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Toxic Effects of Treatment with Cyclophosphamide on Serum Biochemical Markers of Hepatic Function in Albino Rats

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Abstract: Despite being an effective modality of treatment against cancer, chemotherapy is a major factor responsible for the morbidity and mortality caused by cancer. Cyclophosphamide, a widely used chemotherapeutic drug belonging to the Nitrogen Mustard family of alkylating agents, has been documented for many side-effects on the healthy tissues and metabolism of individuals undergoing treatment.

In the present study, an effort has been made to investigate the effects of cyclophosphamide administration on hepatic metabolism of albino rats. Male albino rats were divided into two groups (n= 6 in each). Group 1 (Vehicle control) rats were treated with distilled water and group II rats were intraperitoneally administered with single dose of cyclophosphamide at the dose of 200 mg/kg b wt on day 1 of the experiment. Blood was collected from rats of each group on day 7 via cardiac puncture. Serum was separated and analysed for level of liver function markers and lipid profile parameters. Intraperitoneal administration of cyclophosphamide resulted in a significant increase in serum level of bilirubin and liver enzymes-- Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT) and Alkaline Phosphatase (ALP) (P<0.001), while decreased the level of total protein (P<0.01), albumin (P<0.05) and globulin, when compared to vehicle control group rats. A significant increment in serum level of Total Cholesterol (TC), Low Density Lipoprotein (LDL-c), Triglycerides (TG) and Very Low Density Lipoprotein (VLDL- c) along with a significant decrease in High Density Lipoprotein (HDL-c) was observed following cyclophosphamide administration in group II rats, when compared to control group (P<0.001).

Cyclophosphamide caused severe biochemical alterations resulting in hepatic toxicity, increased lipid peroxidation and redox imbalances in albino rats. Therefore, a routine monitoring of liver function might be advisable for the individuals undergoing chemotherapy as well as before recommending the chemotherapy session.

Keywords: Chemotherapy, Cyclophosphamide, Lipid profile, Serum Glutamic Pyruvic Transaminase, Serum Glutamic Oxaloacetic Transaminase, Alkaline Phosphatase, Lipid Peroxidation, Hepatic toxicity

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Introduction

Chemotherapy is a commonly used treatment modality for various types of cancer. It is based on the administration of antineoplastic agents either singly or in combination to check the growth of highly proliferating tumor cells. The antineoplastic drugs can be alkylating agents (Cisplatin, Cyclophosphamide), antibiotics (Doxorubicin, Bleomycin), antimetabolites (Methotrexate, Azathioprine), Topoisomerase I inhibitors (Topotecan, Rubitecan), histone deacetylase inhibitors (Belinostat, Romidepsin), protein kinase inhibitors (Abemaciclib, Cabozantinib), biological response modifiers (Interferon Gamma) and hormonal agents (antiandrogens, antiestrogens, GnRH analogues) based on their mechanism of action. These drugs act on DNA, RNA or proteins located either in the tumor cells or other elements involved in the carcinogenesis, such as, endothelium, extracellular matrix and cells and molecules of immune system (Espinosa *et al.*, 2003; LiverTox, 2012; Alam *et al.*, 2018).

Being non-selective and cytotoxic in nature, most of the conventional chemotherapeutic agents impose destructive effects on healthy cells with high proliferation rate, such as, cells in bone marrow, digestive tract and hair follicles, leading to immunosuppression, hepatotoxicity, alopecia and other systemic side effects. Liver, being the chief site of metabolism and excretion of endogenous and exogenous compounds, becomes highly susceptible for toxicity caused by them. Hepatotoxicity is one of the main reason behind withdrawal of a drug, as 50% of all acute liver failures and 5% of all hospital admissions are associated with drug- induced hepatotoxicity (Howida, 2016; Singh *et al.*, 2016). Almost all antineoplastic agents cause some degree of hepatotoxicity (LiverTox, 2012).

