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Research Article

CYTOPROTECTIVE EFFICACY OF DRDE-07 AND DRDE-30 AGAINST CISPLATIN INDUCED NEPHROTOXICITY

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ABSTRACT

Aim: The objective of the study was to investigate the cytoprotective action of DRDE-07 and DRDE-30 against cisplatin induced nephrotoxicity in mice.

Subjects and Methods: Randomly bred Swiss female mice (25-30 g) were selected and were divided into five groups (n = 6). Group I treated as control (distil water); Group II were administered Cisplatin at 10mg/kg. And Group III were administered Cisplatin at 10mg/kg and Amifostine (185 mg/kg), Group IV were administered with Cisplatin (10 mg/kg) and DRDE-07 at 249 mg/kg, Group V were administered with Cisplatin (10 mg/kg) and DRDE-30 at 219 mg/kg body weight. Two experiment designs were planned. In first design, drug was given 30 min before administration of cisplatin and followed by three days orally. In second design the drug was given after administration of cisplatin and the rest was same as first design.

Results: Various biochemical parameters had been done on the plasma samples collected from experimental animals exposed to cisplatin through intraperitonial route as well as control and drug groups. It was noticed in the present investigation there was a significant increasement in urea and creatinine value in cisplatin group. it was observed that the pre-treatment of DRDE-07 and DRDE-30 showed efficacy against cisplatin induced nephrotoxicity. the post-treatment is also effective in case of DRDE-07 not for DRDE-30.

Conclusions: It was concluded that the DRDE-07 and DRDE-30 showed much effectiveness when given before the exposure of cisplatin.

Keywords: Cisplatin, DRDE-07, DRDE-30, Cytotoxicity, Cancer, Nephrotoxicity,

1. Introduction:-

Cisplatin, cisplatinum or cis-diamminedichloro-platin (a heavy metal complex) is a most effective anticancer drug used to treat solid tumors such as testicular and bladder, ovarian, breast and lung cancers. It is approved by the FDA in 1978[1]. It was the first member of a class of platinum-containing anti-cancer drugs, which also includes Carboplatin and Oxaliplatin[2]. DNA is the proven primary target of cisplatin, and cisplatin adduct formation effects many DNA-dependent cellular functions, including inhibition of replication and transcription, cell cycle arrest, and DNA damage leading to cell death and apoptosis, but also may results in mutations[3]. Cisplatin has a number of side-effects that can limit its use like – Nephrotoxicity (kidney damage), Neurotoxicity (nerve damage), Nausea and vomiting, Ototoxicity (hearing loss), Electrolyte disturbance, hypomagnesaemia, hypokalaemia and hypocalcaemia[4]. One of the major limitations to the maximization of its therapeutic potential is nephrotoxicity[5]. it is cleared by the kidney by both glomerular filtration and tubular secretion. Cisplatin concentrations within the kidney exceed those in blood suggesting an active accumulation of drug by renal parenchyma cells[6]. Several mechanisms have been considered for cisplatin nephrotoxicity including hypoxia, generation of free radicals, inflammation, lipid peroxidation and apoptosis Also, cisplatin reduces activity of antioxidant enzymes and induced depletion of glutathion (GSH) [7]. Oxidative damage is possibly an important mechanism in the pathogenesis of cisplatin nephrotoxicity due to the increase in the production of excess free radicals and a reduction in the production of antioxidants[8]. As a consequence, it has been hypothesized that antioxidant treatment could be useful in avoiding or reducing nephrotoxicity. Published studies have been shown a protective effect of various antioxidants on nephrotoxicity induced by

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cisplatin[9]. It was studied that Amifostine [S-2(3 amino propyl amino) ethyl phosphorothioate], is a pharmacological antioxidant used as a cytoprotectant in cancer chemotherapy and radiotherapy[10]. Some known cytoprotectants are Amifostine, Dexrazoxane, Leucovorin and Mesna, etc. Amifostine is FDA approved drug for its cytoprotective activity in cancer therapy, reduced myelotoxicity in children and adolescents undergoing chemotherapy for osteosarcoma[11]. Apart from amifostine, a wide variety of other chemical compounds have been evaluated as cytoprotective agents. Amifostine also has lot of drawbacks as cytoprotectant, as it cannot prevent hematologic toxicity induced by melphalan and it is not effective when given orally[12]. It has been studied that DRDE-07 and their analogs mainly DRDE-30 and DRDE-35 have prophylactic efficacy against mustard agents (sulphur mustard and nitrogen mustards). Mustard agent was given dermally after 30 min prior to these analogues. The result shown that some of the analogues were efficient against mustard agents and recovery was seen in biochemical alterations and in histopathological aspect[13,14]. It was also reported that DRDE-07 and its analogues has cytoprotective efficacy against Mechlorethamine (also known as HN-2; one of the nitrogen mustard agents) [15].

This triggers an interest that Amifostine, DRDE-07 and DRDE-30 could be possible nephroprotective agent to reduce cisplatine induced nephrotoxicity. In this study protective efficacy of Amifostine, DRDE-07and DRDE-30 were evaluated against cisplatine induced nephrotoxicity.

