 40 mg/kg), and MEOAA (250 mg/kg and 500 mg/kg)-treated groups of rats. The level of nitric oxide, IL-6, and TNF- $\alpha$  in blood was enhanced in the diseased control group as compared to the control group and markedly decreased in standard drug (Mesna 40 mg/kg) and extract of *A. arvensis* (250 mg/kg and 500 mg/kg)-treated group of rats and catalase enzyme and glutathione peroxide activity markedly reduced in diseased control group as compared to control group and markedly enhanced in standard drug-treated and extract of *A. arvensis* (250 mg/kg and 500 mg/kg) treated group of rats as shown in Table 3.

**3.2. Histopathological Evaluation.** Histopathological studies were carried out with urinary bladder isolated from the control, diseased control, mesna, and MEOAA-treated groups. It was observed that pretreatment with mesna and MEOAA ameliorated the morphological changes induced by CYP (Figure 3).

TABLE 1: Effect of *Anagallis arvensis* edema and hemorrhage of the urinary bladder.

Group	Edema level	Hemorrhage level
Control	0	0
Diseased control	3+	3+
Standard drug-treated	1+	0
MEOAA (250 mg/kg)	2+	1+
MEOAA (500 mg/kg)	1+	0

The values in the table are the mean of 4 animals (MEOAA; methanol extract of *Anagallis arvensis*).

### 3.3. Paracetamol-Induced Hepatotoxicity

**3.3.1. Effect of *A. arvensis* on the Liver Function Test.** It was observed that the levels of bilirubin, ALT, AST, and ALP were significantly increased for the diseased control group when compared to the control group and significantly decreased for standard drug (silymarin 50 mg/kg) and MEOAA (250 mg/kg and 500 mg/kg) treated groups of rats as shown in Table 4.

**3.3.2. Effect of *A. arvensis* on the Blood Lipid Profile.** It was noticed that the level of cholesterol, TG, LDL, VLDL, and total lipids were markedly increased in the diseased group when compared to the control and significantly decreased in standard drug (silymarin 50 mg/kg) and MEOAA (250 mg/kg and 500 mg/kg)-treated groups of rats. However, the level of HDL was significantly decreased in the diseased control group as compared to the control group of rats and markedly increased in MEOAA (250 mg/kg and 500 mg/kg)-treated groups as shown in Table 5.

**3.4. Histopathological Evaluation.** Liver samples were collected from all treated groups and pathological changes were observed after staining with *H* and *E*. Figure 4 presents the protective effect of MEOAA in paracetamol-induced deleterious effects on the liver.

**3.5. GC-MS Analysis.** The GC-MS analysis of plant extract showed the presence of different phytochemicals including 4-ethyl-5-octyl-2,2 bis (trifluoromethyl)-1,3-dioxalane, benzene dicarboxylic acid, and monolinoleoyl glycerol-trimethyl silyl ether as shown in Table 6 (Figure 5).

## 4. Discussion

Medicinal plants are very important for human health. Traditional medicine is used by 80% of the world population [20].

**4.1. Effect of *A. arvensis* on Cyclophosphamide-Induced Cystitis.** The urinary bladder is an empty, muscular organ above and behind the pubic bone in the pelvic cavity [21]. Cyclophosphamide is oftentimes associated with many side effects, the most clinically applicable occasion is the urological toxicity, particularly shown as hemorrhagic cystitis. This is definitely not an immediate impact of CYP; however, it is identified with the development of 4-hydroxy

