



## Toxicity Studies of Triazolo-Thiadiazoles in Ehrlich Ascitic Carcinoma Cells

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### Abstract

Novel treatment approaches are urgently needed for cancer management due to high systemic toxicity and drug resistance in cancer chemotherapy. Three triazolo-thiadiazoles, 6-[3-(4-chlorophenyl)-1H-pyrazol-4-yl]-3-[(2-naphthoxy)methyl] [1,2,4] triazolo [3,4-b] [1,3,4] thiadiazole (CPNT), 6-[3-(4-fluorophenyl)-1H-pyrazol-4-yl]-3-[(2-naphthoxy)methyl] [1,2,4] triazolo [3,4-b] [1,3,4] thiadiazole (FPNT) and 6-[3-(4-chlororophenyl)-1H-pyrazol-4-yl]-3-[phoxymethyl] [1,2,4] triazolo [3,4-b][1,3,4]thiadiazole (CPPT) were tested for their anti-tumor activity in ehrlich ascites carcinoma (EAC) bearing Swiss albino mice. The effect of thiadiazoles in the mean survival time, body weight, hematological and biochemical parameters in cancer induced mice was studied. The inner peritoneal lining of mice was checked for angiogenesis. Histopathology studies of liver, kidney and spleen to assess the toxicity of thiadiazoles were performed. Statistical analyses were performed by one-way ANOVA, followed by Tukey's post hoc test using Graph Pad Prism 5.02. An increase in mean survival time was observed in mice treated with CPNT, 50 mg/kg body weight over control and standard mice. The rise in body weight associated with progression of cancer was controlled on treatment with CPNT. Differed hematological and biochemical parameters in EAC bearing mice were restored towards normal after CPNT treatment. Histopathology studies exhibited only mild hepatic and nephrotoxicity on CPNT treatment. CPNT at an optimal dose of 50 mg/kg body weight inhibited angiogenesis and exhibited excellent anticancer activity with minimal toxic effects *in vivo* against EAC cells when compared to the standard drug cisplatin.

**Keywords:** Thiadiazole, hematology, angiogenesis, histopathology.

### Introduction

Cancer, characterized by uncontrolled cell proliferation remains a major medical challenge worldwide. However most of the chemotherapeutic drugs are toxic at optimum dose and are expensive. Various 1,2,4-triazole derivatives have been found to exhibit significant anticancer properties against wide range of cancers<sup>1-4</sup>. 1,2-pyrazole derivatives also displayed a broad spectrum of biological activities<sup>5,6</sup>. Literature survey on triazoles and thiadiazoles showed that they possess analgesic, anti-inflammatory<sup>5,7</sup>, antiviral<sup>1</sup>, antimicrobial<sup>8,9</sup>, antifungal<sup>10-12</sup>, antibacterial<sup>13</sup>, antitubercular<sup>14</sup> and antitumor<sup>15</sup> activities. Continuous research in the field of medicinal chemistry has led to the discovery of several drugs with excellent therapeutic value. Many research groups around the world are at work to invent newer classes of drugs with higher efficacy, lower cost and lesser toxicity.

*In vitro* MTT assay of CPNT, FPNT and CPPT in hepatocellular carcinoma cells (HepG2) have revealed their excellent cytotoxic behavior<sup>16</sup>. In our present study, we report the *in vivo* anticancer and toxicity studies of the three triazolo-thiadiazoles, in EAC cells. Median survival times (MST) and the changes in the respective body weights were noted. Their toxicity profile was assessed *in vivo*, using hematological, biochemical and histopathology parameters, on completion of fourteen days of

treatment. The inner peritoneal lining was observed for signs of angiogenesis.

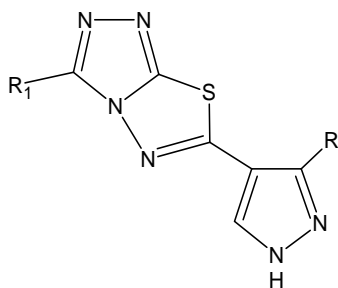
### Material and Methods

**Animals:** The animal studies were carried out in Central Animal Research Facility, Manipal University, Karnataka, India, on eight weeks old adult female Swiss Albino mice (25-30 g). The animal experiments were performed as per the regulations of the Institutional Animal Ethics Committee (IAEC).