Cyclophosphamide ($C_7H_{15}Cl_2N_2O_2P$) (Fig. 1) is an alkylating agent belonging to Nitrogen Mustard family, which is clinically used in chemotherapy for treatment of different types of cancers and in immune related disorders also. It is a pro- drug which needs bio- activation via formation of active

metabolites phosphoramidate mustard and acrolein using hepatic cytochrome P450 system. Although Phosphoramidate mustard is responsible for its anti- neoplastic activity, acrolein exerts side-effects via generation of free radicals and induction of lipid peroxidation (El- Sebaey *et al.*, 2019; Ogino and Tadi, 2022). Cyclophosphamide induced histological alterations in hepatic tissues of albino rats have already been reported in our previous study (Samdershi *et al.*, 2019). In the present investigation, an effort has been made to investigate the effects of administration of cyclophosphamide on serum level of liver function markers and lipid parameters in male albino rats.

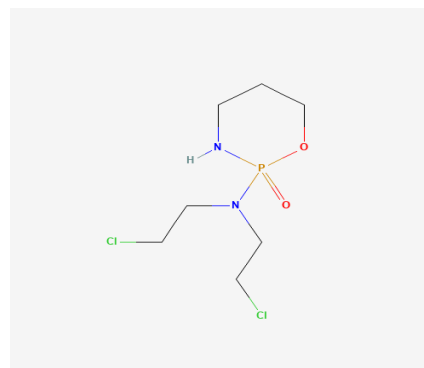


Fig 1: Chemical structure of cyclophosphamide (PubChem).

Materials and Methods

Experimental animal:

Healthy male albino rats (body weight 150- 160 g) were purchased from Jaz Scientific Store, Ranchi and were maintained in plastic cages in animal house of University Department of Zoology, Ranchi University, Ranchi, India. Rats were kept for acclimatization to the standard laboratory conditions (temperature 22- 24 °C and photoperiod of 12 h light/dark) for 7 days prior to the experimentation. Rats were given ad libitum access to standard pellet diet and tap water.

Experimental drug:

Cyclophosphamide (Endoxan-N; 200 mg) was purchased in form of white crystalline powder in injection vials. It was dissolved in distilled water for administration to the rats.

Experimental design:

Grouping of animals and administration of dosage:

Rats were divided into 2 groups with 6 individuals in each. Group I served as vehicle control (VC) in which rats were given distilled water throughout the study. In group II (CYP), rats were intraperitoneally (i.p.) administered with cyclophosphamide at the dose of 200 mg/kg body weight on day 1 of the experiment. Rats were maintained for 6 days. The dose of cyclophosphamide and the experimental duration has been previously reported (Samdershi *et al.*, 2019).

Collection of sample:

On 7th day, blood from rats of each group was collected via cardiac puncture following anaesthesia. Serum was isolated by centrifugation and was further processed for biochemical analyses.

Biochemical analysis:

(A) Liver function test:

Serum collected from rats of each group was analysed for level of total bilirubin, bilirubin direct, bilirubin indirect, total protein, albumin, globulin and liver function enzymes-- Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT) and Alkaline Phosphatase (ALP) using fully automated biochemistry analyser LWC 100 Germany.

(B) Lipid profile:

Serum was also analysed for level of total cholesterol, triglycerides and lipoproteins-- High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL) using fully automated biochemistry analyser LWC 100 Germany.

Statistical analysis:

Values are expressed as Mean \pm Standard Deviation (SD). Data obtained were statistically analysed using Student's *t*- test and values with $P < 0.05$, $P < 0.01$ and $P < 0.001$ were considered significant at degree of freedom 10.

Results

Effects of CYP on serum level of liver function markers:

The effects of treatment with cyclophosphamide on serum level of liver function markers have been summarized in Table 1. After 6 days of single dose administration of cyclophosphamide (200 mg/kg body weight; i.p.), a significant increase ($P < 0.001$) in serum level of total as well as direct and indirect bilirubin was observed when compared to vehicle control group rats (Fig. 2). On the other hand, a significant decrease in serum level of total protein ($P < 0.01$) and albumin ($P < 0.05$) was observed following single dose intraperitoneal administration of cyclophosphamide, when compared to vehicle control group rats. Although cyclophosphamide treatment in group II rats resulted in a decline in serum level of globulin, but the decrease was not significant as compared to that in group I (Fig. 3). Administration of cyclophosphamide resulted in a significant increment ($P < 0.001$) in serum level of liver function enzymes (SGPT, SGOT and ALP) when compared to vehicle control group rats (Fig. 4). Cyclophosphamide induced increase in serum level of bilirubin and liver function enzymes showed the toxic effects of the treatment resulting in hyperbilirubinemia and increased activity of lipid peroxidation and redox imbalance.

