**ORIGINAL ARTICLE**

# Histological Evidence of *Red Velvet Mite Extract* as Hepato-Renal Protective Agent

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## ABSTRACT

**Background:** Dugs formed from animals are in common use today for their effective results in treatment.
**Aim:** The goal was to appraise hepato-renal protective effect Dinothrombium tacnitorium methanolic extract in Albino rats on their histological basis.
**Study design**: It was a randomised control study.
**Methodology**: Aqueous methanolic extract (70% v/v) of Dinothrombium tacnitorium (Dt.Cr) was prepared followed by succeeding evaporation using rotary evaporator. Liver and renal toxicity was done by introduction of $CCl_4$ (2 ml/kg, p.o) in paraffin oil on 7$^{th}$ day of experiment. By the end of project, liver and kidneys were taken out under anesthesia. They were sectioned into 5 µm by microtome. They were stained with Eosin-Hematoxylin dye. They were observed for histo-pathological changes. Administration of methanolic extract of Dinothrombium tacnitorium (300mg/kg body weight/day) orally protected against the $CCl_4$ induced elevation of liver enzymes as well as renal serum markers. All hepatic enzymes and renal markers were elevated in the $CCl_4$ alone treated animals. Administration of methanolic extract to $CCl_4$ challenge protected against the hepato-renal toxic agent.
**Conclusion**: Histological findings suggested that the red velvet mite methanolic extract reduces the histo-pathological changes in both liver and kidneys due to $CCl_4$-induced toxicity in albino rats thus it can work as hepato-renal protective agent.
**Key words**: Dinothrombium tacnitorium, Carbon-tetrachloride, hepatic & renal markers, Albino Rats and Histology.

## INTRODUCTION

Globally, the main organs that are usually affected causing health problems are the liver and Kidneys. Up till now new drugs are not very efficient and easily available for their treatment as well as eradication. According to World Health Organization (WHO) estimatation, 46% of all diseases and 60% deaths globally are because of renal and hepatic diseases. After cancer and heart diseases, liver and renal diseases are the biggest cause for high mortality rate worldwide. The fifth and sixth leading cause of death globally are the hepatic and renal ailments respectively[1].

Liver is the major metabolic and excretory organ whereas kidneys work only as excretory organ. Liver metabolizes carbohydrates and protein. It does fat detoxification and regulation of body homeostasis. Poor drug habits, exposure to environmental toxins and alcohol cause liver diseases along with nephropathies[2]. Hepato and nephro toxic agents include carbon tetrachloride, non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics and carcinogens. They all have different sites ae well as mode of actions[3].

Carbon tetrachloride ($CCl_4$) is commonly employed as hepato-renal toxicity inducing agent among animal models (4). Silymarin, the active constituent of *Silybum marianum*, has antioxidant and hepato-renal protective properties. It regains hepatic antioxidant status and renal functions. Its extract is used as a herbal remedy against hepato-renal toxicity traditionally[5].

In world of today, drugs originated from animals work as an alternative treatment option globally. Among the 252 essential drugs, 8.7% constitute animal origin chemicals[6]. In Pakistan, 31 substances derived from animals constitutes 9% of all traditional medicines[7].

*Dinothrombium tacnitorium (Red valvet mite)* has been used in many medical ailments like paralysis, malaria, urogenital disorders as remedy for centuries[8]. Its antibacterial, antifungal and gastroprotective effects have been established years ago (9). The current project was designed to investigate the hepato-renal protective effect by methanolic extract of *Dinothrombium tacnitorium* in albino rats given $CCl_4$ on the basis of their histological findings.

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## MATERIALS AND METHOD

It was a randomised control study. Reagents used in current research included Diagnostic kits, Silymarin, Carbon Tetrachloride, Hematoxylin Canada balsam, Formalin, Paraffin wax, Xylene, Eosin, Distilled water, Digital electronic balance, Grinder, Vortex mixer, Incubator, Centrifuge machine, Rotary Evaporator. All the chemicals were of analytical grade.