**Synthesis of triazolo-thiadiazoles:** CPNT, FPNT and CPPT were prepared by literature methods<sup>17</sup>. The structures of the three thiadiazoles are given in figure-1.

***In vivo* anticancer studies: Preparation of test solution of triazolo-thiadiazoles:** The solution of thiadiazoles for *i.p.* (intraperitoneal) administration was prepared prior to their injection by suspending them in 0.25 % carboxymethyl cellulose (CMC).

**Induction of Ehrlich Ascites Carcinoma:** Acute toxicity studies were carried out to determine the maximum tolerated dose of triazolo-thiadiazoles as per OECD guidelines, 2001<sup>18</sup>. The therapeutic doses for further studies were selected as 1/10<sup>th</sup> to 1/20<sup>th</sup> of the maximum tolerated dose.



CPPT: R = 4-chlorophenyl, R<sub>1</sub>= phenoxyethyl  
CPNT: R = 4-chlorophenyl, R<sub>1</sub>= 2-naphthoxymethyl  
FPNT: R = 4-fluorophenyl, R<sub>1</sub>= 2-naphthoxymethyl

**Figure-1**  
**Structure of triazolo-thiadiazoles**

The mice were divided into 9 groups of 12 animals each [Group 1- Normal mice; Group 2- EAC-bearing mice (Control); Group 3- treated with single dose of cisplatin (Standard); Group 4- CPPT (50 mg/kg); Group 5- CPPT (150 mg/kg); Group 6- CPNT (50 mg/kg); Group 7- CPNT (100 mg/kg); Group 8- FPNT (50 mg/kg) and Group 9- FPNT (150 mg/kg)]. The ascitic fluid was drawn from donor mice, tested for microbial contamination and tumor viability was determined. From the stock suspension of  $10 \times 10^6$  cells/mL, 0.25 mL (2.5 million cells/mice) was injected *i.p.* to normal mice in order to obtain ascitic tumor (day 0). The dose of cisplatin, given *i.p.* to the standard group on day 1 was fixed at 3.5 mg/kg body weight - calculated from the human dose using suitable conversion factor<sup>19</sup>. The three thiadiazoles in two different concentrations were administered on days 3, 5, 7, 10, 12 and 14 (6 doses) *i.p.* The control group was given 0.25 % CMC *ip.* On day 15, six mice from each group, were sacrificed and hematological, biochemical and histology studies were performed. The remaining six animals from each group were observed daily for 30 days to calculate the mean survival time (MST).

**Mean survival time and increase in lifespan (% ILS):** The total survival days of the animal from the day of tumor induction was counted<sup>19</sup>.

$$\% \text{ Increase of life span} = \left[ \frac{\text{MST of treated group} - \text{MST of control group}}{\text{MST of control group}} \right] \times 100$$

**Body weight:** All the animals were weighed on days 0,3,5,7,10,12 and 14<sup>20</sup>.

$$\% \text{ increase in weight} = \left[ \frac{\text{Animal weight on corresponding day} - \text{Animal weight on day 0}}{\text{Animal weight on day 0}} \right] \times 100$$

**In vivo toxicological assays:** Blood samples of six animals from each group were collected for hematological and biochemical studies on 15<sup>th</sup> day. Hematological parameters including hemoglobin, erythrocyte (RBC) count and leukocyte (WBC) count were determined in peripheral blood sampled from the retro-orbital plexus of mice on day 15<sup>21</sup>. Liver function was assessed by Serum bilirubin, Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic

Transaminase (SGPT), Alkaline Phosphatase (ALP) and protein levels<sup>22</sup>.

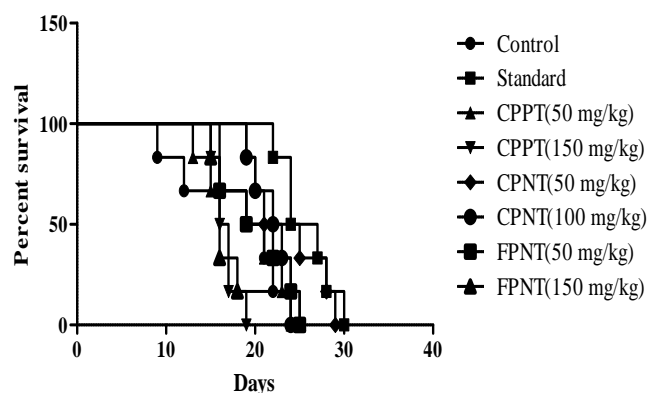
**Angiogenesis:** The animals were sacrificed and the inner peritoneal lining was checked for signs of angiogenesis.