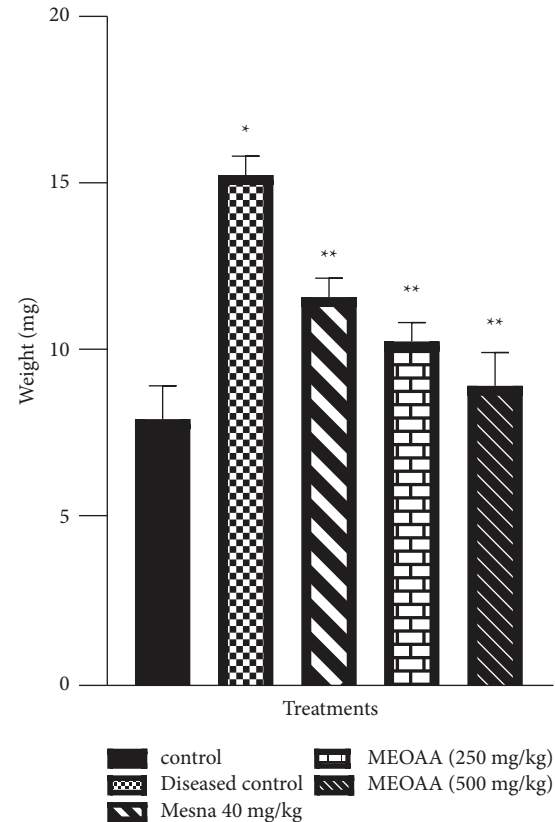


FIGURE 1: Data have been presented as mean  $\pm$  SEM. Evaluation of weight of the bladder for control, diseased control (cyclophosphamide 150 mg/kg), standard drug (Mesna 40 mg/kg)-treated, and methanolic extract of *A. arvensis* (MEOAA) (250 mg/kg and 500 mg/kg)-treated group of rats. (\* $P < 0.05$  compared to the control group, \*\* $P < 0.05$  compared to the diseased control group;  $n = 4$ ).

metabolites, basically acrolein [22]. In spite of the prophylactic administration of mesna, a large number of patients under treatment with cyclophosphamide develop cystitis [23, 24]. Extracts of *A. arvensis* are used in various illness conditions and known for their beneficial effects on the genitourinary system. Our study revealed the beneficial effects of *A. arvensis* in preventing the inflammation evoked by cyclophosphamide administration in rats. In the present study, Gray's criteria were used to differentiate between various levels of edema and hemorrhage in all groups. The score for edema and hemorrhage was markedly elevated in diseased control as compared with the control group. This showed that treatment with cyclophosphamide had increased edema and hemorrhage in the diseased group [14]. Pretreatment with MEOAA (250 mg/kg and 500 mg/kg) reduced significantly edema and hemorrhage score in the cyclophosphamide treated group of rats. These findings were in parallel with a previous study [25]. The weight of the wet urinary bladder was measured to quantify vesical edema in all groups. Pretreatment with the extracts of *A. arvensis* decreased the weight of the bladder in the cyclophosphamide treated group of rats. These results were in parallel with the previous study [25]. The rats were set independently in a container partitioned into 9 squares, for 5 min., and the

