Effect of *Trianthema portulacastrum* extracts on hematologic and hepatic enzymes disorders induced by carbon tetrachloride in rabbits (*Oryctolagus cuniculus*)

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**ABSTRACT**

The study evaluated therapeutic effects of aqueous and ethanolic extracts of *Trianthema portulacastrum* against carbon tetrachloride (CCl₄) induced toxicity in rabbits. The entire sample plant was identified using key, dried, ground, and bioactive compounds were extracted using Soxhlet extractor. Adult rabbits (N = 60, 1 kg body weight) were divided into 5 groups: group I as controls, group II received CCl₄ only, groups III to V received CCl₄ in normal saline but were later orally administered different doses (75, 150 and 225 mg kg⁻¹) of silymarin, aqueous or ethanolic extracts of *T. portulacastrum*. Hematological parameters were investigated 6 days after administration of the ameliorants. The effects of *T. portulacastrum* ethanolic and aqueous extracts were then compared to effects of standard silymarin drug. It was discovered that ethanolic extracts conferred hepatoprotection on rabbits as silymarin did, suggesting that these extracts may be used instead of silymarin to improve animals health exposed to toxicity.

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**1. Introduction**

Carbon tetrachloride (CCl₄) is an industrial solvent that induces hepatotoxicity in animals by initiating oxidative stress and inflammation [1,2], thereby causing injuries to different organs in the body. It has also been found to induce renal injury [3]; and liver necrosis [4]; in mice. CCl₄ use has been shown to increase the activity of liver enzymes such as Alanine aminotransferase (ALT), and Aspartate aminotransferase (AST) due to liver necrosis [5,6]; It also induces acute and chronic tissue and organ damage by activating the cytochrome P450 first-stage system to form the metabolically reacted trichloromethane (CCl₃) and peroxytrichlor methyl (CCl₃OO˙) radicals [7]. These free radicals bind to large molecules such as proteins, fats, and nucleic acids. They also stimulate inactivated CYP2E1 activity [8]; and increase oxo8dG concentrations in animal tissues [9].

*Trianthema portulacastrum* Linn (or *T. monogyna* Linn) is a common weed also known as horse purslane, carpet weed, or giant pigweed that is traditionally valued by Indian and African cultures for its numerous medicinal and bioactive compounds. The plant is grown in most tropical countries including Pakistan, Sri Lanka, Ceylon, and India [10]. It is a dietary plant containing fibres, potassium, protein, sodium, riboflavin, and iron [11]. Extracts of *T. portulacastrum* possess several pharmacological properties including painkiller, antiseptic, anti-pyretic, anti-inflammatory [12,13]. For example, the roots are known to have antipyretic, analgesic, spasmyloytic and anti-inflammatory activity [13]; and are commonly used for the treatment of various diseases such as jaundice and dropsy [14]. The leaves are diuretic and are applied in the treatment of various disorders. For example in Nigeria, the leaves are used to treat gonorrhea [15]. They are also potentially hepato-protective [16,17], anthelmintic [18], antioxidative [19], anti-hypertensive and anti-hypolipidemic [17]. Ethanolic leaf extract of *T. portulacastrum* has been found to be effective against hepatic damage produced by paracetamol and thioacetamide in rats [20] and by aflatoxin B1 [21].

The present study was aimed at evaluating the curative effect of different extracts (aqueous and ethanolic) of *T. portulacastrum* against hemato-hepatotoxicity...
induced by CCl4 in rabbits. The hepato-protective effects of the extracts were compared to those of silymarin, a known active ingredient in drugs for evaluating hepato-protective activity [22]. We hypothesized that extracts of T. portulacastrum will induce hepato-protective effects from CCl4 intoxication to similar levels as in silymarin.

2. Materials and methods

2.1. Silymarin as standard drug

Silymarin is an active ingredient in drugs for evaluating hepato-protective activity [22]. Sample of silymarin drug was obtained from the market.

2.2. Preparation of plant extracts

Trianthema portulacastrum plants were obtained from farmers and identified by plant taxonomists. The entire plant was dried and ground in a mechanical blender. The air-dried and pulverized plant material was dipped for four days in 500 g L⁻¹ of solvent ethanol (95%) and aqueous medium, separately, and stirred on a hot plate for 3 days to dissolve solvents and then filtered using 15 mm Whatman filter paper to obtain the final ethanolic and aqueous extracts. After the evaporation of ethanol and water using rotary evaporator, residues approximately 30 g were kept at 4 °C. The extracts were dissolved in distilled water just before administration.

