Adverse effects on cardiovascular status and lipid levels of albino Wistar rats treated with cisplatin and oxaliplatin in combination with 5 Fluorouracil

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Abstract: The study was designed to comparatively assess direct damages on cardiac tissues and aorta associated with abnormalities in lipid profile and cardiac biomarkers induced by two platinum cytotoxic compounds with and without 5FU (5Fluorouracil) in rats. Albino Wistar rats were treated with 5FU (15mg/kg), cisplatin (0.8mg/kg) and oxaliplatin (0.8mg/kg) in different dosing schedules. The changes in the lipid levels, CPK and Tropinin I levels, following treatment with single and combination schedules of CDDP, 5FU and Oxaliplatin were compared with the control group maintained on normal saline. Changes in LDL and cholesterol levels were highly significant in cisplatin and oxaliplatin treated rats. Myofibrillar loss and vascular wall thickening was seen in cisplatin treatment groups in the acute model of toxicity. The damages were mild but progressive. Tropinin I levels were raised well above diagnostic risk levels in the delayed model of toxicity in the rats treated with oxaliplatin in combination of 5FU, indicative of definite cardiotoxic potential of oxaliplatin in combination of 5FU mimicking the FOLFOX regimen.

Keywords: Oxaliplatin, cisplatin, 5FU, Rats, cardiac, toxicity, lipid levels.

INTRODUCTION

The modest therapeutic efficacy of single agent 5-Fluorouracil (5FU) was increased manifolds by combining first generation platinum compound cisplatin /CDDP (*cis*-diamminedichloroplatinum) and third generation platinum compound oxaliplatin to design optimal schedules for germ cell carcinomas and colorectal carcinoma respectively. Cardiotoxicity of 5FU is rare but lethal adverse effect manifested as MI, cardiogenic shock, pericarditis, CHF and Tako Tsubo syndrome (Deboever *et al.*, 2013; Grunwald *et al*., 2012; Dalzell and Samuel, 2009). The exact etiology of 5FU induced cardiotoxicity is not elucidated (Najam *et al.*, 2013). A study conducted on rabbits reported that 5FU causes myocardial necrosis, thickened intra- myocardial arterioles, apoptosis in distal coronary arteries and myocardial tissues of the epicardium (Tsibiribi *et al*., 2006). Cisplatin generates DNA cross-links and bifunctional and monofuntional DNA adducts, in addition to the generation of superoxide radicals which induce direct cytotoxicity (Bano *et al.*, 2013). On the other hand few and far between cases of oxaliplatin induced cardiotoxicity due to coronary vasospasms manifested as Kounis syndrome, ECG abnormalities, ischemia or the unusual case of myocardial stunning are reported (Chang *et al*., 2011; Cerny et.al.,2008). Since these two platinum compounds are given in combination with 5FU as first line agents in variety of carcinomas, and at the same time are associated with ambiguous effects on cardiovascular status and lipid levels, we opted to define any direct damages on myocardial cells and aorta correlated with changes in lipid levels and cardiac biomarkers in the harvested plasma for each treatment and the control group in an experimental study conducted on rats. Cardiac toxicity of platinum compound (cisplatin/oxaliplatin) in combination with anti-metabolite (5FU) in rodents has not been tested or reported before.

METHODS

Study design

The study was designed in the Department of Pharmacology, University of Karachi and conducted in DUHS (Dow University of Health Sciences), following ethical and institutional approval. All the animal protocols were in compliance of the Helsinki declaration (Rickham, 1964), amended in 1996. The first stage of the study was to develop two models of toxicity, labeled as acute and delayed, based on dosing protocol and rest period between doses. The second stage was to comparatively assess the changes in the levels of cardiac markers and lipid profile within each treatment group in the two models following histopathological assessment of the isolated cardiac tissues and aorta of the animals in the acute model of toxicity.

STATISTICAL ANALYSIS

Data was analyzed on SPSS version 19 with paired sample test, p value<0.05 was considered significant, p

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value<0.01 highly significant and p value<0.001 was considered very highly significant.

Animal protocols

Healthy male albino Wistar rats (weight 220-250gms) were selected from a stock of inbred species produced after 20 generations of brother × sister mating. The animals were kept in the well-ventilated and spacious "Rat House" of DUHS, which is specially designed and well equipped for the conduct of experimental research. The temperature was maintained at 23±2°C. Relative Humidity was kept 65-75%. Humidity less than 40% was avoided at all times to avoid the risk of ring tail or middle ear infection. Light and dark cycle is 10:14 hrs. The lighting was diffused and checked for flickering. Standard polypropylene cages with wire mesh tops were use to keep the animals. Cage racks were positioned to allow proper air exchange and save the animals from draughts. Opaque cages were selected to filter harmful glare and allow the rats to hide from neighboring rats and humans. The bedding (wood shavings) was free of microbial, parasitic or chemical contaminants with appropriate depth and layering (0.5±2 cm). No in-cage shelters were provided. The feed provided to the rats was nutritionally adequate with 40% protein content prepared in the laboratories and was contamination free. Food and tap water was provided *adlibitum*. The animals were adapted to the constant environment for a period of seven days with sufficient rat chow, before the dosing started. The animals were accustomed to 'gentling' during rest period.

