## Evaluation Of Nephroprotective Activity of Cod Liver Oil in Drug Induced Nephrotoxicity

*Vikram V. Nimbalkar, **Sahil A. Maniyar ***Dr. P. M.Gaikwad

*Assistant Professor, **Resident, ***Prof. & Head, Department of Pharmacology

**Corresponding Address :** : D.V.V.P.F'S College of Pharmacy, Vilad Ghat, Ahmednagar (MS) India.414111

**Mail id -** rajevikram@gmail.com

**Mobile No. -** 9970072233

**Abstract :**

Cisplatin (a platinum-compound) is a anti-neoplastic drug used in the treatment of various cancers but eventually results in severe adverse effects namely nephrotoxicity or renal disorder through generation of reactive oxygen species (ROS). The existing drugs can cure most of the diseases. Still there is a never ending search for finding new drugs in the hope that it would yield drugs with lesser side effects and better therapeutic benefit than the existing drugs. This work was researched for evaluation of the Nephroprotective activity of Cod liver oil against cisplatin induced nephrotoxicity. The cod liver oil administered orally (0.5, 1, 2 gm/kg/day) for 14 day, cisplatin was administered at the dose of 7 mg/kg for 1st day intraperritoneally. Cisplain treated group showed increased level of Serum & Urine creatinine, uric acid, urea, which were significantly retrived in group pretreated with cod liver oil. In conclusion the histopathological & biochemical parameter confirmed that the cod liver oil protect against cisplatin induced renal damage, probably through its antioxidant activity.

**Keywords:** Cod liver oil, Cisplatin, Nephrotoxicity

**Introduction :** The kidney is an essential organ required by the body to perform several important functions including the maintenance of homeostasis, regulation of the extracellular environment, such as detoxification, and excretion of toxic metabolites and drugs[1]. Therefore, the kidney can be considered as a major target organ for exogenous toxicants. Nephrotoxicity is a kidney-specific feature in which excretion does not go smoothly owing to toxic chemicals or drugs[2]. Approximately 20% of nephrotoxocity is induced by drugs, but medication of the elderly increases the incidence of nephrotoxicity up to 66% as the average life span increases. Chemotherapy or anticancer medicine has been of limited use due to nephrotoxicity [3,4]. Nephrotoxicity can be diagnosed through a simple blood test. Evaluation of nephrotoxicity through blood tests includes the measurements of blood urea nitrogen (BUN), concentration of serum creatinine, glomerular filtration rate and creatinine clearance. However, these assessments of nephrotoxicity are only possible when a majority of kidney function is damaged[5]. Therefore, discovery and development of biomarkers that can detect kidney dysfunction at the early stage are needed. In this research, we summarize the mechanisms of Cod liver oil as nephroprotective and highlight their constituent in protective action.

**Mechanisms of drug-induced nephrotoxicity :**

General mechanisms that cause nephrotoxicity include changes in glomerular hemodynamics, tubular cell toxicity, inflammation, crystal nephropathy, rhabdomyolysis, and thrombotic microangiopathy [1,6,7,8].

**Materials and Methods:**

**Animals:** The healthy Wister albino rats of either sex weighing between 150-200 g were taken for the study. They were housed under controlled conditions of temperature (23±2oc), humidity (55±5%) and 12h light and 12h dark cycles. The animals were fed with standard pellet diet and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethical Committee as per the CPCSEA guidelines.

**Vide:** PDVVPF's COP/PG04/2016.

**Drug & Chemicals:** Cod liver purchased from Seven seas, ciplatin was purchased from Alkem and other reagent were purchased and all chemicals used in these studies were of analytical grade

**Experimental design :**

Experiments were performed in accordance with the guidelines for the care and use of laboratory animals, laid down by the Committee for the Purpose of Control and Supervision of Experiments in Animals (CPCSEA). The rats were divided into six groups of six animals each. Group 1 (Control) received distilled water (1 ml/kg/d, p.o), for 14 days. Group II (Toxic) received cisplatin (7 mg/kg i.p.) for 1st day. Group III received cisplatin (7 mg/kg i.p.) and Cystone (Std, 75 mg/kg/d p.o.) (Standard Group) for 14 days. Group IV, V and VI received 0.5,1,2 gm/kg/d cod lver oil (p.o.) (Test 1, Test 2 and Test 3) for 14 days. In addition to this, the animals in groups IV, V and VI were co-administrated intraperitoneally once in 1st day with cisplatin in a dose of 7 mg/kg [9,10].