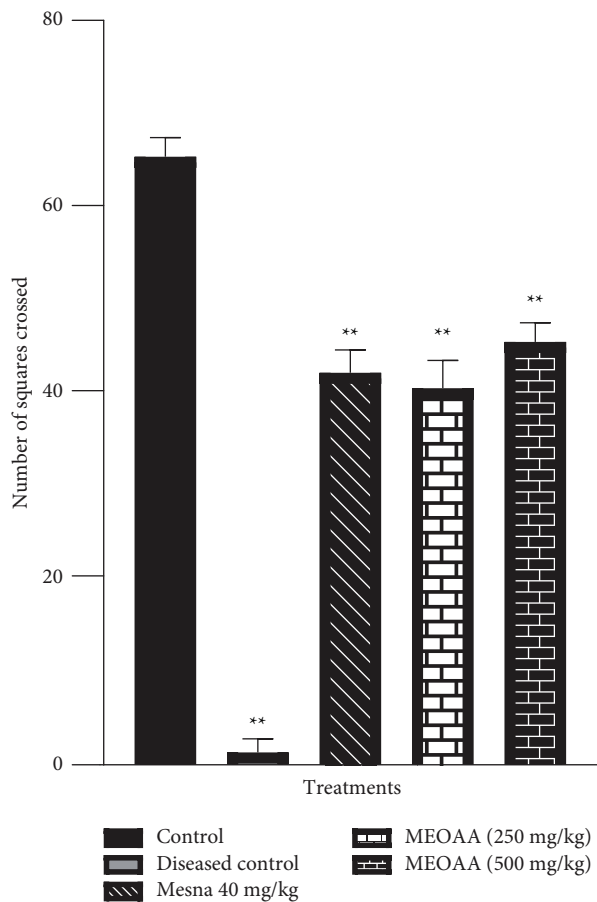


FIGURE 2: Data have been presented as mean  $\pm$  SEM. Determination of a number of squares crossed by control, diseased control (cyclophosphamide 150 mg/kg), standard drug (Mesna 40 mg/kg)-treated and methanolic extract of *A. arvensis* (MEOAA) (250 mg/kg and 500 mg/kg) treated group of rats. (\* $P < 0.05$  compared to the control group, \*\* $P < 0.05$  compared to the diseased control group;  $n = 4$ ).

number of squares crossed with 4 paws was taken as a record of locomotor action [26, 27]. Pain-like crisis produced by the injection of cyclophosphamide was consistently prevented by the oral treatment with MEOAA. Previous studies showed that marked analgesic effect in several models [14, 28]. Evans blue dye was used to determine vesical vascular permeability. The optical density of extracted dye was checked spectroscopically at 600 nm. The permeability of the extracted dye decreased in methanol extract of *A. arvensis* treated group of rats. Previous studies have reported the same findings [25]. The nitric oxide work in the inflammation by the cyclophosphamide cystitis had been emphasized. Nitric oxide is by all accounts delivered by means of cholinergic incitement of mucosal muscarinic receptors, probably on urothelial cells, and this would eventually influence bladder work. Cyclophosphamide fundamentally expanded the statement of the inducible nitric oxide synthase in rat urothelial cells [29]. Among inflammatory mediators, TNF- $\alpha$  and IL-6 are mainly involved in CYP-induced IC and their levels have been enhanced [14]. However, pre-treatment with the extracts of

TABLE 2: Effect of *A. arvensis* on vesical vascular permeability.

Groups	Optical density
Control	0.004 $\pm$ 3.33
Diseased control	0.069 $\pm$ 0.001*
Standard drug-treated	0.005 $\pm$ 0.001**
MEOAA (250 mg/kg)-treated	0.014 $\pm$ 0.001**
MEOAA (500 mg/kg)-treated	0.009 $\pm$ 0.001**

Determination of the vesical vascular permeability from control, diseased control, standard drug (Mesna 40 mg/kg), and methanol extract of *A. arvensis* (MEOAA) (250 mg/kg and 500 mg/kg)-treated groups of rats (\* $P < 0.05$  compared to the control group, \*\* $P < 0.05$  compared to diseased control group;  $n = 4$ ).

*A. arvensis* (250 mg/kg and 500 mg/kg) reduced the level of nitric oxide, TNF- $\alpha$ , and IL-6 in cystitis. Previous studies reported the same findings [14].