Treatments groups and dosing protocol

Thirty six animals were included in both the acute and delayed model of toxicity. The animals were divided further in six groups based on treatments. Six rats were assigned in each treatment group within each model as Group A [2 ml (0.9%NS)], Group B [(5FU) 15mg/kg], Group C [(CDDP) 0.8mg/kg], Group D [(Oxaliplatin) 0.8mg/kg], Group E [(5FU+CDDP) (15+0.8) mg/kg] and Group F [(5FU+Oxaliplatin) (15+0.8) mg/kg]. The drugs were administered IP (intraperitoneal). The injections were carefully made at the midway of the xyphoid and the pelvic bone (lower right quadrant of the abdomen, close to the midline) and caution was taken to avoid the bladder, cecum or liver. Needle (25 needle gauge) was inserted at an angle of 30° for the shaft to reach a depth of 0.5 cm. In the acute model of toxicity the doses were administered on days 1, 5, 10, 15, 20. The blood was sampled by cardiac puncture on day 25 and the heart with attached vasculature (aorta) was excised for histopathological assessment during which the animal was kept deeply anesthesized by using chloroform. Specimen heart submitted in a single container each in formalin, received and coded as respective treatment group, with attached vasculature, was measured and cut surface was marked for apparent lesions and color. Representative section of heart cut and submitted in cassette A, B, C, D, E & F. Similar doses with schedule alteration were given on day 1 and day 5 for 5 weeks with a rest period of 10 days after two weeks of dosing in the delayed model of toxicity. Blood was sampled by cardiac puncture after 30 days following the last dose in the delayed model of toxicity.

Biochemical assessment

Blood drawn by cardiac puncture was collected in anticoagulant tubes, 3ml in Lavender top EDTA (Ethylenediaminetetraacetic acid) tube, 3ml. in Light blue top Citrate tube and 3 ml in Green top Heparanized tube. The blood cells are separated from the plasma by centrifugation for 10 minutes (1000-2000×g) in a refrigerated centrifuge machine. The centrifuge time is kept at 10 minutes to attain designated plasma for serological testing immediately after transferring the plasma into polypropylene tubes with Pasteur pipettes at a temperature of 2-8°C.

The following parameters were assessed by biochemical testing using standardized kits.

1. Cardiac Enzymes (CPK, Tropinin I)
2. Lipid profile (LDL, HDL, TGS, Total Cholesterol and Total lipids)

Light microscopy

After removal of the heart and vasculature, longitudinal section is made through both ventricles from the base to the apex of the heart. The section of the aorta is trimmed from the middle of the last one centimeter caudal segment of thoracic aorta. Tissue samples were fixed in 10% formalin in saline, dehydrated in ascending grades of ethyl alcohol, cleared in xylol and embedded in molten paraplast wax blocks at 57-61°C, 4-5 micron thick section cut were stained by H&E (Hematoxylin & Eosin) and Periodic Acid Schiff (PAS). Prepared slides were assessed for structural evaluation under a bright field light microscope by trained pathologist unaware of the treatments (blinded assessment).

RESULTS

Acute cardiac toxicity in experimental models-biochemical parameters

Table 1 shows the difference in the lipid levels of animals included in each treatment group (B, C, D, E, F) with the animals included in the control group A. The difference in the cholesterol level in each treatment group is very highly significant from the control group ($p<0.001$) except group C ($p<0.01$), in which is the difference is highly significant. Fig. 1 shows a rise in the levels of cholesterol in each treatment group which is the highest in group E followed by group F. Cholesterol levels are comparatively lower than other groups in animals of group C. The rise in the cholesterol levels is seen more in group D as compared to group B. Difference in the TGS level in each treatment group is very highly significant

Table 1: Comparative difference in lipid levels between all treatments groups in experimental model of acute toxicity

Paired Samples Test							
Acute Toxicity			Paired Differences		t	df	p-value
			Mean	Std. Deviation			
Lipid Profile	Cholesterol	Group A - Group B	-25.667	6.088	-10.327	5	0.000
		Group A - Group C	-16.667	7.866	-5.190	5	0.003
		Group A - Group D	-32.000	6.986	-11.221	5	0.000
		Group A - Group E	-63.667	5.241	-29.757	5	0.000
		Group A - Group F	-45.667	10.857	-10.303	5	0.000
	TGS	Group A - Group B	-15.833	4.446	-8.723	5	0.000
		Group A - Group C	-124.000	7.099	-42.784	5	0.000
		Group A - Group D	-78.000	9.055	-21.099	5	0.000
		Group A - Group E	-115.667	6.186	-45.801	5	0.000
		Group A - Group F	-73.833	2.483	-72.829	5	0.000
	Total Lipids	Group A - Group B	-106.167	7.782	-33.415	5	0.000
		Group A - Group C	-164.167	33.654	-11.949	5	0.000
		Group A - Group D	-176.667	55.500	-7.797	5	0.001
		Group A - Group E	-3.000	362.175	-0.020	5	0.985
		Group A - Group F	-139.667	11.201	-30.542	5	0.000
	HDL	Group A - Group B	0.500	1.378	0.889	5	0.415
		Group A - Group C	2.000	4.561	1.074	5	0.332
		Group A - Group D	0.167	6.585	0.062	5	0.953
		Group A - Group E	-5.333	7.118	-1.835	5	0.126
		Group A - Group F	-1.000	7.874	-0.311	5	0.768
	LDL	Group A - Group B	-24.000	3.521	-16.695	5	0.000
		Group A - Group C	-8.667	3.502	-6.061	5	0.002
		Group A - Group D	-16.000	10.863	-3.608	5	0.015
		Group A - Group E	-23.833	6.401	-9.121	5	0.000
		Group A - Group F	-15.167	8.635	-4.302	5	0.008

from the control group ($p<0.001$). Fig. 4 shows a rise in the levels of TGS in each treatment group which is the highest in group C followed by group E. Levels of TGS are comparatively lower than other groups in animals of group B. The rise in the TGS levels is slightly more in group D as compared to group F. Table 1 shows that difference in the lipid level in each treatment group is very highly significant from the control group ($p<0.001$) except in group E in which the difference is not significant from the control group. Difference in the LDL level in each treatment group except group C & D, is very highly significant from the control group ($p<0.001$). In group C & D, the difference is significant ($p<0.05$) but not highly significant. The difference in the HDL in each treatment group is not significant from the control group ($p>0.05$). Fig. 5 shows that the total lipid levels are highest in group D and lowest in group E. Fig. 3 shows that the LDL levels are highest in group B and lowest in group C. Fig. 2 shows that the HDL levels are highest in group E. Table 2 shows that difference in the Troponin I levels in each treatment group is not significant from the control group ($p>0.05$) except group F in which the difference in the levels of Troponin I are significant as compared to the control group ($p<0.05$). The difference in the CPK level in each treatment group is very highly significant from the control group ($p<0.001$).