**Estimation of different parameters :**

At the end of the experiments on 15th day the animals were anaesthetized with anesthetic ether and sacrificed. The blood samples were collected from retro-orbital plexus and left at room temperature for 2 h. The blood samples were centrifuged for 10 minutes at 3000 rpm to separate the serum. The sera were estimated for concentration of serum creatinine, urea,uric acid, urine creatinine, urea, uric acid and also serum & urine sodium,potasium estimated for nephroprotective activity. Kidneys were removed for for histopathological examination [11].

**Histopathological studies of rat kidneys:**

Kidneys of sacrificed animals were identified and carefully dissected out for histopathological studies. After rinsing in normal saline, sections were taken from each harvested kidney. The tissue was fixed in 10% formal-saline, dehydrated with 100% ethanol solution and embedded in paraffin. It was then processed into 4-5m thick sections stained with hematoxylin-eosin and observed under a photomicroscope (magnification power-40X).

**Statistical analysis :** Results were expressed as the Mean ±standard error means S.E.M.). The comparison of data within groups was performed by the analysis of variance using ANOVA test. Significant difference between control and experimental groups was assessed by Dunnett's test. A probability level of less than ($P < 0.05$) was considered significant. Statistical analysis was performed using INTA

**Results:**

**Body & Kidney weight :** There is significant decrease in the body weight & significant increase in the kidney weight of toxic group observed when compared to normal group, where as the significant increase in the body weight& significant decrease in kidney weight showed in the standard, test 1,test 2 & test 3 (table no.1).

**Table-1: Effects on Body weight, kidney weight in normal, Cisplatin, and Cod liver oil treated rats.**

| Group No. | Treatment | Body Weight (gm) | Kidney Weight (gm) |
|---|---|---|---|
| 1 | Vehicle | ..... | 1.403±0.02964 |
| 2 | Cisplatin | 205.54±1.490$^{\#\#}$ | 1.595±0.01747$^{\#\#}$ |
| 3 | Cisplatin + Cystone | 217.94± 1.245$^{**}$ | 1.427±0.02052$^{**}$ |
| 4 | Cisplatin +Cod-liver oil 0.5 gm/kg | 207.91±1.808 | 1.593±0.01334 |
| 5 | Cisplatin + Cod-liver oil 1 gm/kg | 213.13±1.601$^{**}$ | 1.503±0.01576$^{**}$ |
| 6 | Cisplatin + Cod-liver oil 2 gm/kg | 218.67± 1.424$^{**}$ | 1.432±0.01254$^{**}$ |

**N=6,values are expressed as Mean± SEM, comparison were made as follows,# p<0.05,## p<0.01 when compared with normal control,* p<0.05,** p<0.01 when compared with negative control(values are compared on 15th day by one way ANOVA Dunnett t test) N.S.= not significant.**

**Urine Volume:** There is significant decrease in the urine volume of toxic group when compared to normal group where as insignificant increase the test 1 group and in standard, test 1 and test 2there is significant increase in the urine volume of rats when compared to toxic group (table no.2)

**Urine analysis :** There is insignificant increase in urine urea, creatinine (table no.2) & uric acid (table no.3) in toxic group when compared to normal group, where as test 1 showed the insignificant decrease in urine urea, creatinine (table no.2) & uric acid (table no.3) as compared to toxic group & significant increase in the standard, test 2 & test 3 group when compared to toxic group.

There is significant decrease & increase in urine sodium & potassium respectively in the toxic group when compared to normal group, and insignificant increase & decrease in Urine Sodium, potassium respectively in the test 1 group as compared to toxic group where as the significant increase in urine sodium & significant decrease urine potassium in the standard, test 1,test 2 group.(table no.3)

**Table-2: Effects on urine volume, urine urea, urine creatinine in normal, Cisplatin, and Cod-liver oil treated rats.**

| Group No. | Treatment | Urine Volume (ml/day) | Urine urea (mg/dL) | Urine creatinine (mg/dL) |
|---|---|---|---|---|
| 1 | Vehicle | ..... | 39.130±0.717 | 1.552±0.1183 |
| 2 | Cisplatin | 1.251±0.1393$^{\#\#}$ | 59.710±1.145$^{\#\#}$ | 3.514±0.1330$^{\#\#}$ |
| 3 | Cisplatin+Cystone | 2.149±0.1124$^{**}$ | 41.367±1.453$^{**}$ | 1.859±0.0791$^{*}$ |
| 4 | Cisplatin+Cod-liver oil 0.5 gm/kg | 1.554±0.1163 | 57.658±1.200 | 3.167±0.1166 |
| 5 | Cisplatin+Cod-liver oil 1 gm/kg | 1.831±0.1054$^{**}$ | 52.995±1.179$^{**}$ | 2.825±0.1283** |
| 6 | Cisplatin+Cod-liver oil 2 gm/kg | 2.053±0.1156$^{**}$ | 42.838±1.369$^{**}$ | 1.963±0.1762$^{**}$ |

