Ascorbic Acid Attenuates Imbalanced Redox Potential Associated With Cyclophosphamide Administration In Rats.

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Abstract: Oxidative stress is a major etiological factor in the development and progression of cancer as most chemotherapeutic agents also acts through increased production of free radicals resulting in imbalanced cellular redox potential and tissue toxicity. This study evaluated the effect of ascorbic acid in combination with cyclophosphamide in rats. Male Wistar rats weighing averagely 160g were randomly selected into four groups: Group A (control, received 0.2ml normal saline), group B received 0.2ml of cyclophosphamide (4mg/kg body weight at 3 days interval for 21 days intraperitoneally), group C received 0.2ml of ascorbic acid orally (100mg/kg body weight/day for 21 days) and 0.2ml of cyclophosphamide at three days interval for 21 days while group D, received ascorbic acid orally for 21 days. Some biochemical and antioxidants indices were determined using standard methods. Results showed, that rats orally treated with ascorbic acid elicits significant ($p<0.05$) decreases in the levels of white blood cell (WBC), Cholesterol (CHO), Triglyceride (TRG), Urea, Creatinine and Malondialdehyde (MDA) with Alanine aminotransferase (ALT), Alkaline phosphatase (ALP) and Gamma glutamate transferase (GGT) activities as well as significant ($p<0.05$) increases in Reduced glutathione (GSH) Pack cell volume (PCV), red blood cell count (RBC), High density lipoprotein–cholesterol (HDL-C), serum and tissue total protein with Catalase (CAT) and Superoxide dismutase (SOD) activities. Cyclophosphamide treated rats however showed significant decreases ($p<0.05$) in GSH, serum and tissue total protein, PCV, RBC, HDL-C concentrations, CAT and SOD activities with elevated levels of MDA, urea, creatinine, WBC, CHO, TRG, and, ALT, ALP and GGT activities. Interestingly, animals pretreated with ascorbic acid before and during cyclophosphamide administration showed significant increases ($p<0.05$) in concentrations of serum and tissue total protein, GSH, PCV, RBC, HDL-C, CAT and SOD activities with corresponding decreases in WBC, CHO, TRG, urea, creatinine and MDA concentrations as well as, ALT, ALP and GGT activities compared with Cyclophosphamide treated group. Properties exhibited by ascorbic acid are indication of its antioxidative effects which accounts for its ability to attenuates cyclophosphamide- induced cellular redox imbalance, oxidative stress and tissue toxicity probably by activation of cellular anti-oxidative pathways.

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1. Introduction

Cancer is characterized as a disease associated with unsuppressed growth and spread of anomalistic cells which cannot respond to normal regulatory signals that ensure the intercellular co-operation required in multicellular organisms and consequently, result in altered genes that regulate differentiation and cell growth. Cancers are caused by both internal and external factors from insufficient diets, inherited mutations to radiation and tobacco.

Chemotherapy is a primary technique referred to as antineoplastic therapy and is used to eradicate all neoplastic cells via pharmacological drug administration, surgery or radiotherapy. Most of the drugs work best when the cells are in the dividing (exponential) phase, while one of their principal mechanisms are increased oxidative stress, as evident from increased levels of reactive oxygen species, (ROS) free radicals and lipid peroxidation (George et al., 2010). Another problem with antineoplastic drugs is that they are not specifically selective for neoplastic cells and will eliminate normal healthy cells as well, especially those that divide often such that cells responsible for triggering many of the body's crucial immune responses are also affected, due to imbalanced in cellular redox potential consequent of oxidative stress leading to depression of the patient's immune system and increasing their risk of developing viral or bacterial infections (Gonzalez et al., 2005). Common toxicities encountered are haematological,

gastrointestinal, skin and hair follicle toxicity, nervous system toxicity, local toxicity, metabolic abnormalities, hepatic toxicity, urinary tract toxicity, cardiac toxicity, pulmonary toxicity, gonadal toxicity e t c (George et al., 2010).