Delayed cardiac toxicity in experimental models-biochemical parameters

Table 3 shows the difference in the lipid levels of animals included in each treatment group (B, C, D, E, F) with the animals included in the control group A. The difference in the cholesterol level in each treatment group is very highly significant from the control group ($p<0.001$) except group C and D in which the difference from the control group is not significant. Rise in the levels of cholesterol in each treatment group is noted, which is highest in group E followed by group F. Cholesterol levels are comparatively lower than other groups in animals of group C. The rise in the cholesterol levels is seen more in group B as compared to group C and D. Fig. 1 shows that the levels of cholesterol are higher in rats of group F in delayed toxicity model as compared to rats in group F in acute toxicity model.

The difference in the TGS level in each treatment group is very highly significant from the control group ($p<0.00$) except group B and C in which the difference in TGS levels of animals is not significantly different from the control group. Fig. 4 shows a rise in the levels of TGS in each treatment group which is the highest in group D followed by group F, whereas the levels of TGS are comparatively lower than other groups in animals of

Table 2: Comparative difference in cardiac biomarkers between all treatment groups in experimental model of acute toxicity

Paired Samples Test							
Acute Toxicity			Paired Differences		T	df	p-value
			Mean	Std. Deviation			
Cardiac Biomarkers	Troponin I	Group A - Group B	0.018	0.037	1.206	5	0.282
		Group A - Group C	0.012	0.031	0.956	5	0.383
		Group A - Group D	-0.027	0.033	-1.983	5	0.104
		Group A - Group E	-0.005	0.047	-0.259	5	0.806
		Group A - Group F	0.032	0.023	3.414	5	0.019
	CPK	Group A - Group B	-89.833	14.261	-15.430	5	0.000
		Group A - Group C	-56.667	7.581	-18.310	5	0.000
		Group A - Group D	-88.833	13.615	-15.982	5	0.000
		Group A - Group E	-120.000	13.312	-22.081	5	0.000
		Group A - Group F	-279.333	27.303	-25.060	5	0.000

Table 3: Comparative difference in lipid levels between all treatment groups in experimental model of delayed toxicity

Paired Samples Test							
Delayed Toxicity			Paired Differences		t	df	p-value
			Mean	Std. Deviation			
Lipid Profile	Cholesterol	Group A - Group B	-31.667	2.338	-33.175	5	0.000
		Group A - Group C	-8.000	15.837	-1.237	5	0.271
		Group A - Group D	-16.167	51.871	-0.763	5	0.480
		Group A - Group E	-49.667	4.457	-27.295	5	0.000
		Group A - Group F	-48.667	2.733	-43.626	5	0.000
	TGS	Group A - Group B	-12.667	17.143	-1.810	5	0.130
		Group A - Group C	-33.167	32.566	-2.495	5	0.055
		Group A - Group D	-182.833	21.554	-20.778	5	0.000
		Group A - Group E	-50.833	15.145	-8.222	5	0.000
		Group A - Group F	-92.167	18.357	-12.299	5	0.000
	Total Lipids	Group A - Group B	-87.333	41.932	-5.102	5	0.004
		Group A - Group C	-83.333	41.582	-4.909	5	0.004
		Group A - Group D	-222.500	59.119	-9.219	5	0.000
		Group A - Group E	-144.333	67.470	-5.240	5	0.003
		Group A - Group F	-181.167	42.021	-10.561	5	0.000
	HDL	Group A - Group B	4.500	8.044	1.370	5	0.229
		Group A - Group C	0.333	7.090	0.115	5	0.913
		Group A - Group D	2.167	6.014	0.882	5	0.418
		Group A - Group E	6.667	6.802	2.401	5	0.062
		Group A - Group F	-4.333	4.457	-2.381	5	0.063
	LDL	Group A - Group B	-28.667	12.111	-5.798	5	0.002
		Group A - Group C	-30.667	4.131	-18.183	5	0.000
		Group A - Group D	-45.333	27.391	-4.054	5	0.010
		Group A - Group E	-29.333	3.724	-19.295	5	0.000
		Group A - Group F	-29.167	4.446	-16.069	5	0.000

group C. The rise in the TGS levels is more in group E as compared to group B.

The difference in the Lipid level in treatment group D & F is very highly significant from the control group ($p<0.001$). Difference in the LDL level in each treatment group is highly significant from the control group ($p< 0.001$) except groups B & D, in which the difference is significant ($p<0.05$). The difference in the HDL in each treatment group is not significant from the control group (>0.05). Fig. 5 shows that the total lipid levels are highest in group D and lowest in group B and C. Fig. 3 shows that the LDL levels are highest in group D and lowest in group C. Fig. 2 shows that the HDL levels are highest in group F and lowest in group E.