2. Materials and methods:-

2.1. Chemicals:

Cisplatin {cis-diamminedichloroplatin}, O-pthalaldehyde (OPT), glutathione and 4'6-diamidino-2-phenylindole (DAPI) were purchased from Sigma Chemical Company (USA). Other chemicals of high purity were from Qualigens (India) or E-Merck (India).

2.2. Animals:

Randomly bred Swiss female mice (25-30 g) from the institute's animal house facility (Approval No.37/GO/c/1999/CPCSEA11.03.1999) were used for the study. The animals were kept in polypropylene cages with sterilized and dry paddy husk as a bedding material. Free access to food (Ashirwad Ltd, India) and water were allowed until two hours before the experiment. The care and maintenance of animals were as per the approved guidelines of the 'Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)', India. A day before percutaneous exposure, hair on the back of the animals was closely clipped using a pair of scissors. Food and water were allowed two hours after the experiment. All animal procedures were approved by the Institutional Animal Ethical Committee.

2.3. Experimental Design:-

Two experiment designs were planned. In first design, drug was given 30 min before administration of cisplatin and followed by three days orally. In second design the drug was given after administration of cisplatin and the rest was same as first design.

Mice were distributed in following groups:

Group I: Healthy control

Group II: Cisplatin (10 mg/kg)

Group III: Cisplatin (10 mg/kg) + Amifostine (185 mg/kg)

Group IV: Cisplatin (10 mg/kg) + DRDE-07 (249 mg/kg)

Group V: Cisplatin (10 mg/kg) +DRDE-30 (219 mg/kg)

Cisplatin was dissolved in normal saline and sonicated it for 3-5 minutes. After sonication, 10 mg/kg dose of cisplatin was administered through intra peritoneal route. Amifostine, DRDE-07 and DRDE-30 were given orally after 2 hours of cisplatin administration. For amifostine, 185 mg/kg dose was used. For DRDE-07, 249 mg/kg, and for DRDE-30, equimolar dose of DRDE-07 i.e.219 mg/kg was used[16].

2.4. Biochemical evaluation:-

After Cisplatin exposure, animals were kept in a well-ventilated room 24 h for further monitoring and then shifted to the experimental animal room. The body weight was recorded daily. On fourth day, mice were anaesthetized with ether for collection of blood from orbital sinus, and then sacrificed by cervical dislocation for the removal of kidney. Blood samples were collected and

centrifuged at 4000 rpm for half an hour and plasma was separated[17]. Estimation of blood urea nitrogen (BUN) and serum creatinine (SCR) by using the commercial kits was done[18].

2.5. Histopathological evaluation:-

After three days, animals were anesthetized with ether and then sacrificed by cervical dislocation for removal of Kidney organs. Samples of kidney were removed, pruned, blotted and weighed. The organ to body weight index (OBWI) was calculated as a ratio of organ weight multiplied by 100 and divided by body weight. The tissues were fixed in neutral buffered formaline solution for histopathological evaluation. After proper fixation, small pieces were processed by dehydration and embedded in paraffin wax. Various sections (4-6 µm thickness) were prepared and stained with haematoxylin and eosin for light microscopy [19]. From each group, ten slides per organ were prepared. Out of these slides, two slides/ organ/group were selected randomly for the evaluation lesions. From each selected slide, 90 µm2 area was identified randomly and lesions were marked and compared with that of control [20]. The severity of lesions was characterized using LEICA – Qwin - 500 Image Analyzer and converted into percentage [21].

2.6. Statistical analysis:-

Statistical evaluations were made using one-way analysis of variance with Student–Newman–Keul's multiple comparisons test. A probability of 0.05 and less was taken as statistically significant. The analyses were carried out using SigmaStat for Windows version 2.03 (SPSS Inc., USA).