Cancer chemotherapeutic drugs are divided based on several factors including their chemical composition and functions; Alkylating agents directly damage DNA prohibiting tumour cells from further division, antimetabolites interferes with synthesis of DNA and RNA, antitumour antibiotics (anthracyclines) interfere with enzymes involved in DNA replication while topoisomerase and mitotic inhibitors interfere with topoisomerase and stops mitosis (Gonzalez et al., 2005).

Cyclophosphamide, (figure1) is a cytotoxic alkylating agent, a widely used anticancer drug which has been in clinical use for over fifty years with potent antineoplastic and immunosuppressive properties (Tew et al., 1996; Lawson et al., 2008). It is commonly administered in the treatment of malignant lymphomas, solid tumours, leukemia, neuroblastoma, retinoblastoma and carcinomas of the breast, ovary, endometrium and lung, usually in combination with other chemotherapeutic agents (Ahmed and Hombal, 1984; Colleoni et al.,2002). It is administered either orally or parentally over a wide range of dosage which are determined by the underlying disorder. It is an inactive pro drug that requires metabolic activation by the cytochrome P-450 system. The process of its activation produces hydroxylated active metabolites such as acrolein, phosphoramide mustard, and nitrogen mustard, believed to be toxic, or the inactive compound carboxyphosphamide (Sladek, 1988; Tew *et al*., 1996). Its metabolites can react with carboxyl (-C (O)OH), mercapto (-SH), amino (-NH2), phosphate (-PO3H2), and hydroxyl (-OH) groups and can form cross-links with DNA and proteins both resulting in inhibition of DNA replication and cell death by apoptosis as alkylating agents (Connors et al.,1974; Flowers et al., 2000). It is effectively metabolized in the liver and approximately 70-80% of the administered dose is activated by hepatic microsomal mixed-function oxidases (cytochrome P450 enzyme system) to form 4-hydroxycyclophosphamide, which is in equilibrium with its ring-open tautomer aldophosphamide (Friedman *et al*., 1976). Various cyclophosphamide isoenzymes have been demonstrated to be involved in the bio activation of cyclophosphamide in humans, including CYP2A6, 2B6, 3A4, 3A5, 2C9, 2C18 and 2C19, with 2B6 displaying the highest 4-hydroxylase activity. Hydroxy-cyclophosphamide readily diffuses into cells (Boyd *et al*., 1986). Cyclophosphamides are eliminated via the urine in humans and the urinary elimination of cyclophosphamide and its metabolites are almost complete 24 hours after start of treatment. Less than 20% of the administered dose is eliminated unchanged in the urine. Between 30-60% of the total cyclophosphamide dose is eliminated via the kidney as cyclophosphamide or metabolites. The major metabolite found in the urine is carboxy-phosphamide (Boddy *et al*., 1992; Busse *et al*., 1999). A very small fraction of the cyclophosphamide dose is eliminated via faeces and expired air. Differences found in urinary recovery of carboxyphosphamide and phosphoramide mustard between studies may be due to reactions occurring *ex vivo* during sample processing in some studies. Carboxyphosphamide and phosphoramide mustard are unstable in urine at acidic pH (Joqueviel *et al*., 1998). Like other alkylating agents its toxicity is linked to ROS/Free radical generation eliciting oxidative stress to tissues such as the kidney, heart, liver, testis and the brain hence its acute toxicities has been shown to be related to its cytotoxicity (Taniguchi, 2005; Rouissil et al., 2012; Monika et al., 2014; Divya et al., 2015). It is most toxic to rapidly proliferating tissue such as the haematopoietic system, epithelial cells of the gastrointestinal tract, hair follicles (Fraiser et al., 1991; Langford, 1997).

Chemotherapy drugs are combined with a view of inducing rapid cytoreduction and the overlapping of toxicities is considered when drugs are used in optimal dose and schedules. Approaches to the reduction of chemotherapy-induced toxicity include dose reduction, use of alternate drugs or their analogues, growth factors, and cytoprotective agents (Rang et al., 2012). Interestingly, It has been established that free radicals generated by most anti-cancer agents leads to oxidative stress, imbalanced redox potentials which contributes greatly to cellular toxicity. Hence the use of anti-oxidant agents/drugs or food supplements have been encouraged during cancer therapy.