Table 4: Comparative difference in cardiac biomarkers between all treatment groups in experimental model of delayed toxicity

Paired Samples Test							
Delayed Toxicity			Paired Differences		t	Df	p-value
			Mean	Std. Deviation			
Cardiac Biomarkers	Troponin1	Group A - Group B	-0.227	0.202	-2.751	5	0.040
		Group A - Group C	-0.270	0.194	-3.407	5	0.019
		Group A - Group D	-1.162	0.285	-9.968	5	0.000
		Group A - Group E	-0.278	0.101	-6.719	5	0.001
		Group A - Group F	-0.862	0.152	-13.888	5	0.000
	CPK	Group A - Group B	-84.333	5.007	-41.260	5	0.000
		Group A - Group C	-63.167	6.306	-24.536	5	0.000
		Group A - Group D	-203.833	7.250	-68.865	5	0.000
		Group A - Group E	-104.167	6.795	-37.553	5	0.000
		Group A - Group F	-572.167	43.083	-32.530	5	0.000

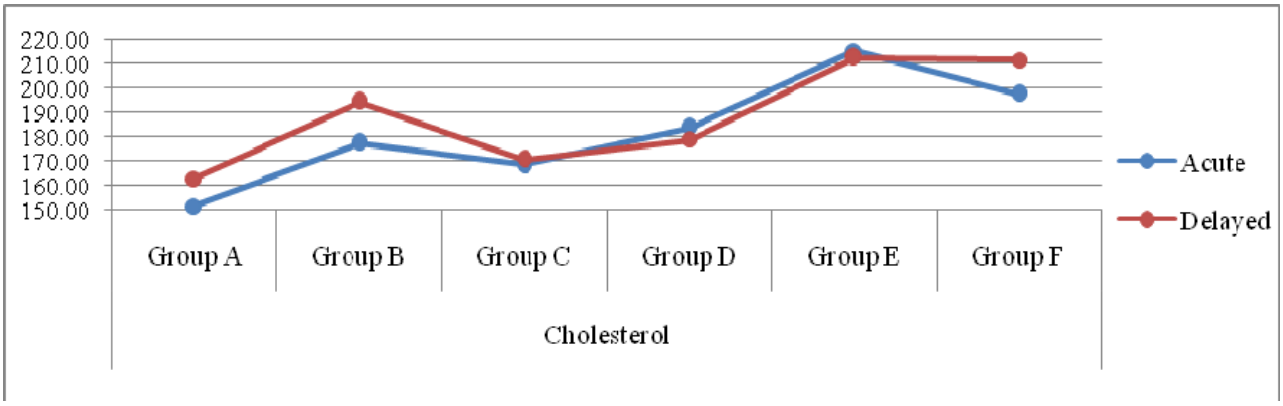

Fig. 1: Cholesterol levels (mg/dl) in acute and delayed experimental model of toxicity

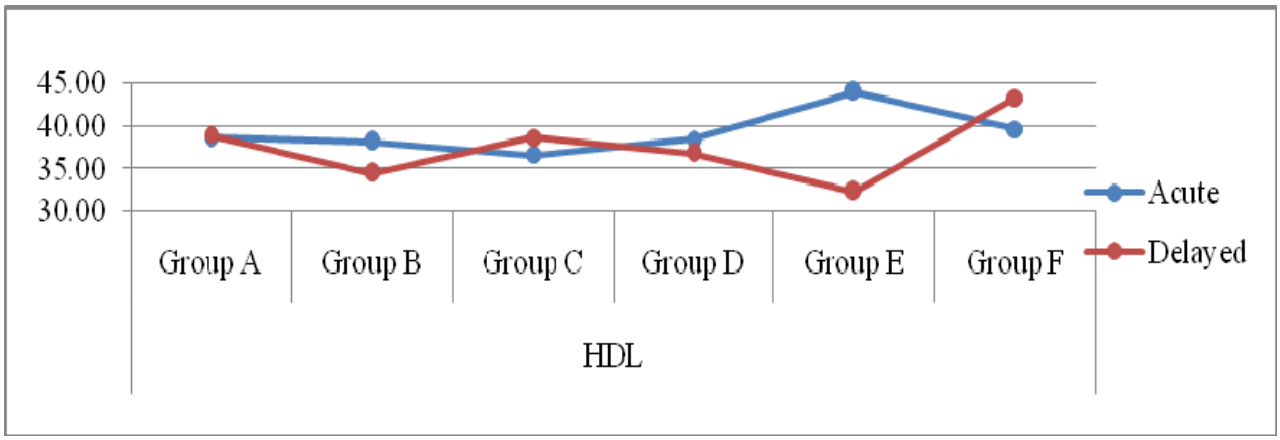

Fig. 2: HDL levels (mg/dl) in acute and delayed experimental model of toxicity

Table 4 shows the difference in the Troponin I levels in treatment group D & F is very highly significant from the control group ($p<0.001$), whereas in group B the difference in Troponin I level is not significant from the control group ($p>0.05$). The difference in the CPK level in each treatment group is very highly significant from the control group ($p<0.001$). Fig. 7 shows the highest level of Troponin I in group D and highest level of CPK in group F (fig. 6).

Cardiac toxicity in experimental models-histopathological

Specimen Heart A (control rat) measured 2.5x0.9 cm with predominantly unremarkable surface. Fig. A1-A3 show normal smooth muscle cardiac myocytes. Normal vasculature of the aorta is seen. Specimen Heart B (5FU treated rat) measured 2.2x1 cm with predominantly unremarkable surface. Fig. B1 shows wavy normal myocardial fibers are seen with no damage. In fig. B2,