2.3. Phytochemical analysis of T. portulacastrum extracts

Trianthema portulacastrum is a food vegetable that contains fibre, potassium, protein, sodium, riboflavin, and iron [11]. The main component of T. portulacastrum Linn. is ecdysterone and other ingredients are trianthenol, 3-acetylrutitic acid, 5,2-dihydroxy-7-methoxy-6,8-dimethyl flavone, lepturomol, 3,4-dimethoxy cinnamic acid, 5-hydroxy-2-methoxybenzaldehyde, P-metoxybenzoate and beta-cyanine [23]. Qualitative and quantitative Phytochemical analysis for the confirmation of bioactive compounds like phenols, steroids, flavonides, carbohydrates, glycosides, alkaloids and Saponins in different extracts of T. portulacastrum was carried out using the standards methods in reports of Santhi and Sengottuvel [24] and Ali and Ibrahim [25] respectively.

2.3. Experimental animals and treatments

Ethical approval was obtained from the ethics committee of Department of life sciences, The Islamia University Bahawalpur, under reference no. 2856/LS. Adult rabbits (Oryctolagus cuniculus; N = 60, 1 kg body weight) were selected and kept in battery cages maintained at 27 ± 1 °C. The rabbits were given a regular diet and a friendly environment for three days to acclimatize. To conduct the experiment, the rabbits were divided into 3 groups (N = 20 each) according to the ameliorants: silymarin (a standard hepato-protective drug), ethanolic or aqueous extracts of T. portulacastrum. Each group was further sub-divided into five groups containing four animals per sub-group. Animals in sub-group I were given normal diet and no CCl4 intoxication (control) or administration of ameliorants, animals in sub-group II received CCl4 intoxication in normal saline (1 ml kg⁻¹ d⁻¹) but without ameliorant, animals in sub-groups III to V received CCl4 in normal saline but were later orally administered different doses (75, 150 and 225 mg kg⁻¹, respectively) of each ameliorant. After 96 h of receiving the CCl4, blood samples were taken through the ear vein of the animals to check the changes in the activity of hepatic enzymes and various hematological abnormalities. Afterwards, the ameliorants were orally administered 6 times at 24 h interval.

2.4. Blood collection

After 6 days of administration of ameliorants, 1 ml of blood sample was collected from all subgroups of rabbits with anticoagulant in complete blood count (CBC) vials and without anticoagulant in serum collection vials. The collected samples were analyzed for activity of enzymes such as ALT (alanine aminotransferase), AST (Aspartate Aminotransferase), ALP (alkaline phosphatase), LDH (lactate dehydrogenase). Plasma Malondialdehyde (MDA) and blood properties such as red blood cells (RBC) and white blood cells (WBC) counts, hemoglobin (Hb) and packed cell volume (PCV) were analyzed as described by Devasagayam et al. [26]; Houwen [27]; Wolford et al. [28] and Caisey and King [29] respectively.

2.5. Statistical analysis

Data were analyzed using ANOVA and Tukey’s test was used to compare the differences among treatment means at a 95% confidence interval. SPSS version 2.0 was used for the analyses.

3. Results

The presence of active phytochemical elements in T. portulacastrum was discovered in the current investigation. The phytochemical active components of T. portulacastrum were analyzed qualitatively and quantitatively from whole plant, with the results shown in Tables 1 and 2.

Administration of CCl4 to the rabbits decreased red blood cell (RBC) count by about 40% than in healthy control animals. However, the application of 225 mg kg⁻¹ silymarin and ethanolic extract of T. portulacastrum at 95% confidence interval significantly
increased RBC count to similar levels in the control animals. Compared to CCl4 stressed animals without ameliorants, aqueous extract of T. portulacastrum at 225 mg kg\(^{-1}\) significantly increased RBC count but was lower than observed in the healthy animals. Exposure to CCl4 increased the white blood cell (WBC) count by about 30% than in healthy animals. However, the application of 75 mg kg\(^{-1}\) silymarin and 225 mg kg\(^{-1}\) ethanolic extracts suppressed WBC count in stressed rabbits to similar levels in the healthy control animals. The packed cell volume (PCV) levels decreased by about 10% after administration of CCl4, compared to healthy animals; but the application of 150 and 225 mg kg\(^{-1}\) silymarin and ethanolic extract and 225 mg kg\(^{-1}\) aqueous extracts increased PCV levels in stressed rabbits, but PCV levels were lower than in the control animals (Table 3).