Catalase is an endogenous antioxidant enzyme and protects the cell from being destroyed or damaged through the reactive oxygen species (ROS). Intracellular ROS and nitric oxide production are activated by acrolein. This further results in the collapse of cellular pathways and causes cell death in the urinary bladder [22]. Pretreatment with the extract of *A. arvensis* (250 mg/kg and 500 mg/kg) enhanced the level of catalase enzyme in cyclophosphamide-induced cystitis. These findings were in accordance with the previous studies [22]. It has been reported that cyclophosphamide decreases the urinary bladder content of glutathione [30]. Cyclophosphamide suppressed the nonenzymatic defense system but also the enzymatic defense mechanism represented by enzymatic glutathione-S-transferase was also suppressed. The glutathione-S-transferase system detoxified the toxic metabolite of cyclophosphamide that is responsible for drug urotoxicity. The diminished glutathione level alongside the diminished glutathione S-transferase action would deny the bladder tissue of a significant safeguard framework and make it more helpless against the malicious impacts of cyclophosphamide [31]. Pretreatment with the extracts of *A. arvensis* enhanced the level of glutathione in cyclophosphamide induced cystitis. Previous studies reported similar findings [32]. The histopathological studies of urinary bladder were carried out. The histopathological examination of the control group is as shown in (Figure 3), rat urinary bladder showed normal transitional epithelial lining and intact mucosa. The interstitial tissue and muscle coats were arranged in a normal pattern. No evidence of any disease, granuloma, or malignancy was seen. Urinary bladder of the rat from diseased control showed focally ulcerated mucosal lining. The interstitial tissue and cleaves of muscle coats contain scattered mixed inflammatory cells. There was focal urinary bladder necrosis. These features are suggestive of interstitial cystitis. The standard drug treated group showed normal contracted transitional epithelium of urinary bladder. The urinary bladder treated with MEOAA (250 mg/kg and 500 mg/kg) presented intact-looking transitional epithelial lining. Congestion in the sub-mucosa was seen. No evidence of any disease, granuloma, or malignancy was seen. These features are suggestive of a mild inflammatory process likely healing cystitis.



TABLE 3: Effect of *A. arvensis* on nitric oxide, catalase enzyme and glutathione peroxide.

Groups	Control (Mean ± SEM)	Diseased control (Mean ± SEM)	Standard drug-treated (Mean ± SEM)	MEOAA 250 mg/kg treated (Mean ± SEM)	MEOAA 500 mg/kg treated (Mean ± SEM)
Nitric oxide (U/ mg)	19.00 ± 0.577	50.66 ± 1.202*	27.66 ± 1.453**	30.33 ± 0.33**	26.66 ± 0.88**
Catalase (U/mg)	23.33 ± 0.66	6.33 ± 1.76*	19.66 ± 0.88**	18.00 ± 0.55**	21.00 ± 0.57**
Glutathione peroxide (U/mg)	26.00 ± 0.57	8.66 ± 0.33*	23.33 ± 0.88**	20.00 ± 0.55**	23.00 ± 0.57**
IL-6 (pg/ml)	30.75 ± 1.49	110.5 ± 1.04*	83 ± 0.04**	73 ± 0.91**	64.5 ± 0.64**
TNF-α (pg/ml)	16.25 ± 1.11	47.75 ± 0.75*	39.5 ± 0.86**	36 ± 0.41**	30.75 ± 0.85**

Determination of nitric oxide, catalase enzyme, glutathione peroxide, IL-6, and TNF-α levels in the blood for control, diseased control (cyclophosphamide 150 mg/kg), standard drug (Mesna 40 mg/kg)-treated and methanol extract of *A. arvensis* (MEOAA) (250 mg/kg and 500 mg/kg)-treated group of rats. (\* $P < 0.05$  compared to the control group, \*\* $P < 0.05$  compared to diseased control group;  $n = 4$ ) (IL-6; interleukin-6, TNF-α; tumor necrosis factor-α).