3. Result:-

3.1. Biochemical evaluation:-

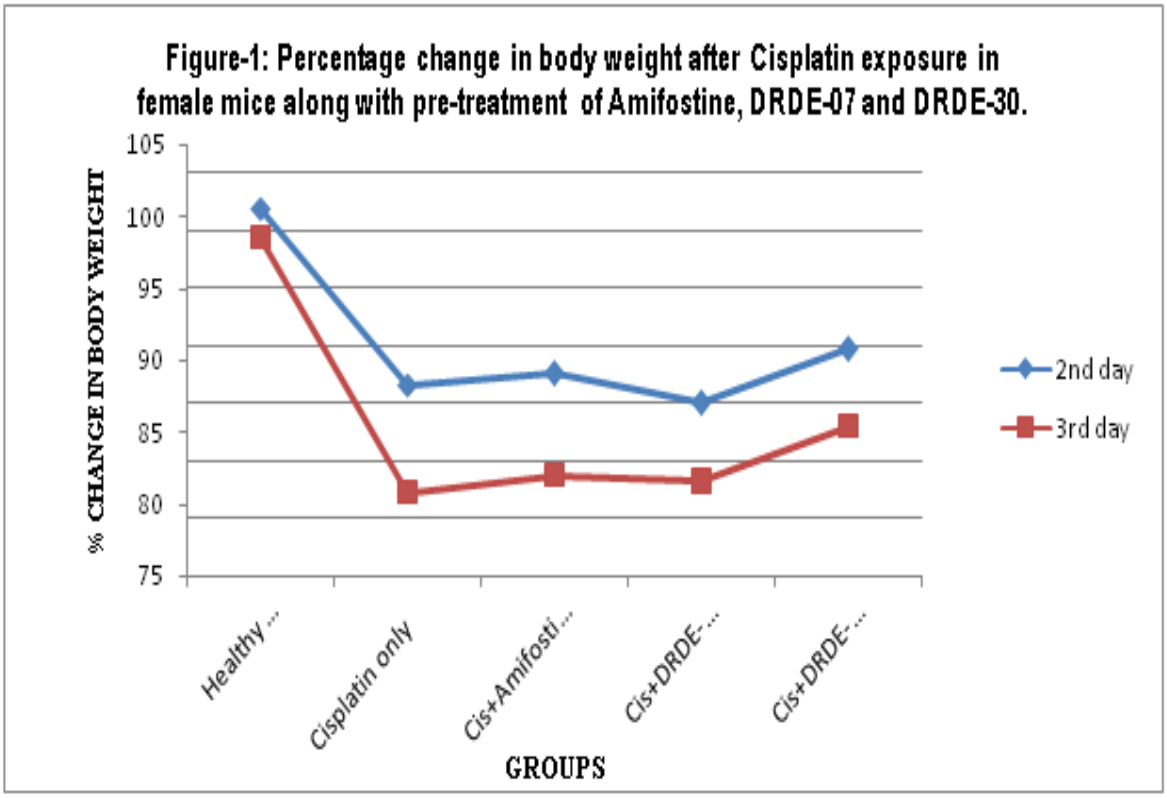

Figure 1: Percentage change in body weight after Cisplatin exposure in female mice along with pre-treatment of Amifostine, DRDE-07 and DRDE-30.

The body weight (BW) showed that the weight is continuously decreased as compared to healthy control group. In cisplatin group, the body weight decreased day by day whereas in amifostine group the BW change was mild. In DRDE-07 and DRDE-30 group the BW was higher in comparison to cisplatin group.

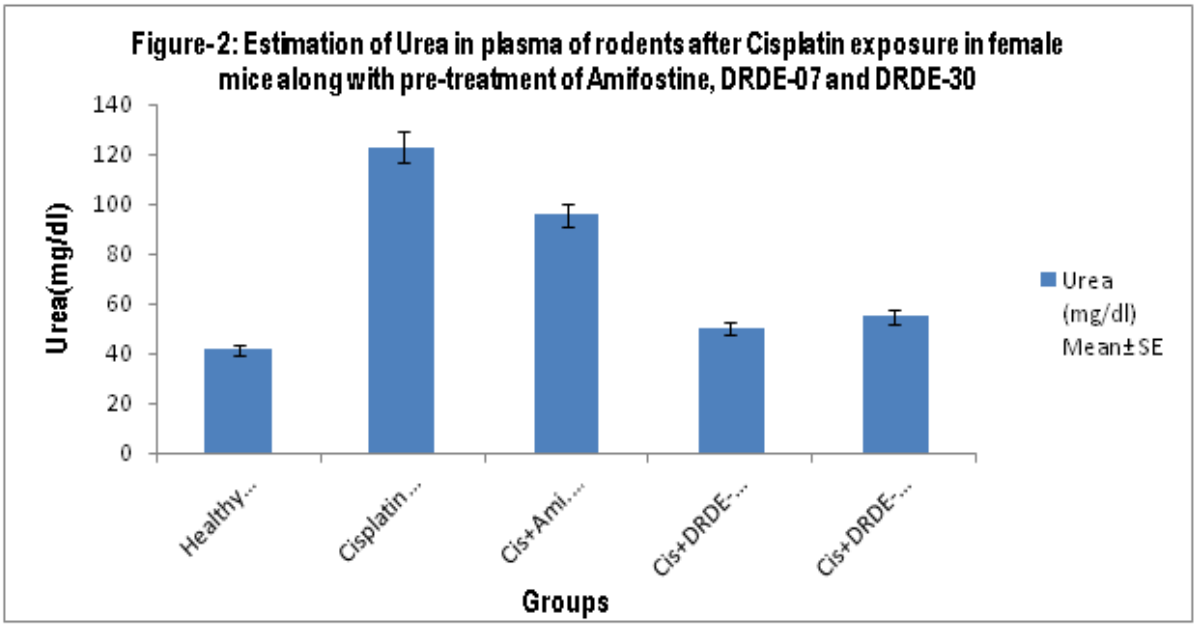

Figure 2: Estimation of Urea in plasma of mice after Cisplatin exposure in female mice along with pre-treatment of Amifostine, DRDE-07 and DRDE-30.