**N=6,values are expressed as Mean± SEM, comparison were made as follows,# p<0.05,## p<0.01 when compared with normal control,* p<0.05,** p<0.01 when compared with negative control(values are compared on 15th day by one way ANOVA Dunnett t test) N.S.= not significant.**

**Table-3: Effects on urine uric acid Na+ and K+ in normal, Cisplatin, and Cod-liver oil treated rats.**

| Group No. | Treatment | Urine Uric acid (mg/dL) | $Na^+$ (meq/L) | $K^+$ (meq/L) |
|---|---|---|---|---|
| 1 | Vehicle | 4.782±0.1302 | 140.33±2.663 | 4.325±0.1634 |
| 2 | Cisplatin | 9.851±0.177## | 126.79±2.185## | 8.637±0.153## |
| 3 | Cisplatin+Cystone | 5.049±0.144** | 130.90±1.216** | 4.767±0.165** |
| 4 | Cisplatin +Cod-liver oil 0.5 gm/kg | 9.236±0.129** | 126.67±2.372 | 8.209±0.1643 |
| 5 | Cisplatin+Cod-liver oil 1 gm/kg | 9.055±0.097** | 136.48±1.550** | 7.851±0.072** |
| 6 | Cisplatin+Cod-liver oil 2 gm/kg | 5.378±0.147** | 138.37±1.113** | 5.096±0.131** |

**N=6,values are expressed as Mean± SEM, comparison were made as follows,# p<0.05,## p<0.01 when compared with normal control,* p<0.05,** p<0.01 when compared with negative control(values are compared on 15th day by one way ANOVA Dunnett t test) N.S.= not significant.**

**Serum analysis:**

There is insignificant increase in serum urea, creatinine & uric acid in toxic group when compared to normal group, where as tet 1 showed the insignificant decrease in serum urea,creatinine & significant increase in uric acid as compared to toxic group & significant increase in the standard, test 2 & test 3 group when compared to toxic group (table no.4).

There is significant decrease & increase in serum sodium & potassium respectively in the toxic group when compared to normal group, and insignificant increase & decrease in serum Sodium, potassium respectively in the test 1 group as compared to toxic group where as the significant increase in serum sodium & significant decrease serum potassium in the standard, test 1,test 2 group(table no.5).

**Table-4: Effects on serum urea, serum creatinine, serum uric acid, in normal, Cisplatin, and Cod-liver oil treated rats.**

| Group No. | Treatment | Serum Urea (mg/dL) | Serum Creatinine (mg/dL) | Serum uric acid (mg/dL) |
|---|---|---|---|---|
| 1 | Vehicle | 31.780±1.442 | 1.060±0.1102 | 3.421±0.1068 |
| 2 | Cisplatin | 50.645±1.621## | 2.244±0.1140## | 6.573±0.1457## |
| 3 | Cisplatin+Cystone | 34.621±1.259** | 1.350±0.1179** | 3.741±0.1156** |
| 4 | Cisplatin+Cod-liver oil0.5gm/kg | 48.601±1.405 | 2.053±0.1100 | 6.355±0.1297 |
| 5 | Cisplatin+Cod-liver oil 1gm/kg | 42.781±1.554** | 1.620±0.1161** | 5.967±0.1084* |
| 6 | Cisplatin+Cod-liver oil 2gm/kg | 33.031±1.585** | 1.250±0.1184** | 3.652±0.1298** |

**N=6,values are expressed as Mean± SEM, comparison were made as follows,# p<0.05,## p<0.01 when compared with normal control,* p<0.05,** p<0.01 when compared with negative control(values are compared on 15th day by one way ANOVA Dunnett t test) N.S.= not significant.**