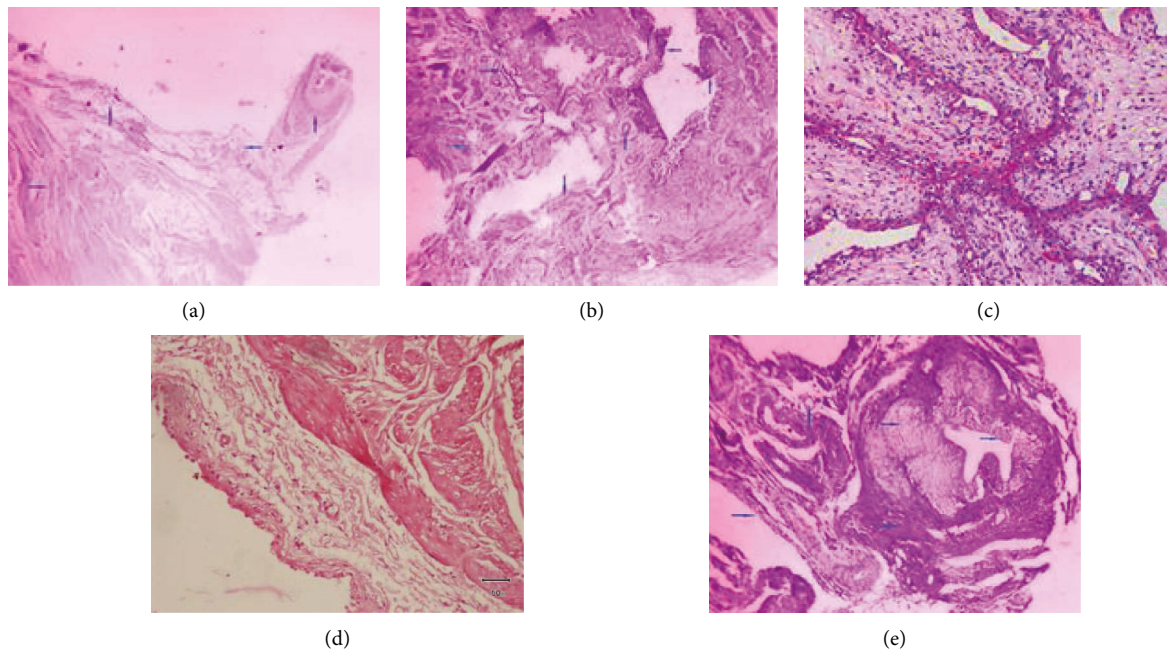


FIGURE 3: Histopathological examination of rat urinary bladder from the control group (a), diseased control (b), standard drug-treated (c), MEOAA (250 and 500 mg/kg)-treated groups (d and e) in the cyclophosphamide-induced cystitis model. (a) shows normal transitional epithelial lining and intact mucosa and (b) shows focally ulcerated mucosal lining. The interstitial tissue and cleaves of muscle coats contained scattered mixed inflammatory cells. There was focal urinary bladder necrosis, (c) normal contracted transitional epithelium of urinary bladder, and (d) shows intact looking transitional epithelial lining. Congestion in the submucosa was seen, (e) intact uniform-looking transitional epithelial lining. The muscle coats were arranged in a normal pattern. No evidence of any disease, granuloma, or malignancy was seen.

TABLE 4: Effect of *A. arvensis* on LFTs.

Groups	Bilirubin (mg/dl)	ALT (mg/dl)	AST (mg/dl)	ALP (mg/dl)
Control	0.46 ± 0.03	52.66 ± 0.31	105.00 ± 1.15	188.00 ± 4.61
Diseased control	0.80 ± 0.058*	58.66 ± 0.66*	147.66 ± 3.38*	267.66 ± 4.97*
Standard drug-treated	0.53 ± 0.033**	53.33 ± 0.33**	102.33 ± 0.88**	121.66 ± 2.18**
MEOAA (250 mg/kg)	0.43 ± 0.030**	51.00 ± 0.55**	107.00 ± 0.57**	177.33 ± 7.42**
MEOAA (500 mg/kg)	0.33 ± 0.032**	52.00 ± 0.57**	104.33 ± 1.85**	103.66 ± 2.17**

Data have been presented as mean ± SEM. Determination of the level of bilirubin, ALT, AST, and ALP in control, diseased control (paracetamol 250 mg/kg), standard drug (silymarin 50 mg/kg), and methanol extract of *A. arvensis* (MEOAA) (250 mg/kg and 500 mg/kg) treated groups of rats (\* $P < 0.05$  compared to the control group, \*\* $P < 0.05$  compared to the diseased control group;  $n = 4$ ).