In Figure 2,the level of urea in plasma in female male mice after exposure of cisplatin was increased in comparison to healthy control group, when the mice was treated with drugs it showed minimum amount of urea in plasma as compare to cisplatin group. Group 4th showed more efficacies as compared to group 3rd and group 2nd.

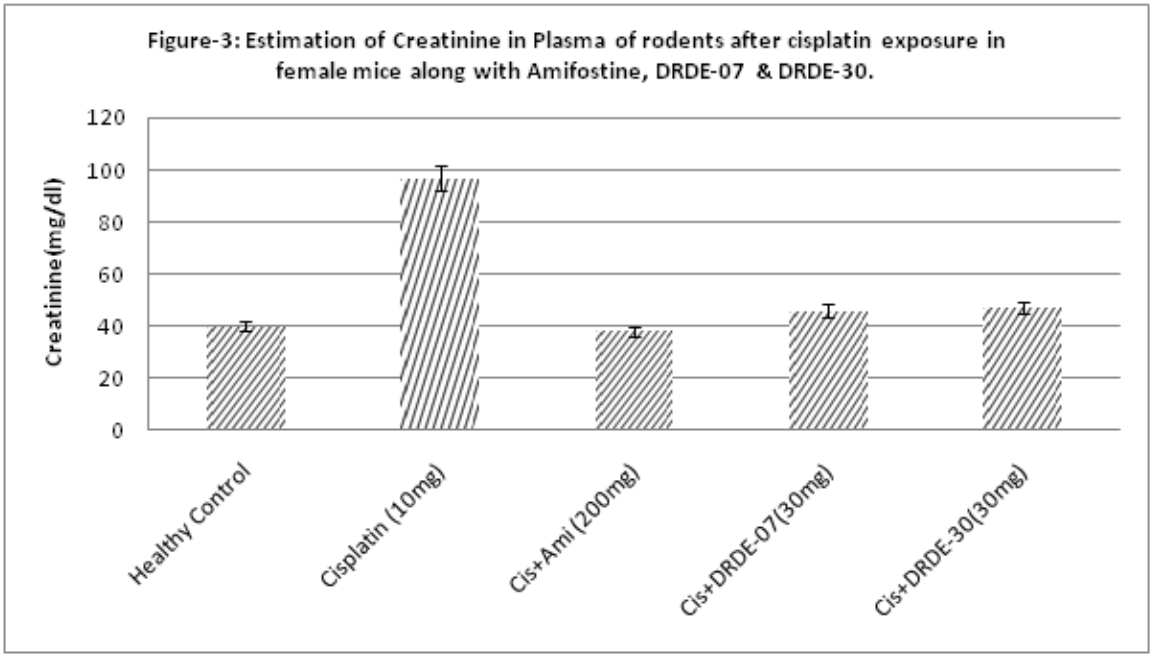

Figure 3: Estimation of Creatinine in plasma of rodents after Cisplatin exposure in female mice along with Amifostine, DRDE-07 and DRDE-30.

In Figure 3, the level of creatinine in plasma in female mice after exposure of cisplatin was increased in comparison to healthy control group. when the mice was treated with drugs it showed minimum amount of creatinine in plasma as compared to cisplatin group. Group 5th showed more efficacy as compared to group 2nd.

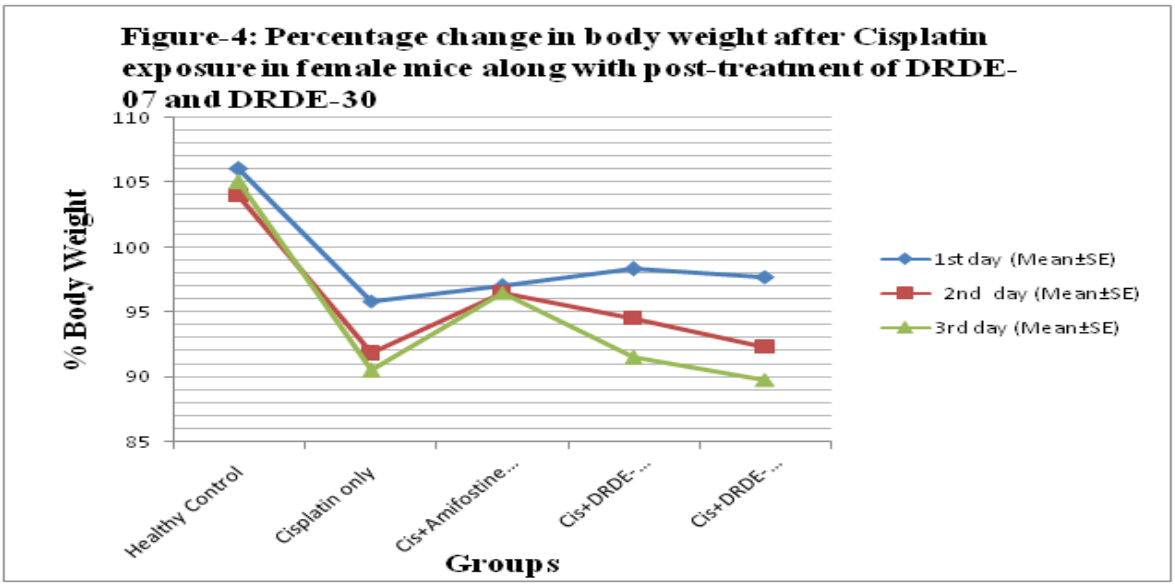

Figure 4: Percentage change in body weight after Cisplatin exposure in male female mice along with post-treatment of DRDE-07 and DRDE-30.