Exposure of the rabbits to CCl4 increased activity of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and malondialdehyde (MDA) by 16%, 25%, 15%, 30% and 35%, respectively than in healthy control animals. However, application of the ameliorants generally decreased activity of these enzymes, compared to CCl4 stressed animals. Generally, application of the ethanolic and aqueous extracts of T. portulacastrum did not vary much compared to the application of silymarin on the various hematological parameters (Table 4).

### 4. Discussion

In this study, we investigated the hepato-protective activity of different doses of silymarin, carbon tetrachloride, in adult rabbits. Results obtained from the leave extracts were compared with those from silymarin, a standard drug for evaluating hepatoprotective activity. According to our results, different doses of the leave extracts (ethanolic and aqueous) showed varying effects on the measured hematological parameters.

Carbon tetrachloride (CCl4) is a hepatotoxin that is known to create oxidative stress and hepatic necrosis.

### Table 1. Qualitative phytochemical analysis of Trienthma portulacastrum extracts.

| Phytochemicals test | Aqueous extract (%) | Ethanol extract (%) |
|---------------------|---------------------|--------------------|
| Alkaloids (Mayer’s Test) | +                   | ++                 |
| Flavonoids (H\(_2\)SO\(_4\) test) | +                   | ++                 |
| Steroids (Liebemann Burchard test) | +                  | +                  |
| Terpenoids (Silkowskii test) | +                  | +                  |
| Phenols (Ferric Chloride test) | +                 | ++                 |
| Saponins (Frothing test) | +                  | ++                 |
| Glycosides (Nitroprusside test) | +                 | ++                 |

Note: ++ = Present in large quantity; + = Present in small quantity.

### Table 2. Quantitative phytochemical analysis of Trienthma portulacastrum extracts in percent of dry weight of extract.

| Phytochemicals test | Aqueous extract (％) | Ethanol extract (％) |
|---------------------|---------------------|---------------------|
| Alkaloids           | 3.3                 | 6.1                 |
| Flavonoids         | 2.1                 | 4.8                 |
| Steroids           | 0.2                 | 0.3                 |
| Terpenoids         | 0.2                 | 0.4                 |
| Phenols            | 0.8                 | 2.2                 |
| Saponins           | 2.5                 | 5.5                 |
| Glycosides         | 0.6                 | 0.4                 |

### Table 3. Effect of different doses of ameliorants on hematological parameters in rabbits after CCl4 intoxication.

| Parameters                | RBC        | WBC        | Hb          | PCV         |
|---------------------------|------------|------------|-------------|-------------|
| Control                   | 7.28 ± 0.05\(^{a}\) | 9.87 ± 0.07\(^{a}\) | 13.19 ± 0.04\(^{d}\) | 41.29 ± 0.08\(^{a}\) |
| CCl4 stress               | 4.25 ± 0.03\(^{bcd}\) | 14.35 ± 0.06\(^{b}\) | 7.35 ± 0.05\(^{b}\) | 36.18 ± 0.07\(^{ab}\) |
| Silymarin                 | 4.02 ± 0.04\(^{ab}\) | 9.82 ± 0.04\(^{a}\) | 13.36 ± 0.04\(^{d}\) | 36.19 ± 0.09\(^{ab}\) |
| 75 mg kg\(^{-1}\)        | 4.46 ± 0.17\(^{de}\) | 15.11 ± 0.02\(^{d}\) | 6.58 ± 0.07\(^{a}\) | 37.18 ± 0.04\(^{c}\) |
| 150 mg kg\(^{-1}\)       | 7.09 ± 0.06\(^{f}\) | 14.15 ± 0.10\(^{b}\) | 7.66 ± 0.15\(^{b}\) | 40.29 ± 0.08\(^{d}\) |
| 225 mg kg\(^{-1}\)       | 3.99 ± 0.05\(^{a}\) | 13.11 ± 0.10\(^{d}\) | 10.09 ± 0.03\(^{c}\) | 35.56 ± 0.13\(^{a}\) |
| Ethanol extract of T. portulacastrum | 4.40 ± 0.18\(^{cd}\) | 12.74 ± 0.18\(^{c}\) | 10.24 ± 0.07\(^{c}\) | 37.18 ± 0.07\(^{c}\) |
| 75 mg kg\(^{-1}\)        | 7.12 ± 0.06\(^{f}\) | 9.77 ± 0.04\(^{a}\) | 13.12 ± 0.08\(^{d}\) | 40.23 ± 0.08\(^{d}\) |
| Aqueous extract of T. portulacastrum | 4.01 ± 0.05\(^{ab}\) | 14.18 ± 0.10\(^{a}\) | 10.10 ± 0.02\(^{c}\) | 35.70 ± 0.29\(^{ab}\) |
| 75 mg kg\(^{-1}\)        | 4.20 ± 0.04\(^{pc}\) | 13.20 ± 0.10\(^{d}\) | 10.12 ± 0.02\(^{c}\) | 36.33 ± 0.19\(^{b}\) |
| 225 mg kg\(^{-1}\)       | 4.66 ± 0.12\(^{a}\) | 11.05 ± 0.06\(^{b}\) | 10.18 ± 0.04\(^{b}\) | 37.28 ± 0.30\(^{c}\) |