**4.2. Effect of *A. arvensis* on Paracetamol-Induced Hepatotoxicity.** Liver assumes an essential part in directing physiological processes. It is associated with various

capacities like digestion, emission, stockpiling, and detoxification of numerous medications, and xenobiotic. Liver illnesses might be brought about by poisonous synthetic

TABLE 5: Effect of *A. arvensis* on blood lipid profile.

Groups	Cholesterol (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Total lipids (mg/dl)
Control	86.00 ± 2.309	81.33 ± 0.88	25.66 ± 0.66	43.66 ± 1.20	19.00 ± 1.73	455.65 ± 5.60
Diseased control	110.00 ± 4.93*	95.66 ± 1.45*	18.66 ± 0.88*	58.66 ± 4.09*	26.33 ± 0.88*	509.31 ± 2.40*
Standard drug treated	82.66 ± 1.76**	74.33 ± 2.18**	24.66 ± 0.33**	46.00 ± 1.00**	15.66 ± 1.20**	443.31 ± 5.04**
MEOAA (250 mg/kg)	84.64 ± 0.33**	75.66 ± 1.20**	26.66 ± 1.20**	37.33 ± 0.66**	16.00 ± 0.57**	440.29 ± 2.33**
MEOAA (500 mg/kg)	77.33 ± 1.76**	70.33 ± 0.33**	28.00 ± 1.52**	29.00 ± 0.57**	14.00 ± 1.52**	418.66 ± 3.93**

Data have been presented as Mean ± SEM. Determination of the level of cholesterol, TG, HDL, LDL, VLDL, and total lipids for control, diseased control (paracetamol 250 mg/kg), standard drug (silymarin 50 mg/kg), and MEOAA (250 mg/kg and 500 mg/kg) treated groups of rats (\* $P < 0.05$  compared to the control group, \*\* $P < 0.05$  compared to the diseased control group;  $n = 4$ ) (MEOAA; methanol extract of *Anagallis arvensis*, TG; triglycerides, HDL; high density lipoproteins, LDL; low-density lipoproteins, VLDL; very low density lipoproteins).