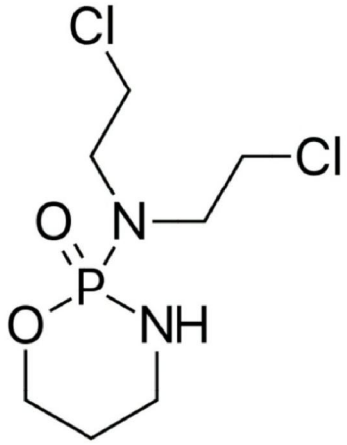

Figure 1: Structure of Cyclophosphamide

Ascorbic acid (Vitamin C) often referred to as antioxidant vitamin (figure 2), have been suggested to limit oxidative damage in humans, thereby lowering the risk of certain chronic diseases (Jelodar et al., 2013). Vitamin C is a potent reducing agent which scavenge free radicals in biological systems, the mono-anion form (ascorbate) is the predominant chemical specie at physiological pH. Ascorbate readily undergoes two consecutive, yet reversible, one-electron oxidations to generate dehydroascorbate (DHA) and an intermediate, the ascorbate free radical (AFR) which is a relatively unreactive free radical with very low reduction potential (Chatterjee,1970; May et al., 2001; Duarte and Lunec, 2005). It directly scavenge oxygen or nitrogen based radical species generated during normal cellular metabolism and this mechanism is based on the ability of ascorbic acid to donate hydrogen atom to lipid radicals, quenching of singlet oxygen and removal of molecular oxygen (Lee et al., 2004).

Vitamin C functions as an antioxidant or pro-oxidant is determined by at least three factors: (1) the redox- potential of the cellular environment; (2) the presence/absence of transition metals; and (3) the local concentrations of ascorbate (Gonzalez *et al.*, 2005). The antioxidant activity of vitamin C is dose-dependent. It has been shown that 100 (mg/kg b/w/day) of vitamin C has antioxidant role while recent studies have also indicated that vitamin C at dose 200 (mg/kg b/w/day) can have antioxidant properties in various tissues of rat (Akbari *et al.*, 2014). Although multiple studies have suggested that the use of antioxidants in combination with chemotherapy and irradiation prolongs the survival time of patients compared with the expected outcome without antioxidant supplements, hence this work investigated the possible effects of such combination using ascorbic acid in rats.

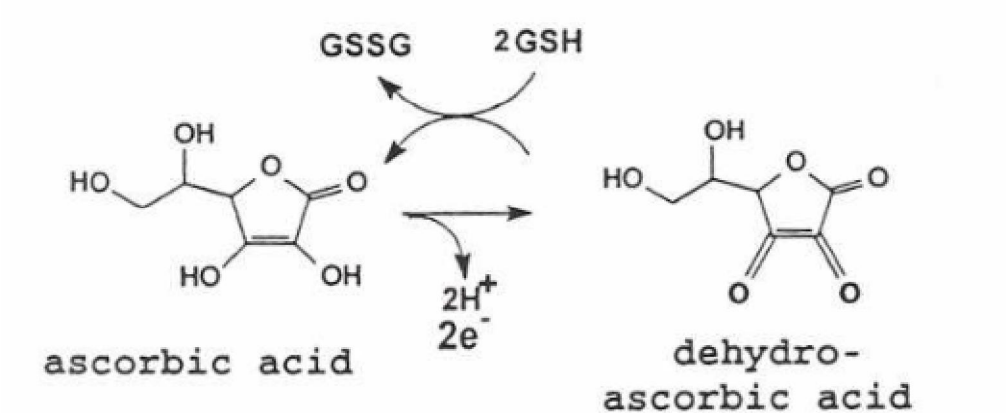

Figure 2: Ascorbic acid and dehydroascorbic acid

2. Materials and Methods.

2.1Materials

Some of the materials used includes, spectrophotometer, thermostatic water bath, centrifuge, analytical weighing balance, beam balance, pH meter, blender, dissecting set and freezers while glassware's include measuring cylinder, volumetric flask, beaker, test tubes, reagent bottles, serum bottles, and conical flask.