Effects of CYP on lipid profile:

Table 2 illustrates the serum level of lipid profile parameters in control and treated group rats. Intraperitoneal treatment with cyclophosphamide at the dose of 200 mg/kg body weight on day 1 of the experiment resulted in a significant increase in serum level of total cholesterol, LDL- cholesterol, triglycerides and VLDL-cholesterol along with a significant decrease in HDL-cholesterol, when compared to vehicle control group rats ($P < 0.001$) (Fig. 5). Cyclophosphamide induced dyslipidemia and hyperlipidemia showed its toxic side effects on the lipid metabolism of individuals undergoing treatment with chemotherapy.

Table 1: Effects of single dose administration of cyclophosphamide (200 mg/kg body weight; i.p.) on serum level of liver function markers in male albino rats

S. No.	Parameters	Groups		t-value	% Change (-) Decrease/ (+) Increase
		Group I (Vehicle Control; Distilled Water)	Group II (CYP; 200 mg/kg body weight; i.p.)		
1.	Bilirubin- Total (mg/dL)	0.69 ± 0.05	2.02 ± 0.18***	15.92	+ 192.75 %
2.	Bilirubin- Direct (mg/dL)	0.53 ± 0.08	1.19 ± 0.25***	5.62	+ 124.52 %
3.	Bilirubin- Indirect (mg/dL)	0.16 ± 0.06	0.83 ± 0.15***	9.27	+ 418.75 %
4.	Total Protein (g/dL)	5.79 ± 0.75	4.21 ± 0.60**	3.69	- 27.29 %
5.	Albumin (g/dL)	3.14 ± 0.53	2.04 ± 0.69*	2.83	- 35.03 %
6.	Globulin (g/dL)	2.65 ± 0.69	2.17 ± 1.18	0.78	- 18.11 %
7.	SGPT/ALT (U/L)	42.67 ± 6.32	65.78 ± 7.54***	5.25	+ 54.16 %
8.	SGOT/AST (U/L)	162.02 ± 15.14	204.63 ± 13.32***	4.72	+ 26.30 %
9.	ALP (U/L)	231.3 ± 15.28	389.33 ± 23.85***	12.47	+ 68.32 %

Values were expressed as Mean ± Standard Deviation (SD); Total number of individuals in each group (N) = 6; df = 10; * P<0.05, ** P<0.01, *** P<0.001, when compared to group I.

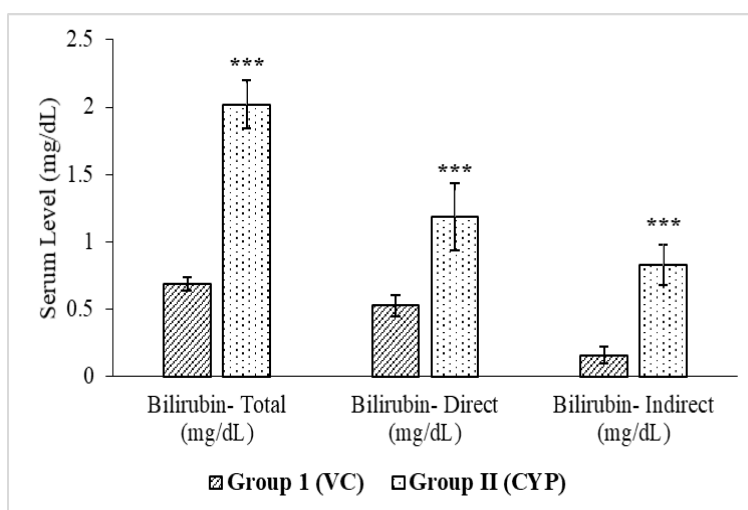


Fig 2: Cyclophosphamide induced alterations in serum level of bilirubin in male albino rats. *** indicates significance at P<0.001.