The body weight (BW) showed that the weight is continuously decreased as compared to healthy control group. In cisplatin group, the body weight decreased day by day whereas in amifostine group the BW change was higher than cisplatin group. In DRDE-07 and DRDE-30 group the BW was not much changed in comparison to cisplatin group.

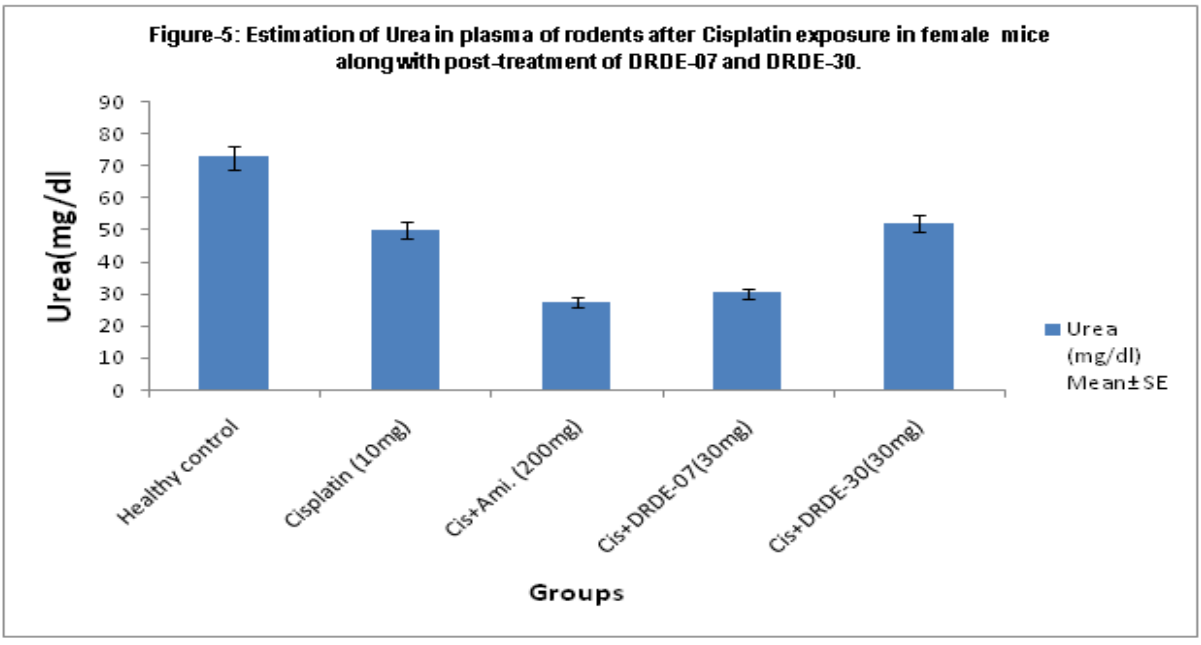

Figure-5: Estimation of Urea in plasma of rodents after Cisplatin exposure in female mice along with post-treatment of DRDE-07 and DRDE-30.

Figure-5 showed the level of urea in plasma in female mice after exposure of cisplatin. It was observed that in cisplatin group urea level was increased in comparison to healthy control group. When the mice were treated with drugs it showed minimum amount of urea in plasma as compare to cisplatin group. Group 3rd showed more efficacy as compared to group 4th and in amifostine group the urea level is least.