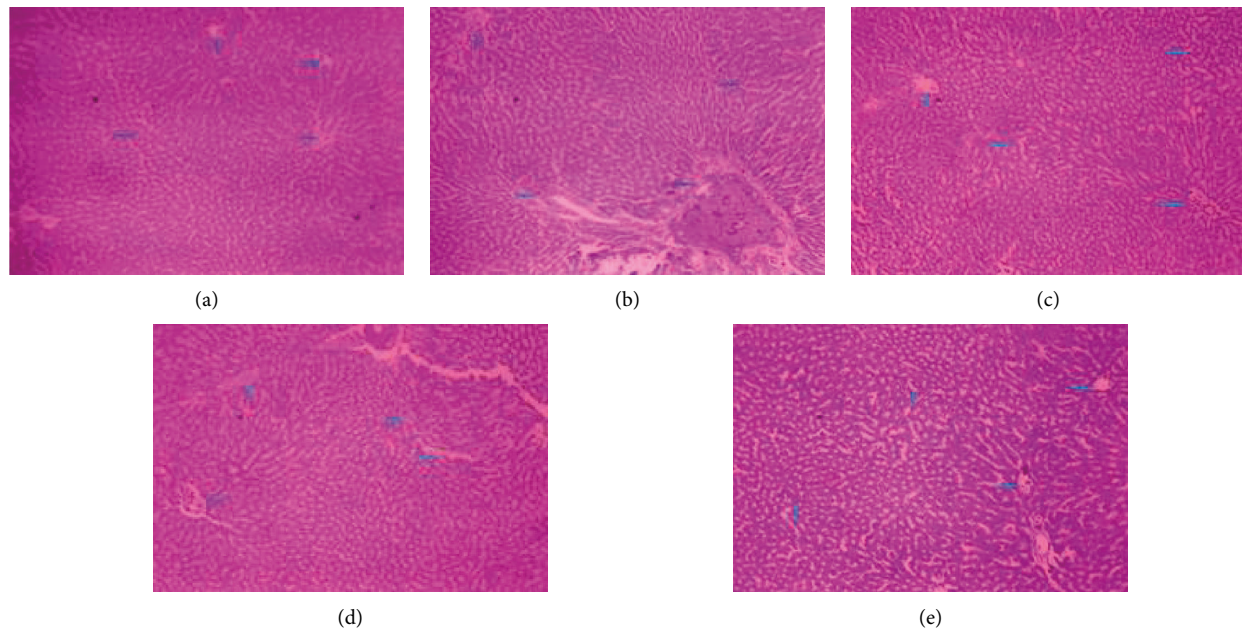


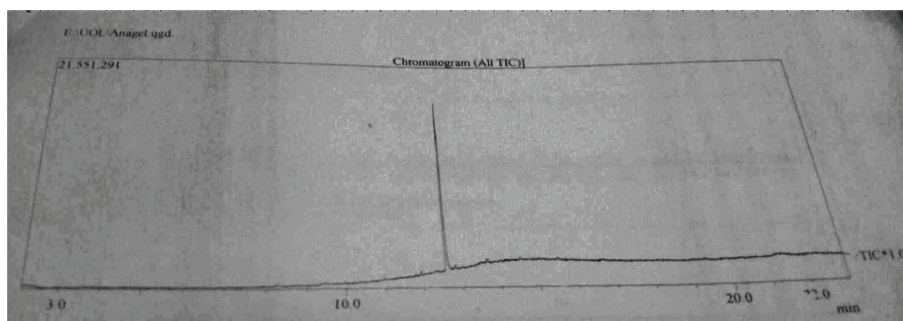
FIGURE 4: Histopathological examination of rat liver from control group (a), diseased control (b), standard drug treated (c), MEOAA (250 and 500 mg/kg) treated groups (d and e) in paracetamol-induced hepatotoxicity. (a) Control group showed normal hepatic architecture. No inflammation, hydropic degeneration, fibrosis, necrosis, atypia, steatosis, or malignancy were seen, (b) diseased rat liver revealed gross hydropic degeneration, steatosis, and necrosis of hepatocytes. However, some areas showed periportal fibrosis with adjacent aggregates of lymphocytes with neutrophilic inflammation, (c) standard drug-treated group showed near to normal parenchymal architecture of hepatic plates with some areas of hepatocytic vacuolation. No atypia and malignancy was seen, (d) MEOAA (250 mg/kg) treatment showed mild degeneration of hepatocytes and central veins dilation. Sinusoids were dilated and congested. Liver tissue showed apoptotic changes. No atypia and malignancy was seen, (e) MEOAA (500 mg/kg) treatment represents normal hepatic architecture. Regeneration of hepatic cells was observed. No atypia or malignancy was seen.

compounds (toxins, chemotherapeutics, peroxidized oil, aflatoxin, carbon tetrachloride, chlorinated hydrocarbons), abundance utilization of liquor, contaminations, and immune system/jumble. Numerous hepatotoxic synthetics harm liver cells by lipid peroxidation and other oxidative harms in the liver. Upgraded lipid peroxidation created during the liver microsomal digestion of ethanol may bring about hepatitis and cirrhosis [10]. Paracetamol is a known antipyretic and analgesic agent and safe when used in therapeutic doses but at high toxic doses produces fatal effects and hepatic necrosis in human, rat, and mice livers. Paracetamol is converted into a metabolite called NAPQI. Then tripeptide glutathione binds to this NAPQI and converts it into a nontoxic metabolite. When there is more and more depletion of the glutathione then the accumulation of the toxic metabolite occurs and results in

hepatotoxicity [33]. NAPQI has a toxic effect on cellular proteins primarily by oxidation [34, 35]. Paracetamol increases serum levels of bilirubin, ALT, AST, and ALP and alters the function and structure of liver cells [36]. The increased level of aminotransferases may be due to the destruction of liver cells and leakage of liver cells in the blood. Pretreatment with the extracts of *A. arvensis* showed protection against the injurious effect of paracetamol and decreased the level of bilirubin, ALT, AST, and ALP in the serum. The findings of the present study agreed with the previous study [15]. Oxidative stress has been accounted for as a significant reason for paracetamol-actuated liver harm, and due to oxidative stress excessive production of free radicals can harm macromolecules as lipids, and the level of total lipids like LDL, VLDL, and cholesterol increased, whereas, the level of HDL decreased. The increased level of

TABLE 6: GC-MS Analysis of methanol extract of *Anagallis arvensis*.