**Extract Preparation:** One kg of Red Velvet Mites was acquired locally. The species *Dinothrombium tacnitorium* identification was done by the zoology department, PU. A

coarse paste of Red Velvet Mites was water logged 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 future use[8].

**Animals:** In the study, male Wistar albino rats (200-250g) were used. They were upheld at a standard temperature, humidity with standard diet and tap water ad libitum. Acclimatization of subjects was done for seven days before the start of study[10].

**Hepato-renal toxicity in animals:** Equal division of animals into different groups was done. $CCL_4$ was given (p.o) to all groups except control group to induce toxicity on 7th day as per schedule. The animals were sacrificed 12 h after the last treatment of $CCl_4$. Blood was collected to analyze it for liver as well as renal enzymes by using standard kit methods (10). Treatment schedules (Table 1)

Table 1: Group Treatment schedule

| Groups | Days | Day 7th |
|---|---|---|
| N.control | D.H20 (4 ml/kg) | D.H20 (4 ml/kg) |
| Intoxicated | D.H20 (4 ml/kg ) | D.H20 (4 ml/kg) +CCl4 (2 ml/kg) |
| Rx. Gr. 1 | Extract 30 mg/kg | Extract 30 mg/kg+ CCl4 (2 ml/kg) |
| Rx. Gr. 2 | Extract 100 mg/kg | Extract 100 mg/kg+ CCl4 (2 ml/kg) |
| Rx. Gr. 3 | Extract 300 mg/kg | Extract 300 mg/kg+ CCl4 (2 ml/kg) |
| S.control | Silymarin 25 mg/kg | Silymarin 25 mg/kg + CCl4 ml/kg) |

**Liver & Kidney Histology:** By the end of project, liver and kidneys were taken out from one animal (each group) under anesthesia. They were washed with normal saline and later were preserved in 10% formalin for complete one day before being embedded in paraffin wax. They were sectioned into 5 µm by microtome. They were stained with Eosin-Hematoxylin dye. They were observed for the following histo-pathological changes:

liver ballooning, degeneration of hepatocytes and nephrons, inflammation, apoptosis, necrosis, fibrosis, renal anatomy and architecture for analysis according to the scoring criteria under microscope.

**Statistical Analysis:** ANOVA with Bonferroni test was employed for analysis of data and Mean ± S.E.M was used for expression of results. Significant (*) result values if $p<0.05$.

The findings of *Dinothrombium tacnitorium methanolic* extract with different doses received to animals showed significant decrease in serum hepato-renal markers level.

**Histo-pathological Analysis of Liver:** The intoxicated group had disorganized hepatic architecture with fatty changes around central vein and necrosis. The normal control group had no such changes (Fig.1). Treatment with different doses of Dt.Cr (30, 100 and 300 mg/kg) exhibited improved architecture of liver as compared to intoxicated group. At the higher dose of 300 mg/kg of Dt.Cr, significantly the normal hepatic architecture was restored. The findings were comparable to standard drug like Silymarin where hepatocytes are radiating around the central vein.

**Histo-pathological Analysis of Kidneys:** The intoxicated group had disorganized renal architecture with severe tubular and glomerular degeneration along with necrosis. The normal control group had no such changes (Fig.2). Treatment with different doses of Dt.Cr (30, 100 and 300 mg/kg) exhibited improved architecture of kidneys as compared to intoxicated group. At the higher dose of 300 mg/kg of Dt.Cr, significantly protection against gross structural disorganization was exhibited. The findings were comparable to standard drug like Silymarin where framework of kidneys was preserved