**Table-5: Effects on Na+ and K+ in normal, Cisplatin, and Cod-liver oil treated rats.**

| Group no. | Treatment | $Na^+$ (meq/L) | $K^+$ (meq/L) |
|---|---|---|---|
| 1 | Vehicle | 143.41±1.249 | 4.569±0.1215 |
| 2 | Cisplatin | 130.50±1.598## | 5.966±0.1175## |
| 3 | Cisplatin+Cystone | 140.41±1.662** | 4.746±0.1410** |
| 4 | Cisplatin+Cod-liver oil 0.5gm/kg | 131.55±1.762 | 5.844±0.1137 |
| 5 | Cisplatin+Cod-liver oil 1gm/kg | 133.13±1.549 | 5.308±0.1365** |
| 6 | Cisplatin+Cod-liver oil 2gm/kg | 139.89±1.800** | 4.921±0.1182** |

**N=6,values are expressed as Mean± SEM, comparison were made as follows,# p<0.05,## p<0.01 when compared with normal control,* p<0.05,** p<0.01 when compared with negative control(values are compared on 15th day by one way ANOVA Dunnett t test) N.S.= not significant.**

**Histopathoological Estimations:**

**Group-I**

Male albino rats with intake of normal distilled water showed normal architecture of renal glomeruli with intact bowmans capsule. Brush bordered cuboidal epithelium lining the proximal convoluted tubules Simple cuboidal epithelium lining the distal convoluted tubules .macula densa is very prominent(fig.1).

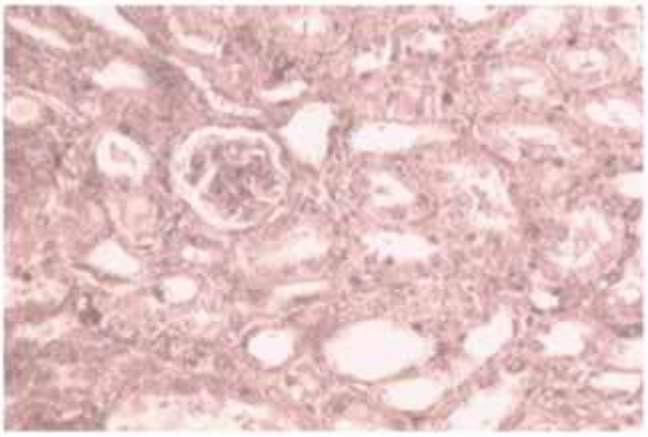

**Fig 1: Group No.1 (Vehicle Control)**

**Group-II**

In the negative control groupII, histopathological findings showed kidney structure distorted by severe necrosis of tubules. The stroma was edematous. The tissue was infiltrated by numerous chronic inflammatory cells. Engorged blood vessels and areas of hemorrhage were seen. Features suggested severe tubular necrosis. Renal histology in the Gentamycin treated group showing severe tubular necrosis (fig.2).

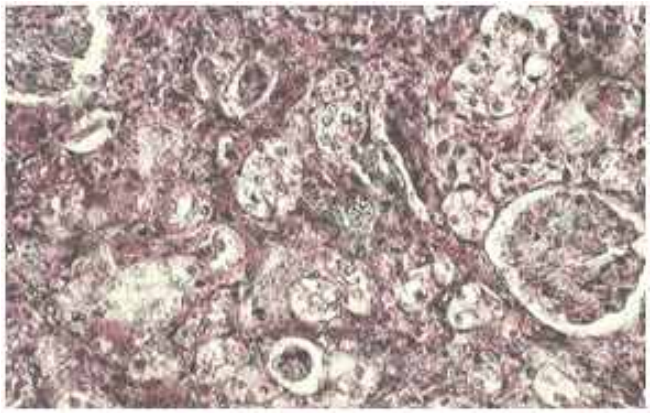

**Fig 2: Group 2 (Cisplatin)**

**Group-III**

In the group III, histopathological findings showed the stroma with a mild degree of edema. There was a mild degree of glomerular congestion. The tissue was sparsely infiltrated by inflammatory cells. Features suggested mild tubular damage. (fig.3)