2.2 Reagents

Some of the reagents and salts includes, Tris buffer, potassium chloride, distilled water, methanol, normal saline, washing buffer, homogenizing buffer. Laboratory kits for the quantitative determination of total protein, cholesterol, Triglycerides, HDL-C, GGT, ALT, and ALP in the serum, trichloroacetic acid (TCA), thiobarbituric acid (TBA), all products of Sigma (USA) and of good analytical grade. Cyclophosphamide, a product of Kwality Pharmaceuticals Limited India and ascorbic acid were obtained from Akol Pharmacy store Ogbomoso, Oyo State Nigeria.

2.3 Experimental Animals and Design

A total of 24 male albino rats of Wistar strain with average weight of 160g were obtained from the animal house of the Department of Medical Laboratory Science, Ladoke Akintola University of Technology, Osogbo, Osun State, Nigeria. All animals were fed on rats pellets and acclimatized for two weeks prior to commencement of the experiment. They were allowed food and distilled water ad libitum before and during the experiment and were handled based on international standard for handling laboratory animals as made available in \my institution. The rats were grouped randomly into four groups (A, B, C, and D,) with six animals in each group. Standard doses were given as 100mg/kg body weight/day for the extract, 100mg/kg body weight/day for ascorbic acid

and 4mg/kg body weight at 3 days interval for cyclophosphamide all made into 0.2ml relative to the weight of the animals. Various group of the experimental animals is shown below.

Group A (Control): each rat in the group was administered with 0.2ml of normal saline by sub-dermal injection for twenty-one days.

Group B (Cyclophosphamide only): each rat in the group was administered with 0.2ml of cyclophosphamide at 3 days intervals for 21 days.

Group C (Ascorbic acid + Cyclophosphamide): 0.2mL of the ascorbic acid was given orally to each rat for 21 days once daily while at 3 days interval, 0.2mL of cyclophosphamide was administered to animals in this group for 21 days.

Group D (Ascorbic acid only): 0.2mL of the ascorbic acid was administered orally to each rat in this group for 21 days once daily.

2.4 Preparation of serum and tissues homogenates

The experimental animals were sacrificed at the end of the administration by cervical dislocation. Blood was obtained through cardiac puncture from the jugular vein of the heart using syringe and needle. The blood sample was transferred into a centrifuge to obtain the serum at 4000rpm for 10minutes. The serum (supernatant) was extracted into the serum bottle, while the packed blood (sediment) was disposed. This process was repeated for each rat in all the four groups and serum obtained were appropriately labeled and later use for some biochemical analyses.

The liver and the kidney were excised and placed in a pre-weighed beaker containing 5mls of washing buffer. They were thoroughly washed in cold washing buffer to remove hemoglobin and stains which may inhibit enzyme activity. All these procedure were carried out at 4^0C. The washed tissues were weighed and transferred to a beaker from which 1g were removed and homogenized using 4mls of homogenizing buffer. The homogenate was stored at 4^0C. This procedure was carried out on all the experimental animals. The homogenates were used for various biochemical analyses.

2.5 Biochemical indices analysed

Serum and tissue proteins were determined by the Biuret (colorimetric) method, based on the principle that proteins gives an intensive violet blue complex with copper salt in alkaline medium while the intensity of the colour formed is proportional to the total protein concentration in the sample (Burtis et al.,1999). The determination of the hematological indices such as the total white blood cell (WBC) counts, red blood cell (RBC) counts and packed cell volume (PCV) were determined by Flow cytometry whose basic principle is the passage of cells in single files in front of a laser so they can be detected, counted and sorted as blood cell components are fluorescently labelled and then excited by the laser to emit light at varying wavelengths after heamolysis. The fluorescence can be measured to determine the amount and type of cells present in the samples using automated machine, while Packed Cell Volume was determined by the Micro hematocrit method using a small quantity of whole blood in a capillary tube and a high-speed centrifuge.