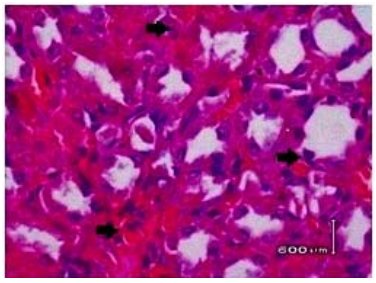

Fig. A1 (H&E Stain) - 40×

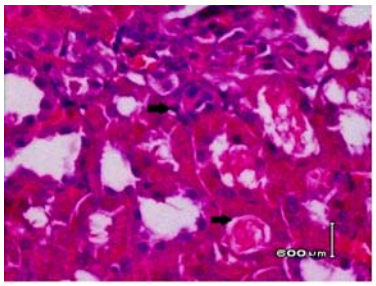

Fig. A2 (H&E Stain) - 40×

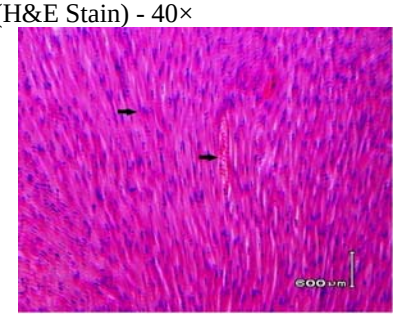

Fig. A3 (H&E Stain) - 10×

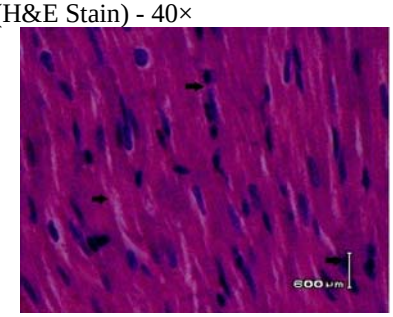

Fig. B1 (H&E Stain) - 10×

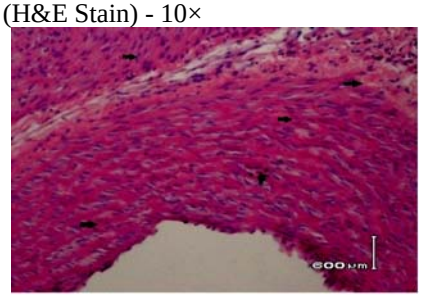

Fig. B2 (H&E Stain) - 10×

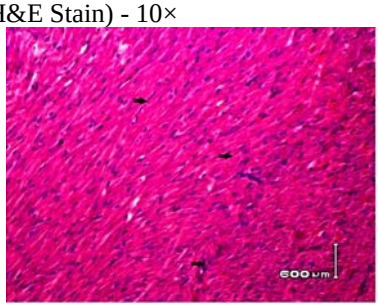

Fig. C1 (H&E Stain) - 10×

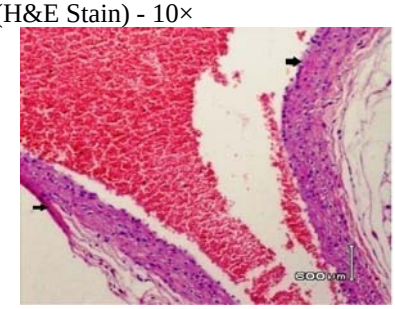

Fig. D1 (H&E Stain) - 10×

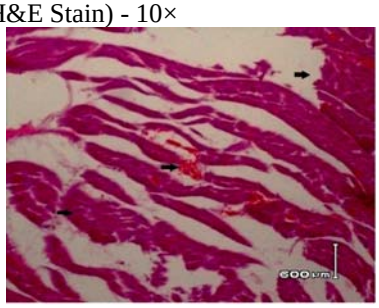

Fig. E1 (H&E Stain) - 10×

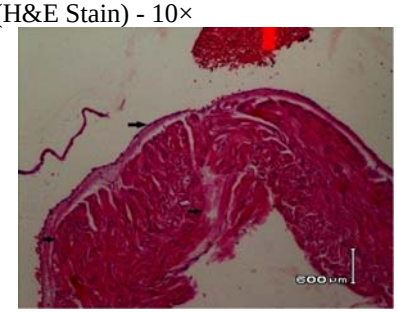

Fig. F1 (H&E Stain) - 40×

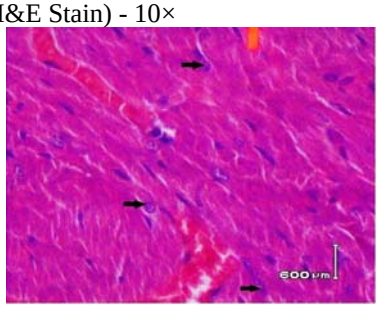

Fig. F2 (H&E Stain) - 10×

Normal vasculature of the aorta is seen. No myofibrillar loss is seen. Normal cytoplasmic vacuoles are visible. No necrosis is seen. Specimen Heart C (Cisplatin treated rat) measured 2.4x1 cm, surface predominantly unremarkable. Specimen heart E (Cisplatin plus 5FU treated rat) measured 2.2x1 cm, surface predominantly unremarkable. fig. C1 shows focal myofibrillar loss. Some intact and fragmented cardiac myocyte are suspected. Fig. E1 shows some pattern of thickening of the vessel walls. The damages are mild but present. Specimen Heart D (Oxaliplatin treated rat) measured 2.4x1 cm, surface predominantly unremarkable. Representative section of