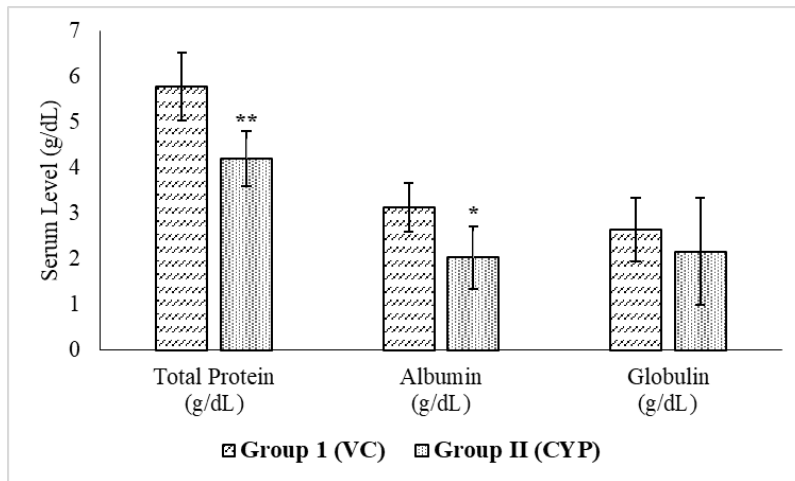


Fig 3: Alterations in serum level of total protein, albumin and globulin following single dose administration of cyclophosphamide in male albino rats. * and ** indicate significance at $P < 0.05$ and $P > 0.01$, respectively.

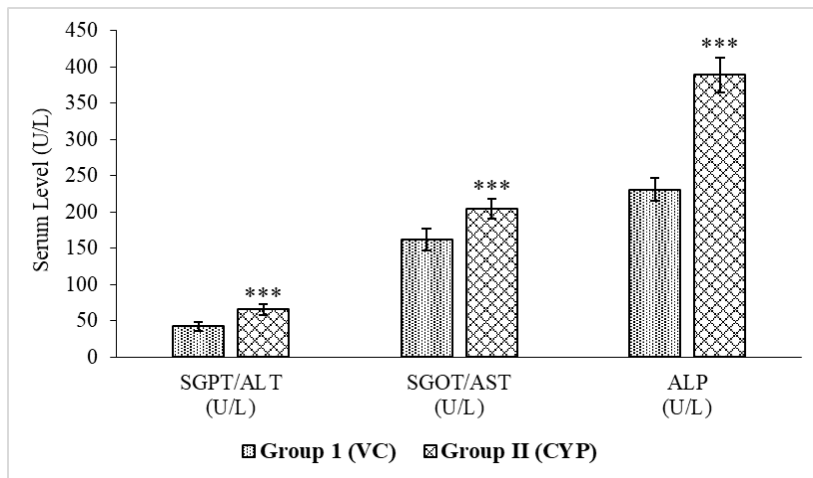


Fig 4: Serum level of liver function marker enzymes (SGPT, SGOT and ALP) in rats treated with chemotherapeutic drug, cyclophosphamide. *** indicates significance at $P < 0.001$.

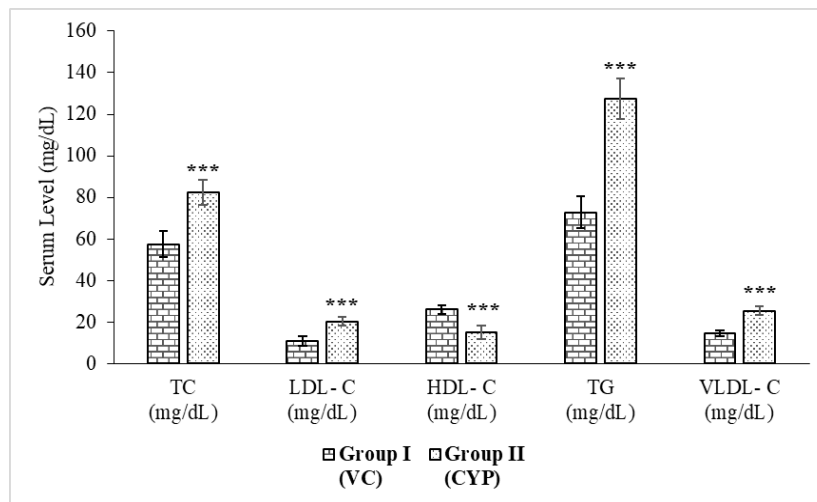


Fig 5: Cyclophosphamide induced alterations in serum level of lipid parameters in male albino rats. *** indicates significance at $P < 0.001$.

Table 2: Effects of single dose administration of cyclophosphamide (200 mg/kg body weight; i.p.) on serum level of lipids and lipoproteins in male albino rats.