Table-2: Hepato-renal serum marker levels in CCl4-intoxicated albino rats

| Groups | AST Levels (IU/L) | ALT Levels (IU/L) | Levels of ALP IU/L | T.B Level (mg/dl) | Serum Creatinine (mg/dl) | Urea (mg/dl) | P-value |
|---|---|---|---|---|---|---|---|
| Control | 64.61±4.44 | 30.40 ±1.37 | 129.3±3.01 | 0.48±0.02 | 0.50±0.05 | 28.10± 2.2 | 0.125 |
| Intoxicated | 258.8±12.22 | 125.8±4.46 | 447.0±6.81 | 2.95±0.11 | 1.79±0.05 | 86.40±4.45 | <0.001* |
| Dt.Cr. (30 mg/kg) + $CCl_4$ | 208.4±16.63 | 109.3±3.03 | 417.2±13.2 | 2.41±0.16 | 1.56±0.04 | 74.63±4.22 | <0.01* |
| Dt.Cr. (100 mg/kg) + $CCl_4$ | 167.2±8.6 | 77.81±3.2 | 277.9±3.52 | 1.29±0.04 | 1.24±0.08 | 46.03±1.6 | <0.001* |
| Dt.Cr.300mg/kg) + $CCl_4$ | 85.63±3.51 | 49.29±1.9 | 179.6±6.04 | 0.61±0.02 | 0.79±0.04 | 35.47±2.99 | <0.001* |
| Silymarin(25 mg/kg) + CCl4 | 71.36±3.96 | 35.42±2.8 | 140.0±3.81 | 0.58±0.06 | 0.65±0.03 | 33.45±2.9 | <0.001* |

*Statistically Significant

Fig. 1: Histopathological sections of liver ; (A) control (B) intoxicated (C) Treatment group1 (D) Treatment group2 (E) Treatment group 3 (F) CCl4+silymarin in Hepatotoxicity model in rats.

