## Original Article

# NEPHROPROTECTIVE EFFECT OF METHANOLIC EXTRACT OF DINOTHROMBIUM TINCTORIUM IN ALBINO RATS

Safeena Sidiq[1], Talha laique [2], Ayesha Ahmad[3], Kashif Butt[4], Jahanzaib Khan[5], Maryam Rashid[6]

**ABSTRACT:**
Modern world has proven scientifically that medicines derived from animals are important tools in treating ailments today.
**Objective:** In current project, goal was to estimate the nephro-protective effects of methanolic extract of Dinothrombium tinctorium against carbon tetrachloride-induced nephrotoxicity.
**Study design:** It was a randomised control study.
**Methodology:** Aqueous methanolic extract (70% v/v) of Dinothrombium tinctorium (Dt.Cr) was arranged followed by subsequent evaporations. Renal toxicity was induced by $CCl_4$ (2 ml/kg, p.o) in paraffin oil on 7$^{th}$ day of experiment. Administration of methanolic extract of Dinothrombium tinctorium (300mg/kg body weight/day) orally sheltered the $CCl_4$ caused elevation of renal serum markers that include urea and creatinine. There was renal markers elevation in the $CCl_4$ alone treated animals.
**Results:** Administration of methanolic extract to $CCl_4$ encounter protection against the renal toxicity.
**Conclusion**: The findings thus suggested that this methanolic extract can be used as nephroprotective agent against $CCl_4$-induced renal toxicity in albino rats.

**Key Words:** Urea, Creatinine, Medicine

## INTRODUCTION:

Renal ailments are threatening human life worldwide. Nephropathies nowadays are a big dilemma for the health professionals. Treatment options are limited as well as not much effective against renal diseases. According to World Health Organization (WHO) estimatation, 46% of all diseases and 60% deaths globally are because of renal hitches. The sixth leading cause of death globally is the renal ailment.[1]

Kidneys are continuously exposed to environmental toxins which eventually lead to various nephropathies.[2] Nephrotoxicants include carbon tetrachloride, non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics and carcinogens. They all have different sites of actions.[3]

In animal models, carbon tetrachloride ($CCl_4$), has extensively been employed to chemically induce renal injury.[4] Silymarin has been reported to have nephroprotective activity against toxins. As a herbal remedy against nephropathies, its extract from the seeds is being used traditionally.[5]

In modern era, Zootherapy provides an alternative treatment option among other known therapies applied globally. Chemicals from animal origin constitutes 8.7% of 252 essential drugs short-listed by the WHO.[6] In subcontinent, 9% of all traditional medicines come from 31 substances of animal origin.[7]

Traditionally, Dinothrombium tinctorium (Red Velvet Mite) extract has been used in the treatment of multiple medical ailments like paralysis, malaria, urogenital disorders and many other medical conditions.[8] It has antibacterial, antifungal and gastroprotective activity that have been established in previous many publications.[9] The current project was proposed to gauge the nephroprotective activity of methanolic extract of Dinothrombium tinctorium against $CCl_4$ induced nephrotoxicity in albino rats.

[1]Department of Pharmacology, Medical Division Islamia University, Bahawalpur.
[2]Department of Pharmacology, Lahore Medical & Dental College, Lahore.
[3]Department of Radio-oncology, ANMOL, Lahore.
[4]Department of Pulmonology, Jinnah Hospital, Lahore.
[5]Department of Ophthalmology, Victoria Hospital, Bahawalpur.
[6]Department of Pharmacology, AMDC, Lahore.

## MATERIAL AND METHODS:

This project was a randomised control trial and was conducted for 07 days at Pharmacology Department of Islamia University, Bahawalpur in 2017. Reagents used in current project included Diagnostic kits, Silymarin, Carbon Tetrachloride, distilled water, Digital electronic balance, Grinder, Vortex Mixer, Incubator, Centrifuge machine, Rotary Evaporator. All the chemicals were of analytical grade. The species Dinothrombium tinctorium identification was done by the zoology department, IUB. With the help of one kg of Red Velvet Mites, a coarse paste of Red Velvet Mites was waterlogged using 70% v/v aqueous methanol. It was carried for 03 days. Crude extract was extracted from filtrate after filtration by using Rotary Evaporator. Final extract was stored till further use[8]. In this study, 36 male albino rats were selected and separated into 06 groups each comprising of six animals. They were supported at a temperature (25±2°C) and humidity (55-55%) along with 12 hour light and dark cycle. Animals were given standard diet and tap water ad libitum. Acclimatization of subjects was done for seven days before the start of study[9]. Acute toxicity testing was carried out on 25 mice of both genders. They were randomly separated into 5 groups with 5 mice in each group. All the animals had overnight fast. Group1 was served with normal saline (10 ml/kg p.o) treated as normal control. 04 treatment groups were given oral methanolic extract of Dinothrombium tinctorium at increasing doses of 0.3, 1, 3, 5 g/kg respectively. Toxic effects like behavior with other animals, alertness, food intake, change in body weight and mortality were monitored strictly from zero hour till day 14. Carbon tetrachloride (2ml/kg p.o) was employed as nephrotoxic agent in male albino rats in order to assess the nephroprotective activity of methanolic extract of Dinothrombium tinctorium.[10] Division of animals with treatment plan during study is summarized in table #I. On 7$^{th}$ day, with a delay of 30 min after the respective treatments, $CCL_4$ was administered to all groups except control group to induce toxicity. Next day blood was collected to analyze it for renal markers by using standard kit methods.[11]