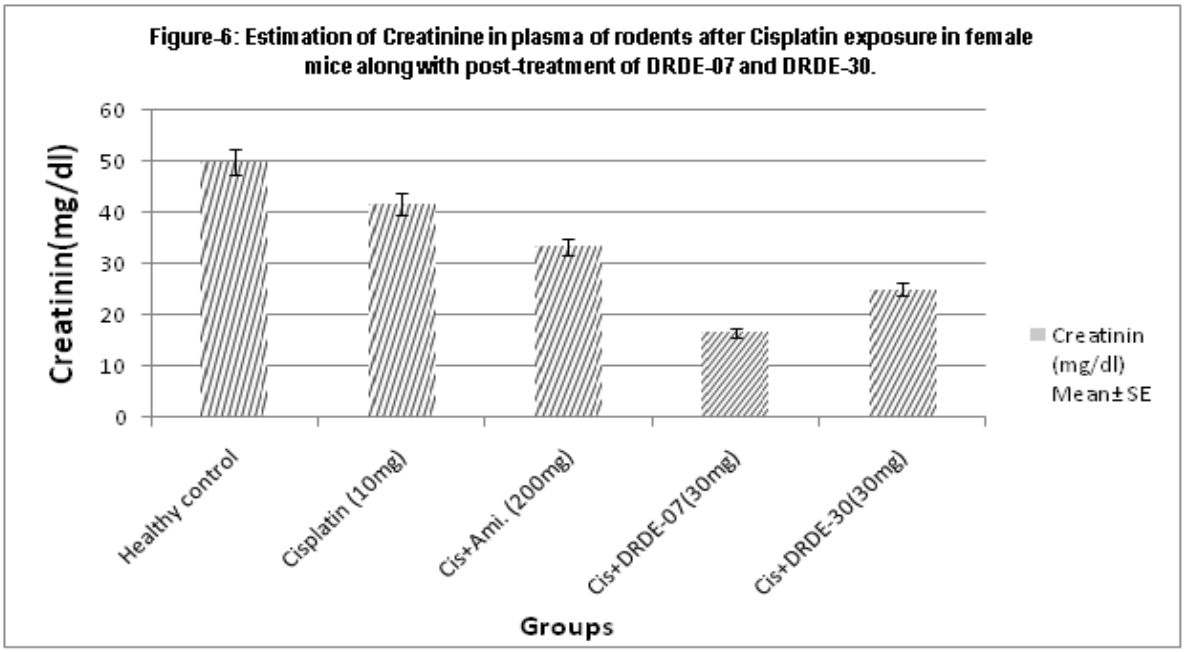

Figure-6: Estimation of Creatinine in plasma of rodents after Cisplatin exposure in female mice along with post-treatment of DRDE-07 and DRDE-30.

In Figure-6, the creatinine level is observed in female mice. After exposure of cisplatin along with Amifostine, DRDE-07 and DRDE-30. It was observed that in cisplatin group the creatinine level increased compare to healthy control whereas in Cisplatin+amifostine group, the creatinine level decrease in comparison to cisplatin group. In group 4th there was lowest creatinine level and in group 5thcreatinine level was higher in comparison to 4th group.

3.2. Histopathology

As seen in table-1, table-2 & figure-7, figure-8, histopathology examination of renal slices from mice treated with cisplatin alone showed different alterations, including acute tubular necrosis and severe desquamation in epithelial cells. When cisplatin administered along with Amifostine, DRDE-07 and DRDE-30, tubular necrosis, haemorrhage, cast formation, swallowing and desquamation were observed in kidney tissue of these groups mildly. No apoptotic bodies, inflammation and glomerular degeneration were observed.

Table-1: Histopathological changes in mice kidney after cisplatin exposure along with pre-treatment with amifostine, DRDE-07 & DRDE-30.

	Haemorrhage	Tubular Congestion	P. Tubular degeneration	Obliteration of chromatin material	Necrosis/ Apoptosis	Eosinophilic debris
Control	-	-	-	-	-	-
Cisplatin	++	++	+	+	+	++
Cisplatin + Amifostine	+	+	-	+	+	-
Cisplatin + DRDE-07	+	+	+	-	+	+
Cisplatin+DRDE-30	+	+	+	+	-	+

-MINIMAL (<10%), + MILD (<20%), ++ MODERATE (<50%) and +++ SEVERE (>50%)