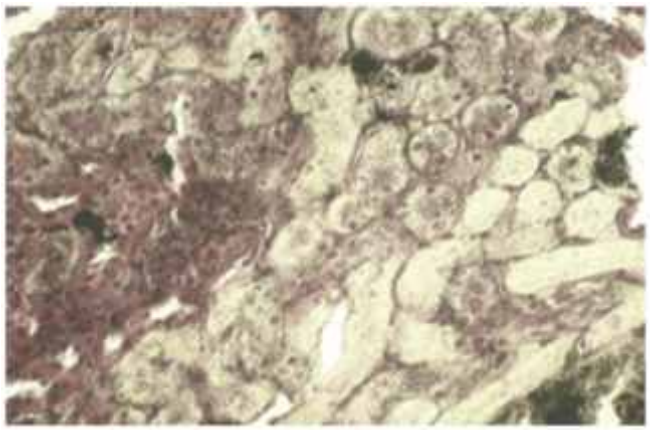

**Fig 3: Group 3 (Cisplatin+Cystone)**

**Group-IV**

In the group IV, histopatological examination showed that there was mild interstitial edema. moderate degree of congestion was also seen in the glomeruli. Numerous engorged blood vessels were seen. Mild tubular changes were noted. The tissue was free from inflammatory cells Renal histology in the A groupIV showing moderate tubular necrosis with significant reversal of inflammatory changes. (fig.4)

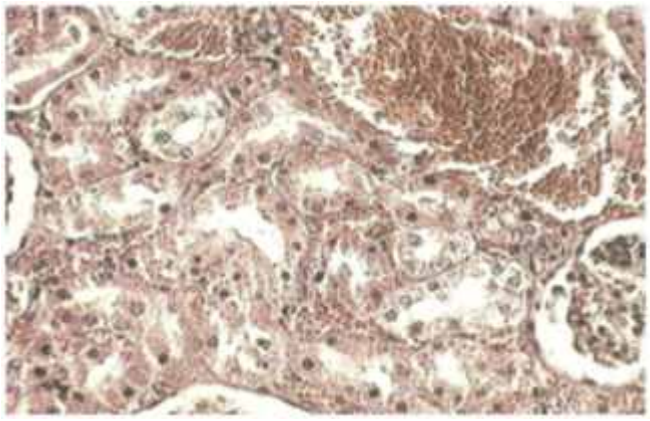

**Fig 4: Group 4 (Cisplatin +Cod liver oil 0.5gm/kg**

**Group-V**

In group V, histopathological findings showed mild interstitial edema, mild degree of peritubular and glomerular congestion and numerous engorged blood vessels. The tissue was free from inflammatory cells. Features suggested mild tubular changes. (fig.5)

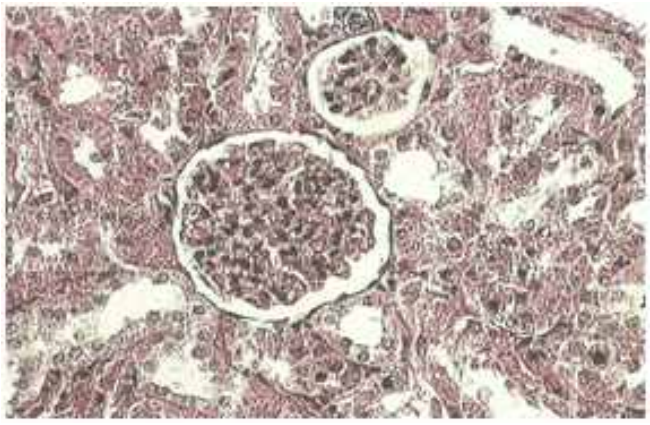

**Fig .5: Group 5 (Cisplatin+ Cod liver oil 1gm/kg)**

**Group-VI**

In group VI histopathology showed mild interstitial edema, mild degree of glomerular congestion and few congested blood vessels. Mild tubular damage was observed. The tissue was sparsely infiltrated by chronic inflammatory cells. (fig.6)

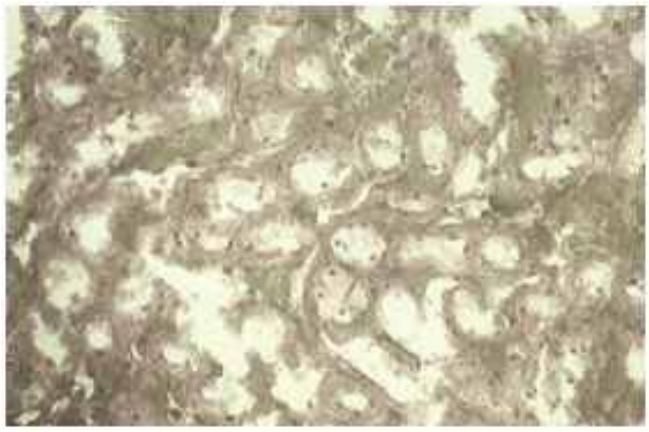

**Fig 6: Group 6 (Cisplatin+ Cod liver oil 2 gm/kg)**

**Discussion :** Cisplatin is a known nephrotoxic agent reported to induce a significant degree of nephro-toxicity at different dose levels. Its nephro-toxic potential was established at a dose level of 80 mg/kg in albino rats [12,13].