Total serum cholesterol concentrations were determined spectrophotometrically based on the principle of enzymatic end point upon enzyme hydrolysis of cholesteryl esters (Trinder,1969) while triglycerides concentration was determined by an enzymatic colorimetric method using standard diagnostic triglycerides kits and working reagents. The quantitative determination of high density lipoprotein (HDL-C) cholesterol was based on HDL-cholesterol (HDL-C) precipitating method of Naito, (1984) and Grove (1979).

Liver and kidney indices such as the activities of serum ALT, GGT and ALP were determined using the method of, Bergmeyer *et al*., (1986), Rec, (1972) and Szasz, (1969) based on the standardized methods by the International Federation of Clinical Chemistry. Also serum urea concentrations were determined according to the method of Berthelot-Searcy as described by Henry, (1991), while creatinine was determined by the method described by Bartels and Bohmer (1972).

Antioxidant indices in the liver and kidney were estimated spectrophotometrically by Thio barbitturic acid-reacting substances (TBARS) as described by the procedure of Varshney and Kale (1990), for the determination of Malondialdehyde (MDA). Determination of GSH concentration was carried out using the method described by Beutler et al., (1963), while SOD and Catalase activities were determined by the method of Mistra and Fridovich (1972) and Aebi, (1984).

2.6 Statistical Analysis

Data obtained was analysed using analysis of variance (ANOVA), value of $p<0.05$ was considered statistically significant.

3.0 Results

See tables.

4.0 Discussions

Cyclophosphamide among other alkylating agents used in cancer treatment has been shown to be cytotoxic while its clinical use is limited due to its ability to damage normal tissue culminating in multiple organ toxicity (Fraiser *et al.*, 1991). It was also reported that its use is associated with oxidative stress and damage resulting from over production of reactive oxygen species (ROS) leading to imbalanced

cellular redox potential (Rouissil *et al*., 2012). In this study, the effects of the use of ascorbic acid as a complement was investigated in cyclophosphamide treated rats with certain biochemical and anti-oxidant indices assessed.

Table 1: Total protein concentrations in the serum, the liver and the kidney homogenates of various treatment groups

Groups	Total protein concentration (Serum) g/dl ± SD	Total protein concentration (liver) g/dl ± SD	Total protein concentration (kidney) g/dl ± SD
Group A (control	9.30±0.09	5.57±0.10	5.74±0.03
Group B (cyclophosphamide, 4mg/kg.bw. only	6.91±0.08*	3.02±0.14*	3.05±0.06*
Group C (ascorbic acid, 100mg/kg.bw+ cyclophosphamide,4mg/kg.bw)	8.08±0.08	4.77±0.09	4.58±0.08
Group D (ascorbic acid 100mg/kg.bw) only.	14.10±0.56*	8.19±0.08*	7.83±0.08*

Values are given as mean and standard deviation of six determinations.
*Values differ significantly from control (P <.05).

Table 2: Results of some haematological indices, White blood cell counts (WBC), Packed cell volume (PCV) and Red blood cell counts (RBC) of various groups.

Groups	WBC Count (x 10^9/L) ± SD	Pack cell volume (PCV) (ng/L)±SD	Red blood cell Count (x10^{12}/L) ± SD
Group A, (Control)	7.85 ±0.36	38.10±0.56	7.16±0.14
Group B (cyclophosphamide, 4mg/kg.bw. only)	11.90±0.36*	31.60±0.56*	5.57±0.13*
Group C (ascorbic acid, 100mg/kg.bw+ cyclophosphamide, 4mg/kg.bw)	8.85±0.12	38.53±1.69	6.92±0.22
Group D (ascorbic acid 100mg/kg.bw) only.	2.75±0.13*	38.53±1.69	10.15±0.14*

Values are given as mean and standard deviation of six determinations.
*Values differ significantly from control (P <.05).

Table 3: Results of the serum lipid profile of various treatment groups

Groups	Total serum cholesterol concentration (mg/dl) ± SD	Serum Triglycerides Concentration (mg/dl) ± SD	Serum HDL Cholesterol concentration (mg/dl) ± SD
Group A, (Control)	37.57±1.62	44.48±3.16	102.24±2.48
Group B (cyclophosphamide, 4mg/kg.bw. only)	72.72±4.22*	80.34±6.43*	74.20±0.59*
Group C (ascorbic acid, 100mg/kg.bw+ cyclophosphamide, 4mg/kg.bw)	55.25±1.24*	67.18±6.49*	83.91±1.55*
Group D (ascorbic acid 100mg/kg.bw) only.	23.35±1.44*	26.93±0.94*	122.40±1.91*

Values are given as mean and standard deviation of six determinations.
*Values differ significantly from control (P <.05).