**Histopathology studies:** The liver, kidney and spleen were removed; slides prepared, staining was done and viewed under light microscope of 40x magnification. Thiadiazole induced hepatotoxicity, nephrotoxicity and splenic toxicity were checked<sup>23,24</sup>.

**Statistical analysis:** The statistical analyses were done by one-way ANOVA, followed by Tukey's post hoc test using Graph Pad Prism 5.02. Results were expressed as the mean  $\pm$  S.E.M and the deviations were considered significant with a p value < 0.05.

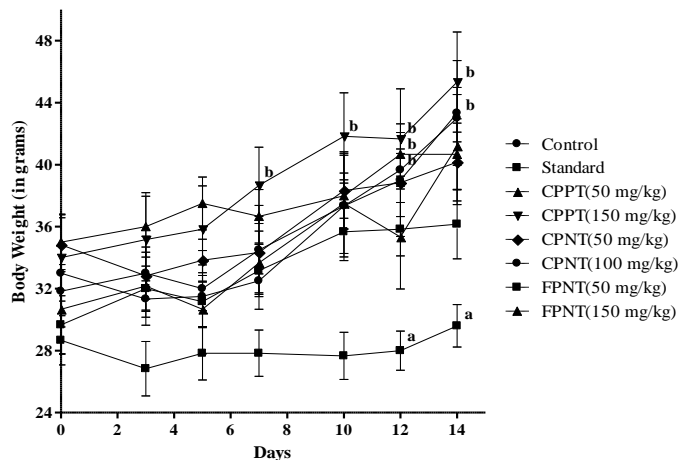
## Results and Discussion

CPNT, at an optimal dose of 50 mg/kg showed an enhancement in the mean survival time by 33 %. FPNT demonstrated a similar response in smaller magnitude, whereas CPPT in both concentrations was found to be inactive. Cisplatin significantly enhanced MST of mice. The detailed data showing the mean survival time is depicted in figure-2.



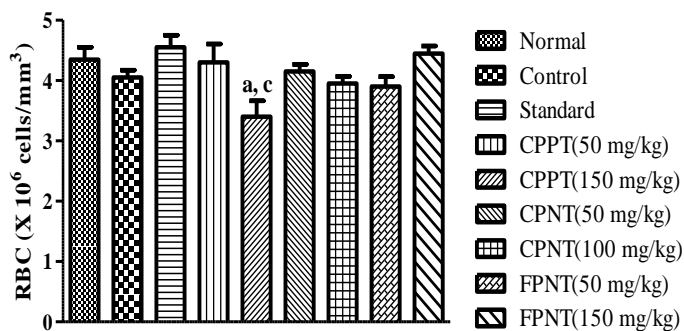
**Figure-2**  
**Kaplan Meier's estimate of survival of EAC bearing mice**

Body weight increase by 15.33 % in CPNT (50 mg/kg) treated mice as opposed to 36.12 % observed in the control mice was indicative of its efficacy in bringing down the progression of cancer. The graph representing the body weight changes of animals are shown in figure-3.



**Figure-3**  
Body weight changes. <sup>a</sup>P < 0.05 equated to control and <sup>b</sup>P < 0.05 equated to standard

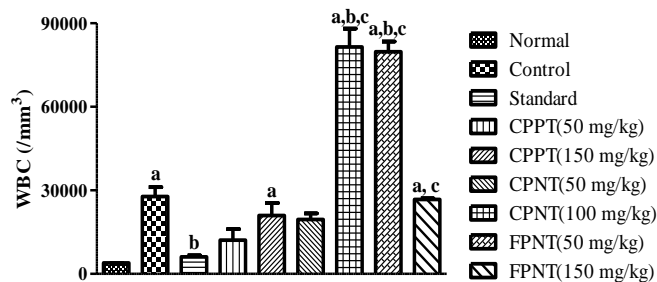
The altered hematological parameters were restored to normal on treatment with CPNT (50 mg/kg). However, none of the thiazidiazoles could normalize the elevated WBC count. CPNT (50 mg/kg) alone, was able to bring down the WBC count to an appreciable extent, though not back to normal. The detailed data obtained for each hematological parameter is given in figure-4, figure-5 and figure-6.