Note: Different letters in the same row show the statistically significant difference (\(p < 0.05\)).
by the formation of free radicals [30]. Its main target organ is the liver, but could also damage other vital organs such as the lungs, heart, and brain [31]. CCl₄ is metabolized (in the liver) by cytochrome P450 2E1 to produce highly reactive trichloromethyl (CCl₃˙) and peroxymethyl (CCl₃OO˙) radicals, both of which are capable of covalently binding to proteins or lipids of cell membranes and organelles, to initiate lipid peroxidation, causing damage to cell membrane, of cell membranes and organelles, to initiate lipid peroxidation, and ultimately liver failure [34].

In comparison to healthy control rabbits, CCl₄ intoxication resulted in considerably higher MDA, a biomarker for lipid peroxidation. Plant extracts (ethanolic and aqueous) and silymarin at dosages of 75, 150, and 225 mg kg⁻¹ reduced the increased MDA formation to a normal level (p < 0.05) in our study. Among the plant extracts, a high dose of ethanolic extract (225 mg kg⁻¹) produced results comparable to those shown in healthy animals. These results coincide with the study of Bishayee et al. [35], who demonstrated that *T. portulacastrum* extract has certain chemical constituents that are capable of inhibiting lipid peroxidation. Administration of ethanolic extracts of *T. portulacastrum* leaves has also been reported to suppress lipid peroxidation in laboratory animals caused by several hepatotoxins such as aflatoxin [21], 7,12-dimethylbenz(a)anthracene [13], paracetamol and rifampicin [36].

The lipid peroxidative degradation of the liver cell plasma membrane produces a variety of enzymes (such as ALT, AST, ALP, LDH). An estimation of the activities of these serum marker enzymes can be used in the diagnosis of hepatic diseases. High levels of these serum enzymes could be indicative of cellular leakage and loss of functional integrity of the cell membrane in the liver [3].

In our study, CCl₄-intoxicated animals showed significantly high levels of ALT, AST, ALP, LDH, and compared to the control animals. Silymarin, as well as the plant extracts, significantly suppressed the elevated levels of the serum enzymes. However, notably, ethanolic extract of *T. portulacastrum* with a dose of 225 mg kg⁻¹ significantly reduced the high serum levels to similar levels in the control animals (p < 0.05). This reduction in the levels of the serum enzymes could indicate that these substances exerted a protective role on the structural integrity of hepatocyte cell membranes by stimulating the regeneration process after being damaged by the hepatotoxin [37]. Also, the reduction in the ALP levels could suggest that these substances (silymarin and leave extracts) restored the stability of the binary function previously damaged by CCl₄ intoxication. These findings are in agreement with previous studies where a significant reduction in the activity of serum enzymes was noted after the supply of alcoholic plant extracts [21,36,38,39].

Administration of silymarin and the plant extracts showed varied responses to the measured biochemical parameters. For example, Silymarin at high dosage (225 mg kg⁻¹) increased RBC and PCV, but low dosage (75 mg kg⁻¹) decreased WBC and increased hemoglobin to similar levels in the control animals. Whereas ethanolic extract at high dosage (225 mg kg⁻¹) significantly normalized all these biochemical parameters to similar levels in the control, compared to silymarin and the aqueous extract (p < 0.05). However, administration of extracts of *Teucrium polium* [40] and *Solanum nigrum* [41] were also found to significantly normalize these parameters in CCl₄ stressed rats. Blood parameters are good indicators of a stressed condition in animals when exposed to toxicants [42].