S. No.	Parameters	Groups		t- value	% Change (-) Decrease/ (+) Increase
		Group I (Vehicle Control; Distilled Water)	Group II (CYP; 200 mg/kg body weight; i.p.)		
1.	TC (mg/dL)	57.5 ± 6.35	82.25 ± 6.05*	6.31	+ 43.04 %
2.	LDL-C (mg/dL)	10.72 ± 2.31	20.34 ± 2.09*	6.90	+ 89.74 %
3.	HDL-C (mg/dL)	26.1 ± 2.18	15.08 ± 3.23*	6.33	- 42.22 %
4.	TG (mg/dL)	72.72 ± 7.61	127.47 ± 9.56*	10.02	+ 75.29 %
5.	VLDL- C (mg/dL)	14.54 ± 1.52	25.49 ± 1.91*	10.03	+ 75.31 %

Values were expressed as Mean ± Standard Deviation (SD); Total number of individuals in each group (N) = 6; df = 10; * indicates significance at P<0.001 when compared to group I.

Discussion

As being the chief site of metabolism of drugs and xenobiotics, liver becomes highly susceptible to toxicity caused by them (Howida, 2016). In the present study, serum level of liver function markers and lipid profile has been analysed to determine the extent of hepatic toxicity induced by chemotherapeutic drug, cyclophosphamide.

While doing toxicity studies with drugs and xenobiotics, estimation of serum level of bilirubin, total protein, albumin, globulin and liver function enzymes (SGPT, SGOT and ALP) have been used as liver function markers. These markers are used to predict the alterations in structure and function of liver, metabolism of protein, excretion of bile and membrane integrity. Bilirubin is the end product of heme catabolism. It is transported to liver in unconjugated form, loosely bound to albumin (Indirect bilirubin), where it is conjugated (Direct bilirubin) to bilirubin glucuronidase and is secreted into bile and gut. Bilirubin is water insoluble in nature, so it needs to be conjugated with water soluble proteins for its excretion. Elevated level of bilirubin may indicate an obstruction of bile flow or a problem in the

processing of the bile in the liver (Lala *et al.*, 2021).

Liver is the main site of protein metabolism. Decline in total protein, albumin and globulin can denote a decrease in their synthesis, reflecting a diseased condition of liver. Lower level of albumin can be related with inhibition of transport of unconjugated bilirubin to the liver. A decreased amount of globulin may reflect weakened immune status of the individual. Serum level of aminotransferases (AST and ALT) and ALP is considered as markers of hepatocellular injury. These enzymes are produced in hepatocytes and regulate physiological processes by catalysing trans-amination reactions to facilitate metabolism of xenobiotics and other macromolecules. Aminotransferases like AST and ALT participate in gluconeogenesis by catalysing the transfer of amino groups from aspartic acid or alanine to ketoglutaric acid to produce oxaloacetic acid and pyruvic acid, respectively (Lala *et al.*, 2021). As hepatocellular injury or loss of hepatocyte membrane integrity trigger the release of these enzymes into the circulation, increased serum level of these enzymes allow direct identification of damages in the liver.

In the present study, administration of cyclophosphamide (200 mg/kg body weight; i.p.) resulted in a significant elevation of serum level of total bilirubin, direct bilirubin and indirect bilirubin as well as liver enzymes (AST, ALT, ALP) in male albino rats when compared to vehicle control group. In agreement with the present findings, El- Sebaey *et al.* (2019) reported a significant elevation in serum level of liver function markers in rats treated with cyclophosphamide at the dose of 50 mg/kg body weight intramuscularly once a week for 4 consecutive weeks. They further explained that the elevation might be due to activity of metabolites of cyclophosphamide that induced hepatic cellular damage and lipid peroxidation leading to leakage of hepatic cytosolic enzymes (AST, ALT, ALP) into the circulation. These results were further confirmed by the histological studies done on hepatic tissues, which have been previously reported by Samdershi *et al.* (2019). Likewise, elevation of serum level of hepatic enzymes and bilirubin along with a decline in total protein and albumin following administration of cisplatin, a platinum based alkylating agent, have been reported by Afsar *et al.* (2017) and Ogbe *et al.* (2020). Chemotherapeutic drugs, such as, doxorubicin, methotrexate and ifosfamide were also documented with hepatotoxic effects in terms of elevation in serum level of bilirubin and hepatic enzymes (Abdulmohsin *et al.*, 2018; Afsar *et al.*, 2019; Ilyas *et al.*, 2021). Findings of present study is further supported by a retrospective cohort study made by Wondimneh *et al.* (2021) on biochemical profile changes in pre- and post-chemotherapy treatment of cancer patients attended at Ayder Comprehensive Specialized Hospital, Mekelle, Northern Ethiopia. They documented a non-significant increase in level of AST and ALT in post- chemotherapy compared to pre-chemotherapy. They further argued that the non- significant increase in hepatic enzymes might be due to inflamed condition of the liver, severe scarring of the liver and death of liver tissues following chemotherapy sessions (Wondimneh *et al.*, 2021).