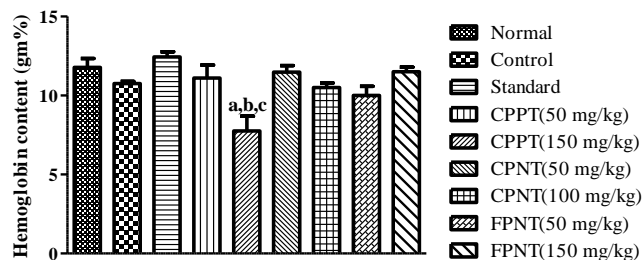
**Figure-4**  
Erythrocyte count. <sup>a</sup>P < 0.05 equated to normal and <sup>c</sup>P < 0.05 equated to standard

The elevated liver enzymes, characteristic of chronic liver disease, were reverted to the normal range in the CPNT (50 mg/kg) treated group, reflecting its efficacy and lesser toxicity as compared to the standard, as seen in figure-7.

In the case of CPNT (50 mg/kg) treated mice, the protein and bilirubin levels were almost in normal range as shown in figure-8 and figure-9.



**Figure-5**  
Leucocyte count. <sup>a</sup>P < 0.05 compared to normal, <sup>b</sup>P < 0.05 to control and <sup>c</sup>P < 0.05 to standard



**Figure-6**  
Hemoglobin count <sup>a</sup>P < 0.05 compared to normal, <sup>b</sup>P < 0.05 to control and <sup>c</sup>P < 0.05 to standard

Formation of new blood vessels was observed in the case of control mice. The peritoneal lining of CPNT (50 mg/kg) treated mice showed only very mild angiogenesis as seen in figure-10.

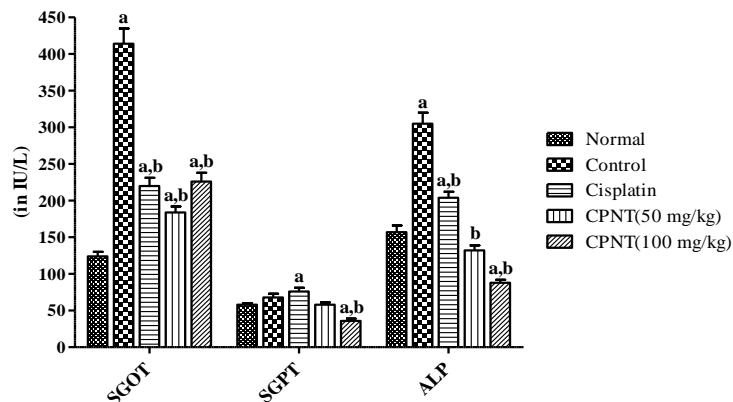
The standard group showed hepatocellular infiltration, congestion and mild central vein dilatation. In the CPNT (50 mg/kg) treated group, only mild central vein dilatation was observed as seen in figure-11. The renal histology of the cisplatin treated group showed signs of glomerular congestion with infiltration and tubular necrosis as depicted in figure-12. The CPNT (50 mg/kg) treated group however, showed no such signs. The standard group also showed loss of splenic architecture and congestion as presented in figure-13. The CPNT (50 mg/kg) treated group showed mild loss of splenic architecture, with minimum congestion.

## Conclusion

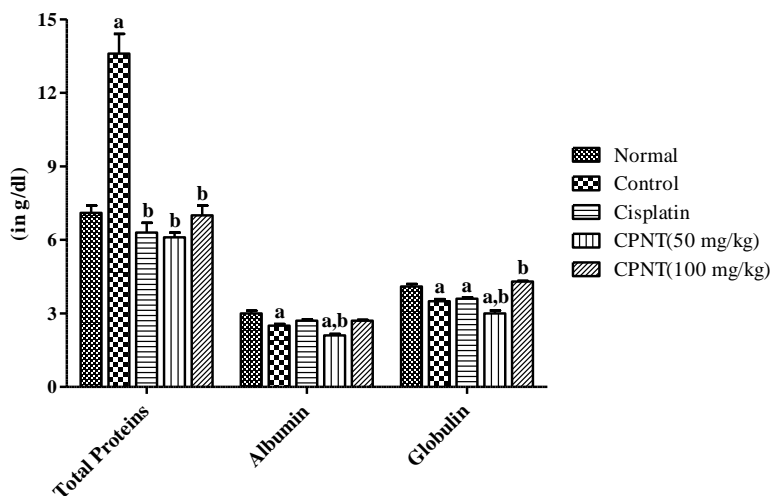
The results of this study suggested that CPNT, at an optimal dose of 50 mg/kg body weight in mice is an effective chemotherapeutic agent with moderately less toxicity. The increased lipophilicity of the molecule due to the presence of naphthoxy methyl and pyrazole substituents must have improved its bioavailability. In addition, the lipophilic property and electron withdrawing effect of chlorine might have added to the effectiveness of the compound as antineoplastic agent. Moreover, in CPNT, the less steric hindrance created by the bulky chlorine atom present in the *para* position of the phenyl group might have given a particular conformation which could