**Table-1:** Group Treatments for Calculation of Nephroprotective Action

| Sets | Days (1-6) | Day (7) |
|---|---|---|
| **Normal Control** | Distilled water 4 ml/Kg | Distilled water 4 ml/Kg |
| **Intoxicated** | Distilled water 4 ml/Kg | Distilled water 4 ml/Kg + CCI4 (2 ml/Kg) |
| **Rx. Set 1** | Dt. Cr 30 mg/Kg | Dt. Cr 30 mg/Kg + CCI4 (2 ml/kg) |
| **Rx. Set 2** | Dt. Cr 100 mg/Kg | Dt. Cr 100 mg/Kg+CCI4 (2 ml/kg) |
| **Rx. Set 3** | Dt. Cr 300 mg/Kg | Dt. Cr 300 mg/Kg + CCI4 (2 ml/kg) |
| **Control set** | Silymarin 25 mg/Kg | Silymarin 25 mg/Kg + CCI4 (2 ml/Kg) |

ANOVA with Bonferroni test was employed for analysis of data by using SPSS computer program and Mean ± S.E.M was used for expression of results. Significant (*) result values if $p<0.05$.

## RESULTS:

Prepared extract, Dt. Cr, was screened for its phytochemical constituents as below in table-2.

**Table-2:** Phytochemical constituents of Dinothrombium tinctorium

| Biochemical Constituents | |
|---|---|
| Alkaloids | +++ |
| Carbohydrates | ++ |
| Flavonoids | ++ |

(+ Sign indicates the presence and (-) sign indicates absence and number of signs shows the intensity)

Results of renal biomarkers showed significant decrease in their serum levels among groups treated with Dinothrombium tinctorium extract with different doses.

**Table – 3:** Serum Creatinine & Urea Levels in $CCI_4$-intoxicated albino rats.

| Group Allocation | Serum Creatinine (mg/dL) | Urea (mg/dL) | p-value |
|---|---|---|---|
| Control (D/W 4ml/Kg) | 0.50±0.05 | 28.10±2.2 | <0.125 |
| Intoxicated ($CCI_4$ 2 ml/Kg) | 1.79±0.05 | 86.40±4.45 | <0.001* |
| Dt.Cr (30 mg/Kg) + $CCI_4$ | 1.56±0.04 | 74.63±4.22 | <0.01** |
| Dt. Cr (100 mg/Kg) + $CCI_4$ | 1.24±0.08 | 46.03±1.6 | <0.001** |
| Dt. Cr (300 mg/Kg) + $CCI_4$ | 0.79±0.04 | 35.47±2.99 | <0.001** |
| Silymarin (25 mg/Kg) + $CCI_4$ | 0.65±0.03 | 33.45±2.9 | <0.001** |

*Statistically Significant

Acute toxicity studies showed that the extract used in study was practically non-toxic. It was also non nephrotoxic at selected given doses since the biochemical markers were in normal range.

## DISCUSSION:

There are less number of modern medicine available for cure of renal diseases. Hence, the people have moved towards traditional treatment options for many years. Dinothrombium tinctorium was picked in current study due to its old-fashioned use in medical ailments.[9]

In current project, $CCl_4$-induced nephrotoxicity was carried out in male Wistar albino rats to observe its effects as nephroprotective agent. Our work was in line with previous studies who used same agent for induction.[10] Paradoxically, gentamicin was the inducing agent in other studies.[12]

Nephrotoxicity is impaired renal functions produced due to nephrotoxin drugs or other noninfectious agents.[13] Silymarin was used as control drug in current project to relate different strengths of Dinothrombium tinctorium extract as nephroprotective agent. It was used as standard drug in many old publications so our work was in line with past researchers.[5] Serum urea and creatinine levels were analyzed as biochemical renal markers.

Acute toxicity studies were carried out in current project in 25 mice. Strict surveillance for toxic behaviours for 24 hours and then daily for 14 days was conducted in our project. In other studies acute toxicity assay was done but for 24 hours and then daily for just 7 days.[11] Protocol adopted in current study regarding number of animals and groups was similar as adopted in one animals study to see different hepatoprotective effect of Fumairia indica plant extract but some modifications were made in our setting.[14] Different doses of extract were given to treatment groups in current study. In one previous work the plant extract at a dose of 50,100, 200 and 400 mg/kg body wt. exhibited orally to observe its nephroprotective effects. The extract at a dose of 30,100 and 300 mg/kg body wt. administered orally in current project to treatment groups respectively.[11]

**Limitations:**

Our study had a number of limitations like financial constraint and less resources. No histopathological study of renal tissue was done. Only renal function tests were done to assess nephroprotective effect of extract in present study. No similar study is available for comparison. It observed methanolic extract from animal origin as nephroprotective agent.

## CONCLUSION:

The findings indicate that the methanolic extract of Dinothrombium tinctorium can be used as nephroprotective agent in albino rats.

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