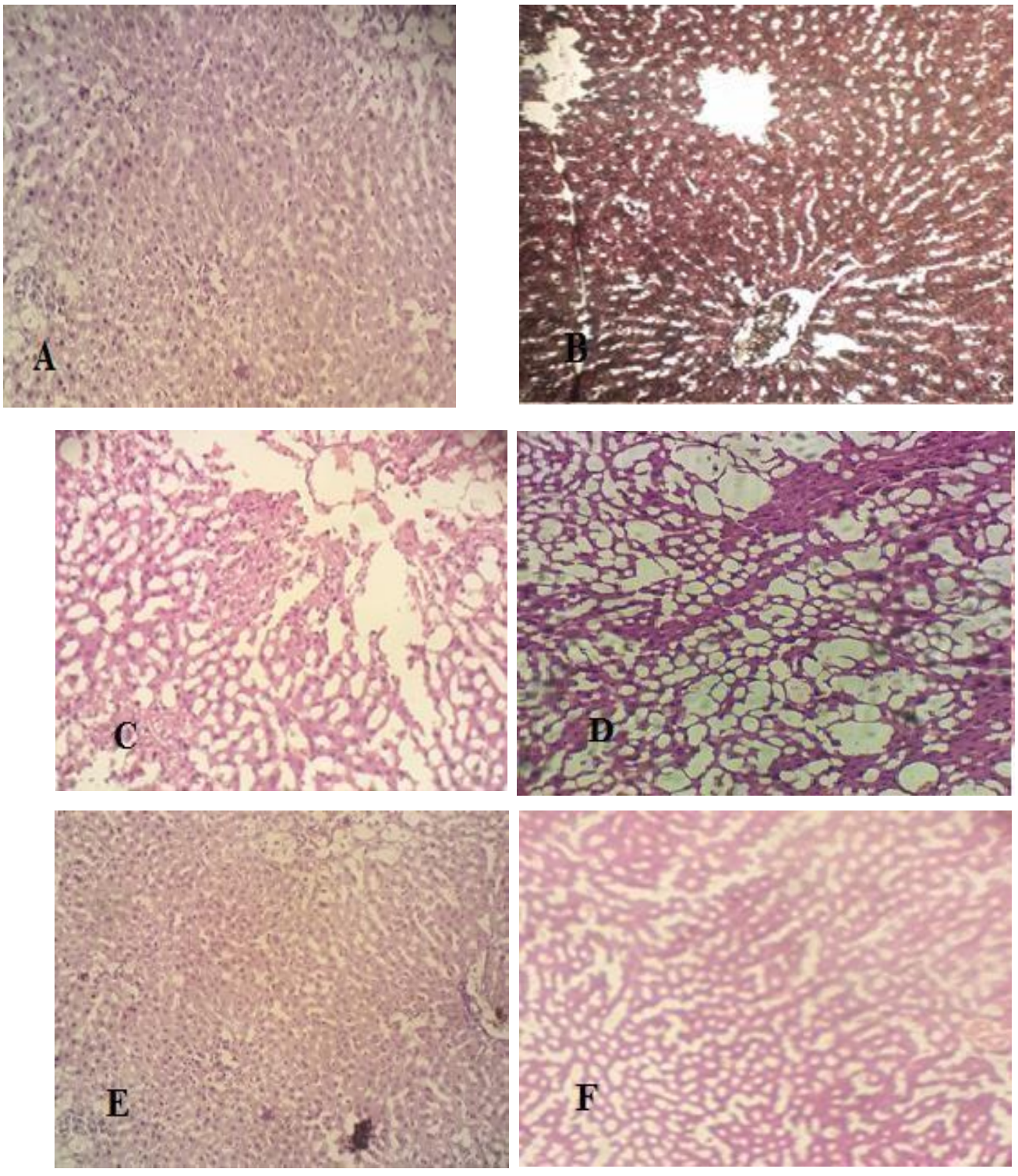
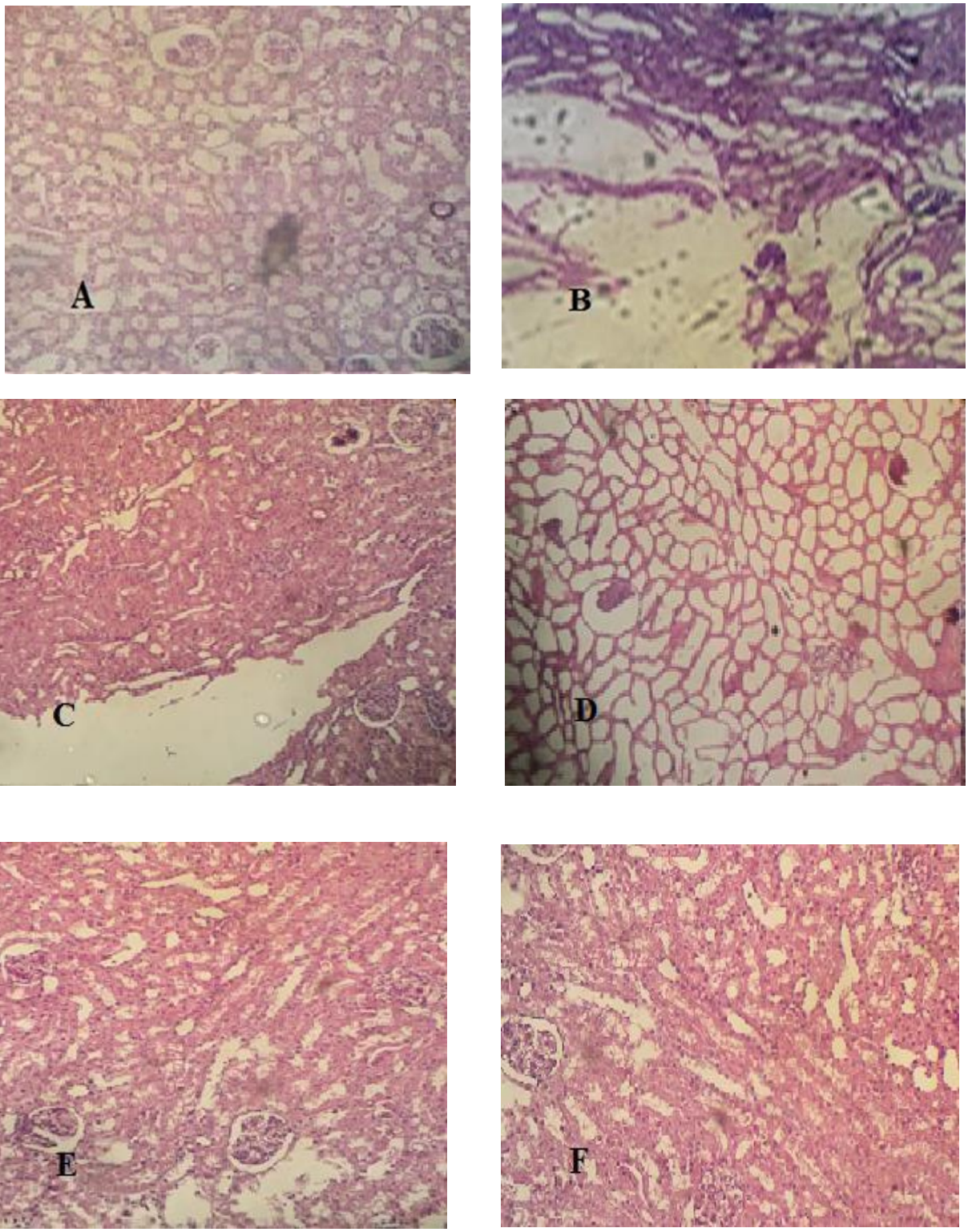