increase the binding affinity and interaction of the molecule with appropriate receptors. The antitumor activity of this triazolo-thiadiazole should be tested in other tumor cell lines as

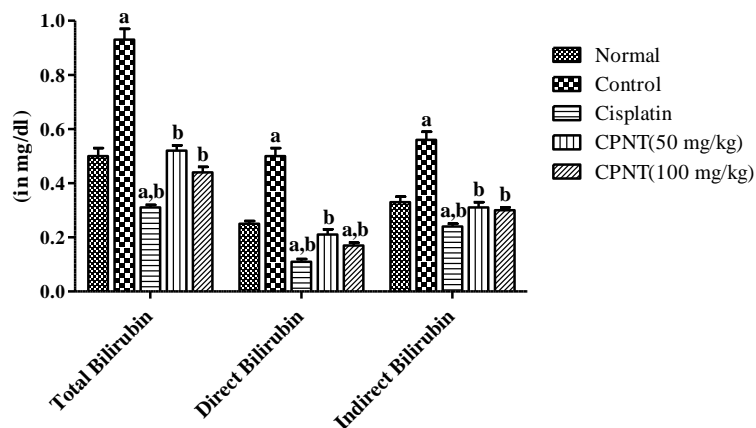
well, as it might potentially expand the clinical utility of this molecule in other cancers.



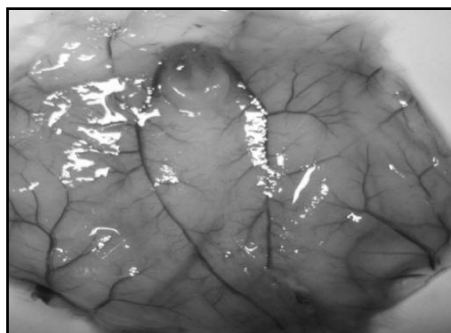
**Figure-7**  
 Liver enzyme levels. <sup>a</sup>P < 0.05 compared to normal and <sup>b</sup>P < 0.05 to control.



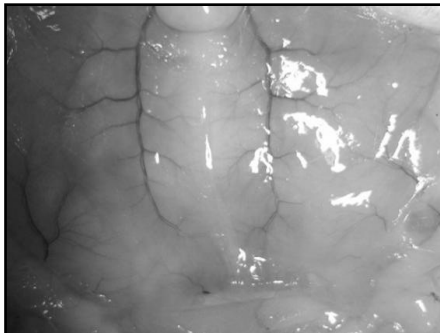
**Figure-8**  
 Protein levels. <sup>a</sup>P < 0.05 equated to normal and <sup>b</sup>P < 0.05 to control.



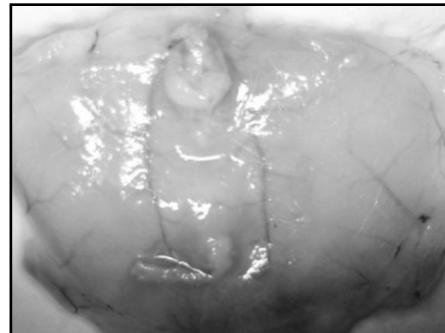
**Figure-9**  
 Bilirubin levels. <sup>a</sup>P < 0.05 compared to normal and <sup>b</sup>P < 0.05 to control



Control

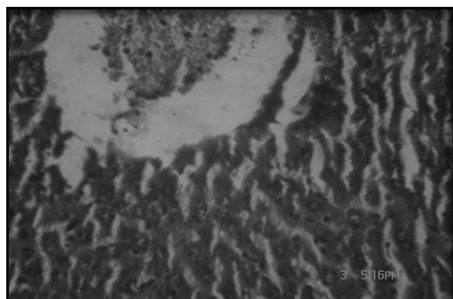


Standard

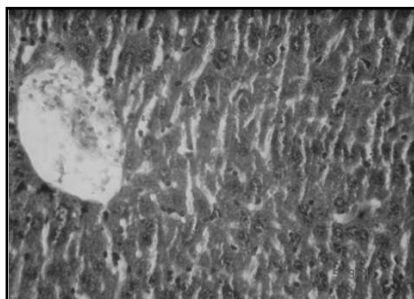


CPNT (50mg/Kg)