There was no change in the normal behavioral pattern of animals and no sign and symptoms of toxicity were observed and no mortality was observed till 24h. Cod liver oil were safe up to a maximum dose of 2 gm/kg Body weight. The biological evaluation was carried out at doses of 0.5,1 and 2 gm/kg b.w by oral route. Urine urea, creatinine, uric acid, potassium and Serum creatinine, urea, uric acid, potassium was found to be significant ($P<0.001$) increased in rats treated with only cisplatin, whereas treatment with the cod liver oil reversed the effect of cisplatin indicating nephroprotective activity. (Table No.1,2,3,4,5). The impairment in kidney functions is accompanied by an increase in urine & serum creatinine urea and uric acid level. It is one of the essential compounds for maintaining cell integrity participation in the cell metabolism. The significant and progressive weight loss in cisplatin treated rats may possibly be due to the injury of renal tubules and the subsequent loss of the tubular cells to reabsorb water, leading to dehydration and loss of body weight.. The cod liver oil showed dose depended protective effect. Cod liver oil might have exhibited nephroprotective activity by the virtue of its antioxidant activity.

**Conclusion :** Taking into consideration the results obtained in the present investigation, it can be concluded that Cod-liver oil has a definite Nephroprotective activity; hence it could be use in the treatment of kidney disorders like kidney dysfunction.

**Acknowledgment :** The authors are thankful to Dr. S.H. Khaparde, Department of Pathology, P.D.V.V.P.F's Medical College for histopathological studies.

**References :**

1) Ferguson, M. A., Vaidya, V. S. and Bonventre, J. V. (2008) Biomarkers of nephrotoxic acute kidney injury. Toxicology 245, 182-193.

2) Finn, W. and Porter, G. (2003) Urinary biomarkers and nephrotoxicity. Clinical Nephrotoxins (2nd ed), pp. 621-655.

3) Galley, H. F. (2000) Can acute renal failure be prevented? J. R. Coll. Surg. Edinb. 45, 44-50.

4) Kohli, H. S., et.al, (2000) Treatment-related acute renal failure in the elderly: a hospital-based prospective study. Nephrol. Dial. Transplant. 15, 212-217.

5) Kirtane, A. J et.al,. (2005) Serum blood urea nitrogen as an independent marker of subsequent mortality among patients with acute coronary syndromes and normal to mildly reduced glomerular filtration rates. J. Am. Coll. Cardiol. 45, 1781-1786.

6) Zager, R. A. (1997) Pathogenetic mechanisms in nephrotoxic acute renal failure. Semin. Nephrol. 17, 3-14.

7) Schnellmann, R.G. and Kelly, K.J. (1999). Pathophysiology of nephrotoxic acute renal failure, in atlas of diseases of the kidney vol 1. (R. W. Schrier, Ed.), pp 15.1-15.14.

8) Schetz, M., Dasta, J., Goldstein, S. and Golper, T. (2005) Drug-induced acute kidney injury. Curr. Opin. Crit. Care 11, 555-565.

9) Azhar, M.U., et .al. (2003) Nephroprotective effect of kulthi (Dolichos biflorus) in gentamicin treated rats, Journal of Science and Pharmacy, 5 (2), 50- 53.

10) Sreedevi, Y., Latha, J.P., Bharathi, K., (2011) Protective effect of fruits of Pedalium murex against gentamicin induced nephrotoxicity in rats. International Journal of Phytopharmacology, 2(1), 27-36.

11) Hare, R.S. (1950) Endogenous creatinine in serum and urine, Proc. Soc.Exp. Biol. Med, 74, 148.

12) Borrmann, H. Romeis, B. Mikroskopicheskaia technique. Moscow, 1954 “Mikrotome in Wissenschaft und Forschung.” In Medizintechnik. Berlin, 1958, 102–112.

13) Beauchamp D, Gourde P, Bergeron, MG. Sub-cellular distribution of gentamycin in proximal tubular cells, determined by immune gold labeling. Antimicrobial Agents and Chemotherapy.