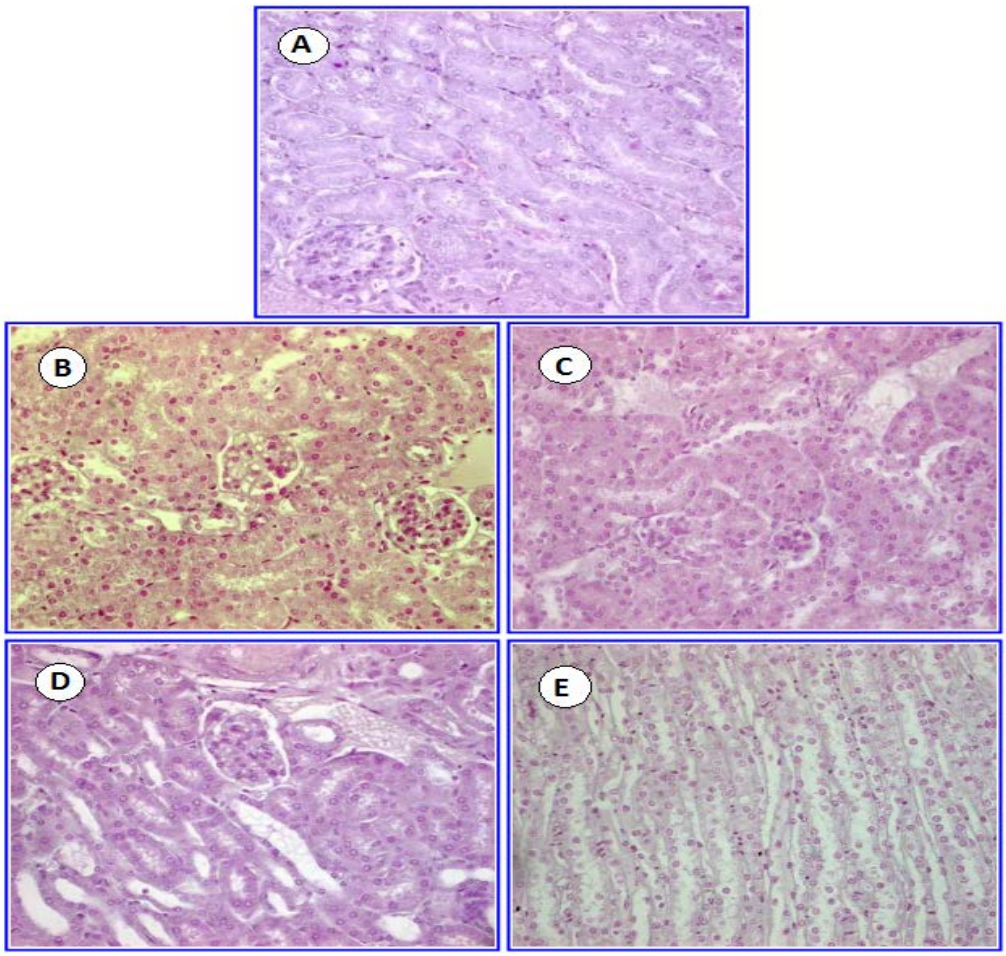

Figure-7: Photomicrograph of control, cisplatin exposed nephritic histopathology, 3 days post exposure in mice along with pre-treatment with amifostine, DRDE-07 & DRDE-30.

H&E, 40X. A Control, B Cisplatin, C Cisplatin+Amifostine, D Cisplatin+DRDE-07, E Cisplatin+DRDE-30

Table-2: Histopathological changes in mice kidney after cisplatin exposure along with post-treatment with amifostine, DRDE-07 & DRDE-30.

	Haemorrhage	Tubular Congestion	P. Tubular degeneration	Obliteration of chromatin material	Necrosis/ Apoptosis	Eosinophilic debris
Control	-	-	-	-	-	-
Cisplatin	++	++	+	+	+	++
Cisplatin + Amifostine	-	+	-	+	+	-
Cisplatin + DRDE-07	+	++	+	-	+	+
Cisplatin+ DRDE-30	+	+	-	+	+	+

-MINIMAL (<10%), + MILD (<20%), ++ MODERATE (<50%) and +++ SEVERE (>50%)