Fig 2: Histopathological sections of Kidneys; **(A)** control **(B)** intoxicated **(C)** Treatment group1 **(D)** Treatment group2 **(E)** Treatment group3 **(F)** CCl4+silymarin in nephrotoxicity model in rats.



## DISCUSSION

Today's medicines have lot of limitations for curing hepato-renal diseases. Medical cure of ailments either from herbal or animal origin has fascinated patients towards them for many years as reported from literature review. The current project was carried out on Red velvet mite methanolic extract, *due to* its traditional uses in medical history[9].

In current project, $CCl_4$-induced hepato-renal toxicity was done in male Wistar Albino rats to evaluate methanolic extract of Dt.Cr. for its hepato-renal protective property (11,12). Our work was in line with previous studies who used same agent for induction. Paradoxically, paracetamol was the inducing agent in other studies[10].

Hepatotoxicity are linked with improper liver functioning because of any reason[13]. Silymarin was used as standard in our study to compare different strengths of *Dinothrombium tacnitorium* extract for its hepato-protective action. It was used as standard by many researchers so our work was in line with past researchers[5].

Protocol adopted with some modifications in current project regarding number of animals and groups were kept same to see hepato-renal protective effect of *Dinothrombium tacnitorium* extract as was done in one previous animals study who observed different hepato-protective effect of *Fumairia indica* plant extract[14].

Different doses of red velvet mite extract were given to treatment groups in current study for hepato-renal protection. Extract of Fabaceae leaves as hepato-protective agent at a dose of 50,100 and 400 mg/kg body wt. was explored in one research article. Paradoxically, the extract of *Dinothrombium tacnitorium* at a dose of 30,100 and 300 mg/kg body wt. given orally in current project to treatment groups respectively[10].

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[15].

In the current project, histology of liver and kidney tissues showed treatment at different doses (30, 100 and 300 mg/kg) of Dt.Cr caused marked changes in preventing the CCl4 induced epithelial injury in both organs. It restored the normal hepatic and renal architecture. Our work and histological findings were in line with one study who looked for hepato-renal effects of Stevia rebaudiana and Aspartame in diabetic rats[16].

Our study had a number of limitations like financial constrains and less resources. LFTs, RFTs and histo-logical slides of liver and kidney tissues were done to evaluate hepato-renal protective effect of extract in present study.

## CONCLUSION

Histological findings suggested that the red velvet mite methanolic extract reduces the histo-pathological changes in both liver and kidneys due to $CCl_4$-induced toxicity in albino rats thus it can work as hepatorenal protective agent.

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