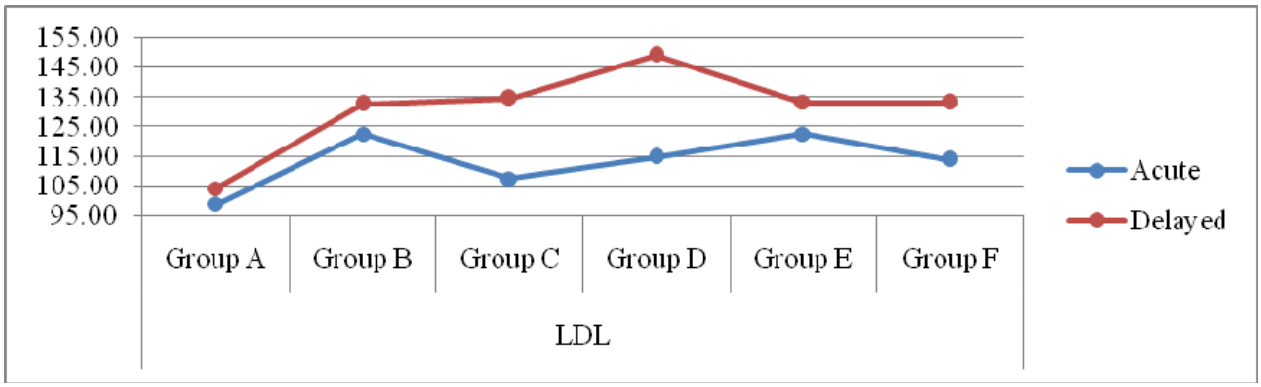

Fig. 3: LDL levels (mg/dl) in acute and delayed experimental model of toxicity

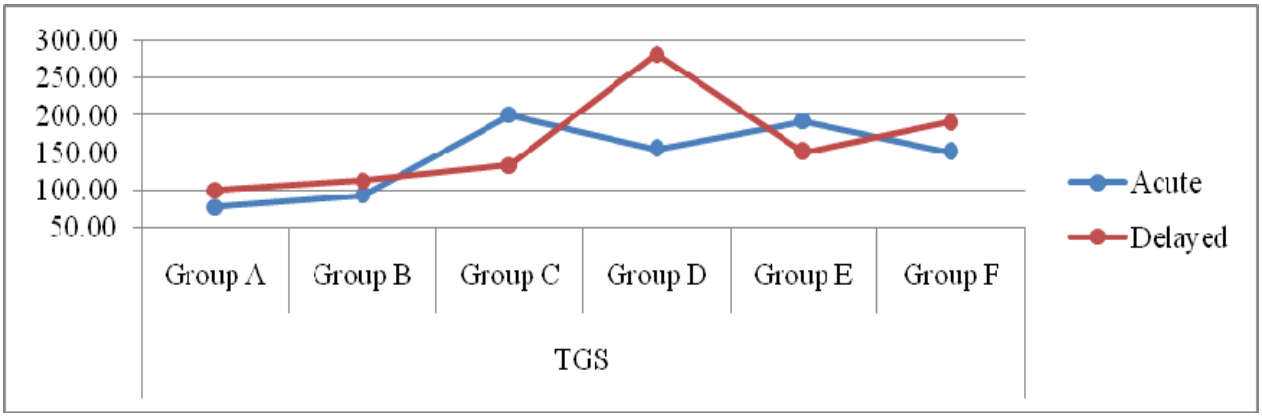

Fig. 4: TGS levels (mg/dl) in acute and delayed experimental model of toxicity

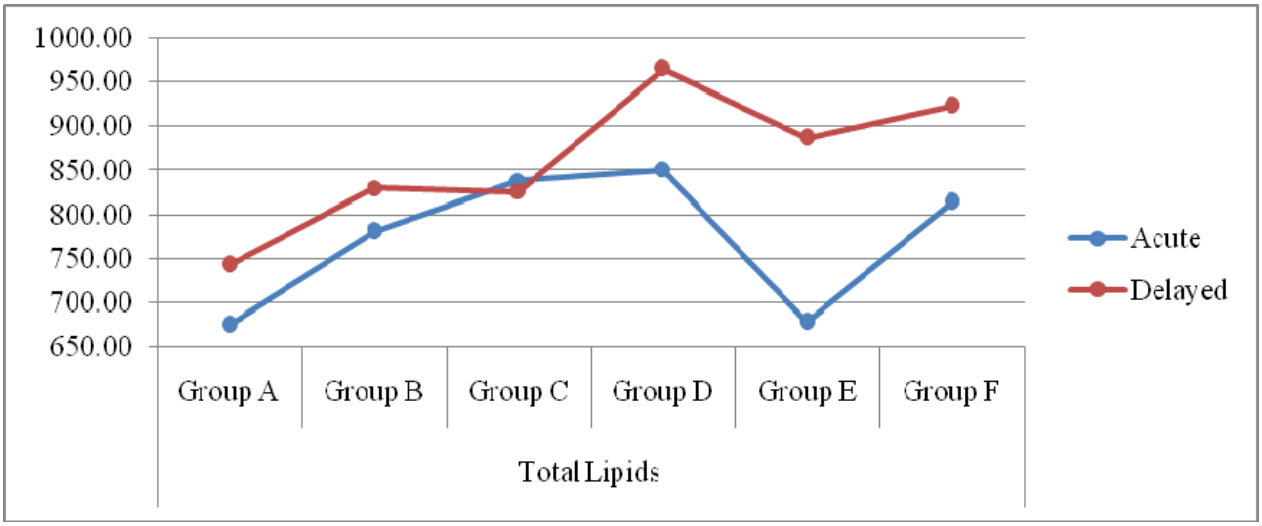

Fig. 5: Total lipids levels (mg/dl) in acute and delayed experimental model of toxicity

heart cut and submitted in cassette D. Specimen Heart F (Oxaliplatin plus 5FU treated rat) measured 2.4x1.1 cm surface is indicative of probable fibrosis. Fig. D1 shows no damage to the vessel walls, any medial thickening or architectural loss. No collagen fibrils or abnormality in endothelial cells. Figs. F1 and F2 show that the myocardium has no damage and is normal and no damages are imparted to the endothelial cells in the aorta.

DISCUSSION

The difference in the cholesterol level in each treatment group in acute toxicity model is highly significant from the control group (table 1). Fig. 1 shows a rise in the levels of cholesterol in every treatment group which is very highly significant in Oxaliplatin treated group and the group treated with 5FU in combination with