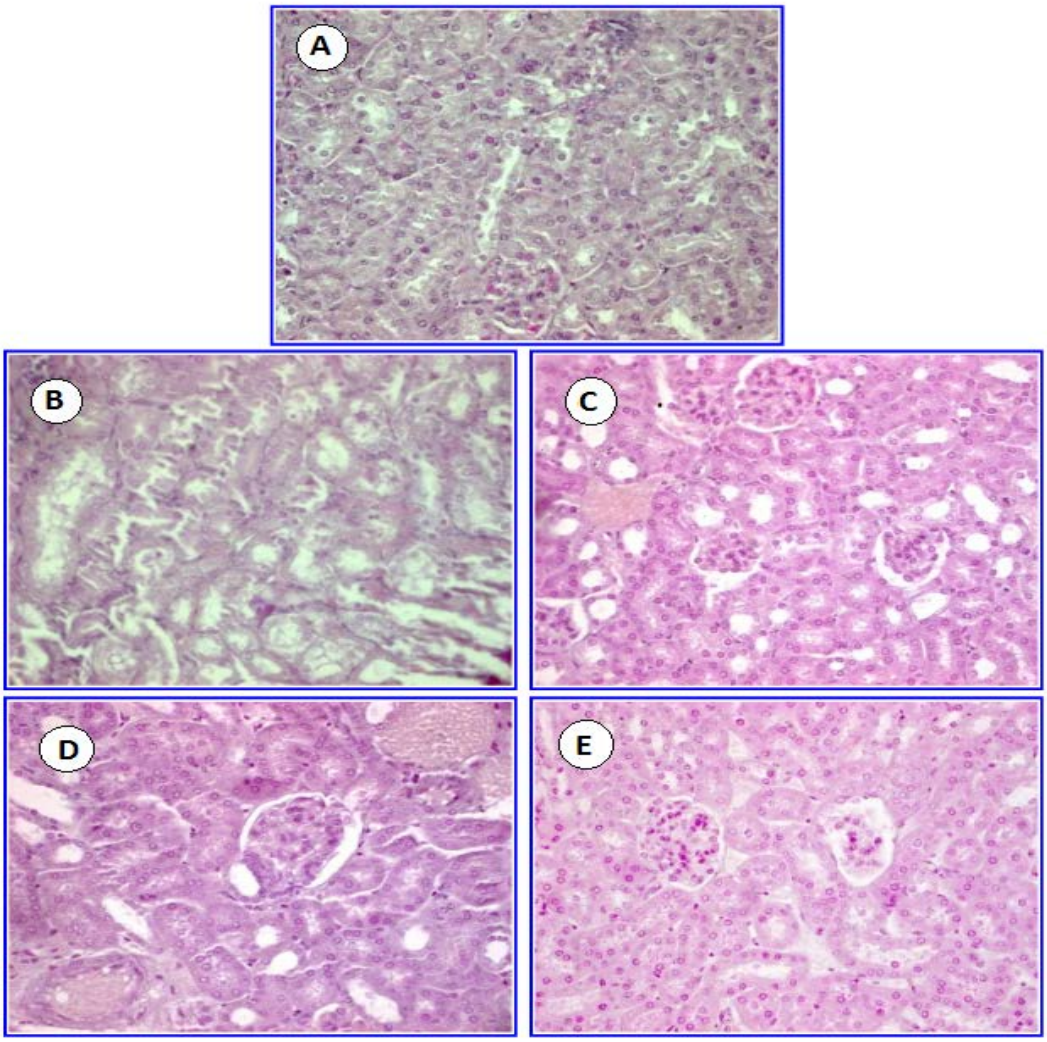

Figure-8: Photomicrograph of control, cisplatin exposed nephritic histopathology, 3 days post exposure in mice along with post-treatment with amifostine, DRDE-07 & DRDE-30.

H&E, 40X. A Control, B Cisplatin, C Cisplatin+Amifostine, D Cisplatin+DRDE-07, E Cisplatin+DRDE-30.

5. Discussion:-

Cisplatin is a chemotherapeutic agent for cancer but it have several side-effects e.g., ototoxicity, nephrotoxicity, etc by which its use is being limited. in this study we have evaluated DRDE-07 and DRDE-30 for its cytoprotection against cisplatin induce nephrotoxicity. we used female mice for this study. In this experiment we used two design ; in first design, we have given drugs before half an hour of cisplatin and in second design the drugs was given after 2 hours of cisplatin exposure.

Apoptosis may represent an early event in the pathogenesis of cisplatin nephrotoxicity[22]. In cultured renal proximal tubular cells, apoptosis is induced over a few hours after exposure to cisplatin[23].COX-2 inhibitor produces adverse effects on renal function particularly during low salt intake in animals and is occasionally associated with acute renal failures in humans, highlighting the important role of this enzyme in renal physiology[24,25]. It was shown in figure 1st and 4ththat the drugs are prophylactically active but not therapeutically effective in cisplatin nephrotoxicity. When we have done pre-treatment with drug in group 3rd, 4th and 5th as above shown the body weight improved as compared to group 1st which has given only cisplatin. But when we given drugs after 2 hours of

cisplatin through intraperitonial. It was not effective as in pretreatment.

6. Conclusion:-

It was concluded that the DRDE-07 and DRDE-30 showed much effectiveness when given before the exposure of cisplatin.

In the present study, various biochemical parameters had been done on the plasma samples collected from experimental animals exposed to cisplatin through intraperitonial route as well as control and drug groups.It was noticed in the present investigation there was a significant increasement in urea and creatinine value in cisplatin group.

Urea is the major metabolite product of protein catabolism. The urea level may be increased due to pre-renal causes (increased protein catabolism, some chronic liver diseases) or renal causes (acute or chronic renal diseases post renal obstruction to urine flow). In this study the urea level is increased due to renal obstruction which is caused by cisplatin. In study first, the urea level was decreased in group 4^{th} and 5^{th} which showed that they work as cytoprotectant. In study second, the urea level was least in group 4^{th} and in group 5^{th} there was no significant difference shown.

It was concluded from above studies that the urea level decreased when it is pre-treated with DRDE-07 and DRDE-30 and post-treated with DRDE-07.
In chemical terms, creatinine is a spontaneously formed cyclic derivative of creatine. Creatinine is chiefly filtered out of the blood by the kidneys (glomerular filtration and proximal tubular secretion). There is little-to-no tubular reabsorption of creatinine. If the filtering of the kidney is deficient, creatinine blood levels rise. Therefore, creatinine levels in blood and urine may be used to calculate the creatinine clearance (CrCl), which reflects the glomerular filtration rate (GFR). A rise in blood creatinine level is observed only with marked damage to functioning nephrons
In figure 4^{th} the creatinine level decreased in comparison to cisplatin group. When DRDE-07 and DRDE-30 was given orally before half an hour of cisplatin there was 50% decreasement in creatinine level by which it is cleared that both drugs shows 50% effectiveness against cisplatin induce nephrotoxicity.

In figure 6^{th} ,when DRDE-07 and DRDE-30 was given after 2 hours of exposure the DRDE-07 showed more efficacy in comparison to DRDE-30 in cisplatin induced nephrotoxicity.

By the whole experiment it was concluded that the pre-treatment of DRDE-07 and DRDE-30 showed efficacy against cisplatin induced nephrotoxicity. the post-treatment is also effective in case of DRDE-07 not for DRDE-30.

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