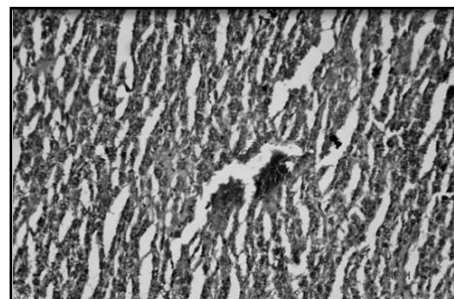
**Figure-10**  
Inner peritoneal lining of mice views



Control

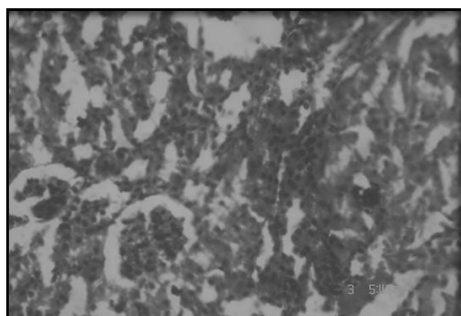


Standard

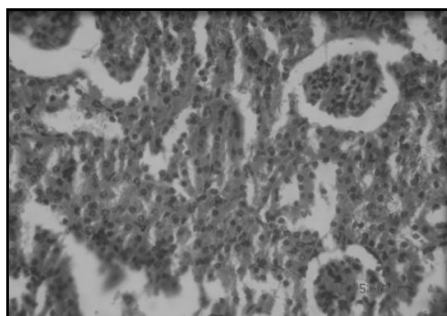


CPNT (50 mg/kg)

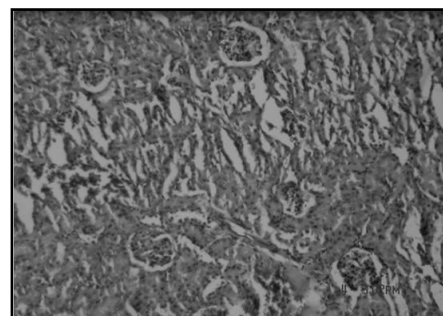
**Figure-11**  
Histology of liver



Control

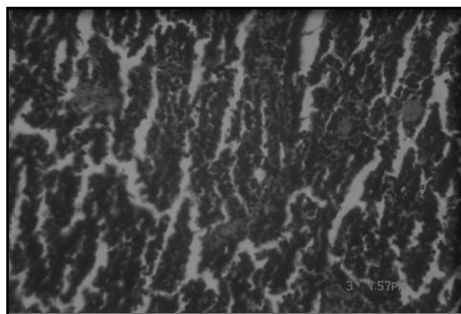


Standard

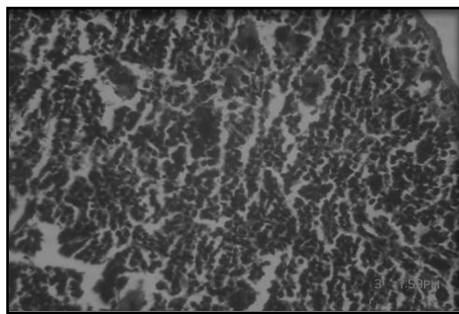


CPNT (50 mg/kg)

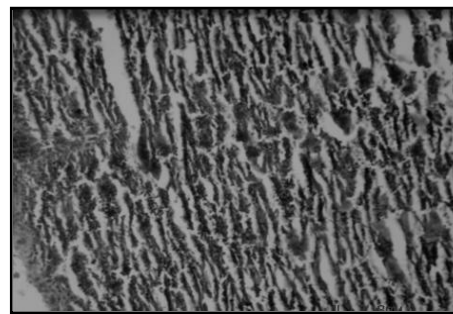
**Figure-12**  
Histology of kidney



Control



Standard



CPNT (50 mg/kg)

**Figure-13**  
Histology of spleen

## Acknowledgement

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