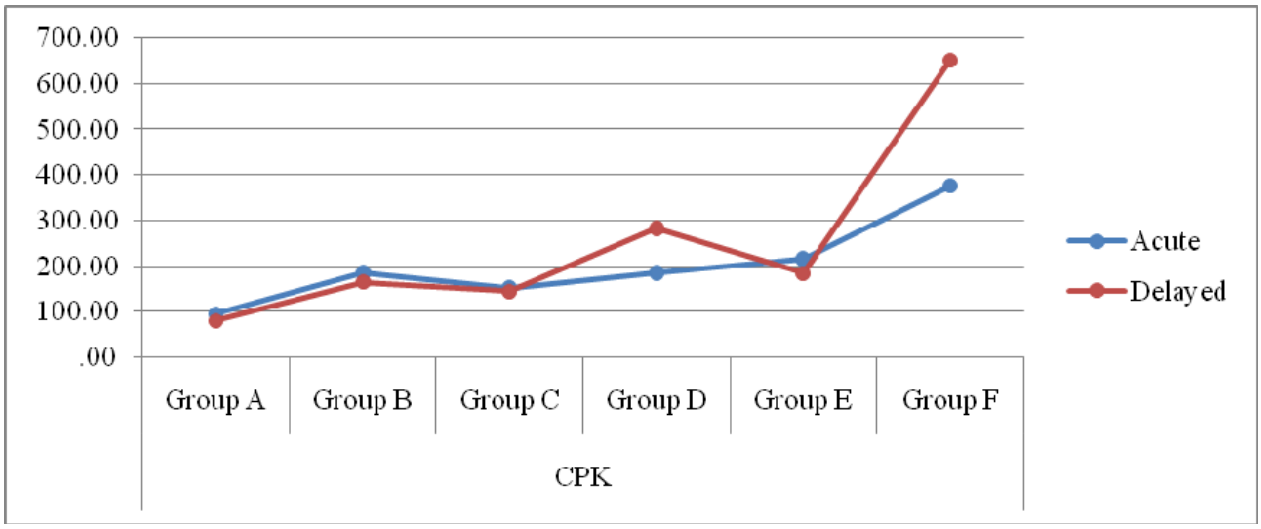

Fig. 6: CPK levels (U/L) in acute and delayed experimental model of toxicity

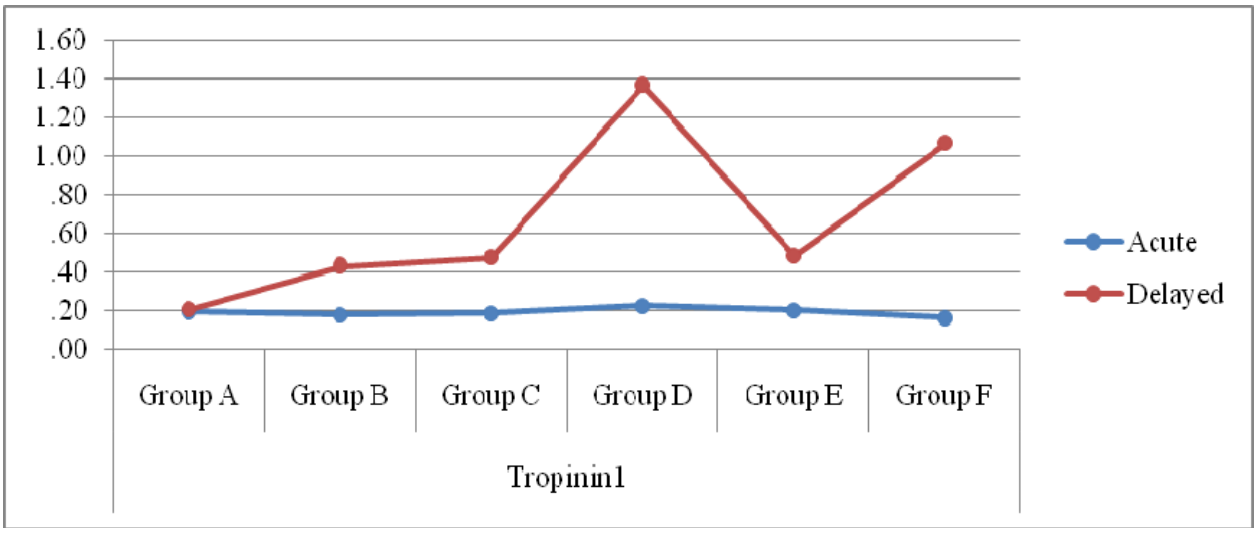

Fig. 7: Tropinin I levels (ng/ml) in acute and delayed experimental model of toxicity

Oxaliplatin. This indicates that Oxaliplatin raises the level of cholesterol in rats whereas the 5FU addition to the same dose of Oxaliplatin also implicates the cholesterol elevating property of Oxaliplatin. Based on studies reporting cholesterol-lowering effect of 5FU (Stathopoulos *et al*, 1995), we expected the cholesterol levels to fall in the rats, on the contrary we measured elevated levels of cholesterol in all the treatment groups as compared to the control group. The rise in the cholesterol levels is seen more in-group treated with Cisplatin + 5FU as compared to group treated with 5FU alone. Cholesterol levels are higher in rats of group F (5FU+Oxaliplatin) in delayed toxicity model as compared to rats in group F (5FU+Oxaliplatin) in acute toxicity model. In the model of delayed toxicity, it is important to note that the difference in the cholesterol level in each treatment group is highly significant from the control group, except Cisplatin treated group and Cisplatin + 5FU treated group in which the difference from the control group is not significant (table 3). The First generation platinum compound (Cisplatin) has a less effects on cholesterol levels as compared to the third generation platinum compound (Oxaliplatin) after treatment in the acute model of toxicity. Hypercholesteremia is considered to be a delayed adverse effect of cisplatin treatment especially in patients of testicular cancer (Koc *et al* 2011; Raghavan *et al*., 1992). Since similar effects are seen on the cholesterol levels of rats in both Group E (cisplatin treated) and Group F (oxaliplatin treated) in the delayed model of toxicity, we think that the risk of hypercholesteremia in long term follow up patients treated with oxaliplatin might be similar to that reported in long term follow up patients treated with cisplatin. However this needs to be investigated further in clinical settings to be confirmed. Chemotherapy associated defects on the androgen levels might lend a hand to this theory.