Because silymarin and ethanolic extract of *T. portulacastrum* had identical findings, it’s likely that this

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**Table 4.** Effect of doses of ameliorants on serum biochemical parameters in rabbits after CCl₄ induced liver necrosis.

| Parameters | ALT (U/L) | AST (U/L) | ALP (U/L) | LDH (U/L) | MDA (μM) |
|-----------|-----------|-----------|-----------|-----------|-----------|
| Control   | 55.36 ± 0.07a | 67.28 ± 0.08b | 146.60 ± 0.15b | 227.20 ± 0.14b | 0.27 ± 0.01ab |
| CCl₄ stress | 66.10 ± 0.16a | 89.39 ± 0.18a | 172.20 ± 0.14g | 318.10 ± 0.12d | 0.41 ± 0.02f |
| Siilymarin | 75 mg kg⁻¹ | 63.12 ± 0.15f | 75.32 ± 0.19f | 167.30 ± 0.20f | 275.60 ± 0.07f | 0.35 ± 0.01de |
|            | 150 mg kg⁻¹ | 59.31 ± 0.14c | 72.77 ± 0.15g | 150.20 ± 0.17c | 242.40 ± 0.18c | 0.31 ± 0.02bc |
|            | 225 mg kg⁻¹ | 54.61 ± 0.14a | 68.44 ± 0.18b | 145.20 ± 0.16a | 225.30 ± 0.14a | 0.27 ± 0.01a |
| Ethanol extract of *T. portulacastrum* | 75 mg kg⁻¹ | 59.95 ± 0.22d | 76.05 ± 0.17f | 167.6 ± 0.18f | 280.40 ± 0.16f | 0.36 ± 0.02de |
|            | 150 mg kg⁻¹ | 60.24 ± 0.16d | 74.35 ± 0.08d | 155.50 ± 0.04d | 260.50 ± 0.14d | 0.34 ± 0.01cd |
|            | 225 mg kg⁻¹ | 54.48 ± 0.19f | 68.37 ± 0.19b | 145.30 ± 0.08a | 225.20 ± 0.14a | 0.27 ± 0.02a |
| Aqueous extract of *T. portulacastrum* | 75 mg kg⁻¹ | 64.45 ± 0.17d | 85.36 ± 0.13f | 167.60 ± 0.14f | 312.40 ± 0.31f | 0.37 ± 0.02e |
|            | 150 mg kg⁻¹ | 63.10 ± 0.22d | 86.69 ± 0.12f | 167.50 ± 0.18f | 305.60 ± 0.17f | 0.34 ± 0.01de |
|            | 225 mg kg⁻¹ | 62.42 ± 0.19d | 86.25 ± 0.14d | 163.00 ± 0.19f | 302.50 ± 1.03f | 0.35 ± 0.02de |

Note: Different letters in the same row show statistically significant difference (p < 0.05).
herbal extract contains the same active ingredient as silymarin. Silymarin is the active ingredient in *Silybum marianum* tinctures, and it’s made up of flavonolignans and flavonoids [43]. The flavonoids account for 4.8% of the dry weight of the ethanolic extract of *T. portulacastrum*, according to the phytochemical analysis. Both silymarin and ethanolic extracts of *T. portulacastrum* may include flavonoids as the primary active ingredient. In particular, the protective effect of an ethanol extract from *T. portulacastrum* at a dose of 225 mg/kg is like that of silymarin, indicating that the alcoholic extract may also be a potential liver-protective factor.

5. Conclusion

The results of our present study confirm the hepatic effect of ethanol extraction from *T. portulacastrum* on experimental-induced liver damage in animal modelling systems. The beneficial effects observed from the extracts are believed to be related to reversing the toxin-induced changes in plasma membrane enzymes. From this study, it is also evident that silymarin and ethanolic extract of *T. portulacastrum* at a dose of 225 mg kg⁻¹ exhibit similar ameliorating effects in reducing CCl₄-induced liver damage in rabbits. It is generally accepted that silymarin exerts its protective response through membrane-stabilizing procedures that block or inhibit lipid peroxide. Since the enzyme leakage is effectively controlled and other biochemical markers react in the same way as silymarin, it seems likely that there is an active principle for this herbal extract. In particular, the protective effect of an ethanolic extract from *T. portulacastrum* at a dose of 225 mg/kg is like that of silymarin, indicating that the alcoholic extract may also be a potential liver protective factor.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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