Lipid profile includes estimation of lipids and lipoproteins. Liver plays a central role in lipid metabolism, as it is the centre for lipoprotein uptake, formation and export to the circulation (Arvind *et al.*, 2019). Lipids, such as, cholesterol and triglycerides, are insoluble in water and they need to be transported in association with water soluble lipoproteins in the circulation. Lipoproteins are important for absorption and transport of dietary lipids by the small intestine, transport of lipids between liver and peripheral tissues (Feingold, 2021). Very Low Density Lipoprotein (VLDL) plays an important role in transport of triglycerides and cholesterol esters to peripheral tissues (Feingold, 2021). Low Density Lipoprotein (LDL), the bad cholesterol, transports cholesterol from the liver to the peripheral tissues. Increased level of LDL- cholesterol can accumulate in arterial walls and initiate the formation of atherosclerotic plaques (Elshourbagy *et al.*, 2014). The rate of production and clearance of LDL is regulated by number of LDL receptors in the liver (Feingold, 2021). High Density Lipoprotein (HDL), the good cholesterol, helps in removal of unesterified (free) cholesterol from peripheral cells and delivers it to the liver through interaction with hepatic HDL receptor, a process known as reverse cholesterol transport (RCT). Additionally, HDL possess antiatherosclerotic, anti-inflammatory, antithrombotic, antioxidative and endothelial protective effects (Elshourbagy *et al.*, 2014). Dyslipidemia is characterized by increase in serum level of lipids (cholesterol and triglycerides) LDL and VLDL along with a decrease in HDL (Elshourbagy *et al.*, 2014).

In the present study, a significant increase in serum level of cholesterol, triglycerides, LDL and VLDL was observed in rats intraperitoneally treated with cyclophosphamide, when compared to control group ($p < 0.001$; Table 2). Additionally, a significant decrease in serum level of HDL was also observed in rats treated with cyclophosphamide. Chemotherapy induced dyslipidemia and hyperlipidemia reported in the present study is in agreement with the previous reports by Desouky *et al.* (2015) and Moirangthem *et al.* (2016) who postulated that the

hypercholesterolemia, hypertriglyceridemia and increase in LDL-cholesterol following treatment with chemotherapeutic drug might be due to hepatic dysfunction in metabolism of lipids and lipoproteins. Free radicals generated by metabolites of cyclophosphamide may induce increase in biosynthesis and decrease in utilization of cholesterol and decreases the activity of lipoprotein lipase, leading to increase in level of cholesterol as well as triglycerides (Moirangthem *et al.*, 2016). Chemotherapy induced alterations in lipid profile parameters were also documented by Afsar *et al.* (2017, 2019) and Ogbe *et al.* (2020), while working with cisplatin and doxorubicin, widely used chemotherapeutic drugs in treatment of various types of cancers. They also advocated for the correlation of dyslipidemic condition with hepatic dysfunction and cardiovascular risks.

Conclusion

Administration of chemotherapeutic drug, cyclophosphamide, resulted in alterations of serum level of liver function markers as well as lipid profile, indicating for severe hepatotoxicity, altered lipid metabolism, increased lipid peroxidation and redox imbalances caused by the treatment. So, in the light of toxicities caused by chemotherapeutic drugs, a regular monitoring of liver function and lipid metabolism is advisable for individuals undergoing chemotherapeutic sessions as well as before scheduling for the chemotherapy to avoid hepatic, cardiovascular and other related adversities.

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