No.	Rt	Molecular wt. (g/mol)	Molecular formula	Phytocomponents	Uses
1	11.92	350	C <sub>15</sub> H <sub>24</sub> F <sub>6</sub> O <sub>2</sub>	4-Ethyl-5-octyl-2,2 bis (trifluoromethyl)- 1,3-dioxalane	Antioxidant [17]
2	12.59	390	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	1,2-Benzenedicarboxylic acid	Antioxidant [18]
3	12.83	498	C <sub>27</sub> H <sub>54</sub> O <sub>4</sub> Si <sub>2</sub>	Monolinoleoyl glycerol trimethylsilyl ether	Antioxidant, anti-inflammatory, antimicrobial [19]

FIGURE 5: GC-MS chromatogram of the methanol extract of *Anagallis arvensis*.

total lipids was due to impaired fat metabolism [15]. Pre-treatment with the methanol extracts of *A. arvensis* (250 mg/kg and 500 mg/kg) decreased the level of LDL, VLDL, cholesterol, and total lipids and the level of HDL increased in paracetamol induced hepatotoxicity. These findings were in parallel with a previous study [15, 37].

Histopathological examination of rat liver from the control group revealed normal hepatic architecture. However, the diseased rat liver revealed gross hydropic degeneration, steatosis, and necrosis of hepatocytes. The result of the liver of standard drug treated group showed near normal parenchymal architecture of hepatic plates with some areas of hepatocytic vacuolation present. In places hepatocytic hyperplasia is prominent and suggestive of the compensatory phenomenon. No atypia and malignancy was seen. The methanol extract of *A. arvensis* (250 mg/kg)-treated group presented mild degeneration of hepatocytes and central vein dilation. The sinusoids are dilated and congested. The liver tissue showed apoptotic changes and rats treated with methanol extract of *A. arvensis* (500 mg/kg) revealed near to normal hepatic architecture and slightly dilated central veins. There were moderate grade inflammatory cells infiltrated present adjacent to hepatic plates. The regeneration of hepatic cells was observed.

GC-MS analysis of methanol extract of *A. arvensis* shown the presence of antioxidant and anti-inflammatory phytochemicals including 4-ethyl-5-octyl-2, 2 bis (trifluoromethyl), benzene dicarboxylic acid, and monolinoleoyl glyceroltrimethyl silyl ether [17–19].

## 5. Conclusion

*Anagallis arvensis* has been shown to have uroprotective properties by lowering NO, TNF-, IL-6, and oxidative stress, while its hepatoprotective effect includes antioxidant and

membrane-stabilizing properties on hepatocytes. These effects could be attributed to the antioxidant and anti-inflammatory phytochemicals found in *A. arvensis*.

## Data Availability

The data related to findings will be available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Authors' Contributions

Ushna Shabbir, Joham Javed, and Sana Ismail collected the data. Irfan Anjum and Muhammad Naveed Mushtaq presented the concept and designed the study protocols. Muhammad Nasir Hayat Malik, Ayesha Pervaiz, Anika Tariq, and Saeeda Noreen isolated the tissues and performed histopathological studies for both activities. Misbah Wazir, Zeenat Islam, Muhammad Majid, and Sehrish Mansha analyzed all data and prepared graphs with statistical analysis, while Dr. Zaka Ur Rehman helped in performing GC-MS analysis of plant extract and its analysis. All authors have read the original manuscript and agreed to submit it.

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