The triglyceride (TGS) levels are lower in rats treated with Oxaliplatin alone or in combination with 5FU as compared to the rats treated with Cisplatin alone or in combination with 5FU in acute model of toxicity (Fig. 4). This data shows that risk of steatosis is high in Cisplatin treatment as compared to Oxaliplatin treatment. Triglyceride levels in rats treated with 5FU alone and Cisplatin alone are somehow similar to the control group in delayed model of toxicity. On the other hand the TGS levels are significantly high in rats treated with Oxaliplatin alone and in combination with 5FU in the delayed toxicity model, which is at contrast with the effects of Oxaliplatin on TGS in the acute toxicity model. High TGS levels associated with steatosis, which is a known toxicity of Oxaliplatin (Regimbeau 2013). It is observed in our animal model of toxicity that steatosis is a delayed toxic manifestation in rats rather than an acute toxic effect of Oxaliplatin.

The LDL levels are markedly raised in the delayed toxicity model of rats treated with Oxaliplatin alone and Oxaliplatin in combination with 5FU than the acute toxicity model. The LDL levels are also high in Cisplatin treatment group but significantly lower than the Oxaliplatin treatment group.

We found dangerously high levels of cardiac biomarkers in the rats treated with Oxaliplatin alone or in combination with 5FU, which were prominent in the delayed toxicity model indicating the belated impact of the cumulative dose. CPK levels were highest in the group treated with the combination of 5FU + Oxaliplatin (mean value 651.50U/L) (Fig. 6). We can assume from these results that the risk of cardiovascular adverse events lies in the patient treated as delayed effects rather than acute manifestations as the damages on the cardiovascular system are progressive. Risk of oxaliplatin induced delayed cardiotoxicity is similar to delayed cardiotoxic events reported with cisplatin in follow up patients (Bano *et al.,* 2012; Haugnes 2010; Sagstuen 2005; Huddart 2003; Strumberg 2002). This is supported by the finding that the Tropinin I levels were raised high above the diagnostic risk level in the rats treated with oxaliplatin alone (group D) and oxaliplatin with 5FU (group F), measured after the last dose in the delayed model of toxicity. The Tropinin I levels crossed the diagnostic risk levels in the rats included in the delayed toxicity model treated with Oxaliplatin alone (1.37ng/ml) or in combination with 5FU (1.07ng/ml). The Tropinin I levels, which may be indicative of an event of MI, CHF or CVA (cerebero vascular accident) are not variably affected (fig. 7) in the acute model of toxicity.

Fig. A1, fig. A2 and fig. A3 show normal smooth muscle cardiac myocytes. Normal vasculature of the aorta is seen. fig. B1 shows wavy normal myocardial fibers are seen with no damage. In Fig. B2, normal vasculature of the aorta is seen. No myofibrillar loss is seen. Normal cytoplasmic vacuoles are visible. No necrosis is seen. Fig. C1 shows some focal myofibrillar loss. Some intact and fragmented cardiac myocytes are suspected. Fig. E1 also shows some pattern of thickening of the vessel walls. Cytotoxic damages are thus seen in both the groups of cisplatin treated rats, with and without 5FU. The damages are mild but present. A proposed mechanism of cisplatin induced cardiotoxicity is due to increased oxidative stress and apoptosis (EL-Alawady, 2011). Ma *et al*., (2010) reported depressed cardiomyocyte contraction and mitochondrial abnormalities, enhanced endoplasmic reticulum stress and associated apoptosis as manifestations of Cisplatin induced cardiac injury. Vascular damage and platelet aggregation with enhanced thromboxane formation leading to adverse cardiac implications is also reported in patients subjected to Cisplatin therapy (Togna *et al.,* 2000), which can be assessed by further investigating the effects of CDDP treatment on platelet levels.

Fig. D1 (oxaliplatin treated rats) shows no damage to the vessel walls, any medial thickening or architectural loss. No collagen fibrils or abnormality in endothelial cells. Fig. F1 and fig. F2 (oxaliplatin plus 5FU treated rats) show that myocardium has no apparent damage and is normal, no damages are imparted to the endothelial cells in the aorta as well. We came across a rise in CPK levels of Oxaliplatin treated rats which are now confirmed after biopsy, were not due to cardiac damage but probably due to damages to the brain and predominantly the skeletal muscles. Signs of any direct toxicity on myocardial cells and walls of the aorta after Oxaliplatin treatment are absent as compared to mild myocardial toxicity marked by myofibrillar loss, which is only indicated in Cisplatin treated rats. The microscopic assessment was made after acute dosing only, in which the Tropinin I levels were not markedly elevated in rats. On the other hand Tropinin I levels significantly elevated in rats treated with oxaliplatin in the delayed model of toxicity suggest possible damages (coagulative necrosis) in the cardiac tissues, which can be investigated in a future study, however with the biochemical evidence at hand, we predict such damages to ensue as delayed adverse effects of oxaliplatin treatment. We also believe that assessment of the oxaliplatin induced cardiac toxicity should not be solely relied on abnormalities in CPK levels which are more correlative and suggestive of skeletal muscle and brain damages in our experimental models rather than direct cardiac damages.

CONCLUSION

Lipid levels in the rats treated with cisplatin and oxaliplatin are elevated as compared to control group treated with normal saline. The delayed effects on triglyceride and cholesterol levels are more pronounced in

the rats treated with cisplatin and oxaliplatin with 5FU. The effects on HDL levels are negligible in treatment groups. CPK levels are highest in rats treated with oxaliplatin with 5FU in delayed dosing schedule. Tropinin I levels are also raised above diagnostic risk level in rats treated with oxaliplatin or cisplatin with 5FU in delayed dosing schedule. Higher risk of cardiac toxicity is manifested as delayed effect rather than an acute reaction with oxaliplatin treatment. No acute damages on endothelial surface or myocardial cells are seen in rats treated with oxaliplatin. There is evidence of myofibrillar loss and vessel wall thickening in cisplatin treated rats in the acute model of toxicity. The biochemical abnormalities in the oxaliplatin treated rats in the delayed model of toxicity indicate that the damages to the heart and the vessels are possible and can be investigated by Histopathological assessment in future studies.

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