Table 4: Serum Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP) and Gamma-Glutamyl aminotransferase (GGT) activities in various treatment groups.

Groups	ALT±SD (U/L)	ALP ± SD (U/L)	GGT±SD (U/L)
Group A, (Control)	25.09±0.64	92.23±1.67	2.01±0.22
Group B (cyclophosphamide, 4mg/kg.bw. only)	36.95±1.05*	121.01±2.45*	5.51±0.31*
Group C (ascorbic acid, 100mg/kg.bw+ cyclophosphamide,4mg/kg.bw)	29.83±1.14*	112.85±1.55*	4.85±0.25*
Group D (ascorbic acid 100mg/kg.bw) only.	14.52±1.39*	73.84±1.35*	1.31±0.11*

Values are given as mean and standard deviation of six determinations.
*Values differ significantly from control (P <.05).

Table 5: Serum Urea and Creatinine concentrations in various treatment groups.

Groups	Serum Urea Concentration (mg/dl)	Serum Creatinine Concentration (mg/dl)
Group A, (Control)	23.36±1.13	0.80±0.04
Group B (cyclophosphamide, 4mg/kg.bw. only)	31.22±1.04	2.52±0.16*
Group C (ascorbic acid, 100mg/kg.bw+ cyclophosphamide,4mg/kg.bw)	28.97±0.82	1.75±0.03*
Group D (ascorbic acid 100mg/kg.bw) only.	12.62±0.84	0.57±0.11

Values are given as mean and standard deviation of six determinations.
*Values differ significantly from control (P <.05).

Table 6: Malondialdehyde (MDA) and Reduced Glutathione (GSH) concentrations, Superoxide dismutase (SOD) and Catalase (CAT) activities in the Liver homogenate of various treatment groups.

Groups	MDA Concentration (U/mg protein)±SD	GSH Concentration (U/mg protein)±SD	SOD Activity (U/mgProtein) ±SD	CAT Activity (U/mgProtein) ±SD
Group A, (Control)	414.5±5.42	18.87±0.298	8.37±0.02	0.67±0.002
Group B (cyclophosphamide, 4mg/kg.bw. only)	861.2±2.669	11.14±0.133	2.39±0.009	0.204±0.002
Group C (ascorbic acid, 100mg/kg.bw+ cyclophosphamide, 4mg/kg.bw)	678.3±0.512	15.67±0.095	5.96±0.04	0.404±0.002
Group D (ascorbic acid 100mg/kg.bw) only.	196.81±0.14	25.41±0.32	13.08±0.03	1.08±0.01

Values are given as mean and standard deviation of six determinations.
*Values differ significantly from control (P <.05).

Table 7: Malondialdehyde (MDA) and Reduced Glutathione (GSH) concentrations, Superoxide dismutase (SOD) and Catalase (CAT) activities in the Kidney homogenate of various treatment groups.

Groups	MDA Concentration (U/mg protein)±SD	GSH Concentration (U/mg protein) ±SD	SOD Activity (U/mgProtein) ±SD	CAT Activity (U/mgProtein) ±SD
Group A, (Control)	210.86±0.293	15.63±0.095	6.07±0.06	0.589±0.001
Group B (cyclophosphamide, 4mg/kg.bw. only)	390.6±0.37	8.47±0.018	2.04±0.047	0.218±0.002
Group C (ascorbic acid, 100mg/kg.bw+ cyclophosphamide, 4mg/kg.bw)	270.1±0.34	11.91±0.08	4.36±0.059	0.398±0.002
Group D (ascorbic acid 100mg/kg.bw) only.	105.48±0.43	23.11±0.26	10.14±0.04	0.91±0.02

Values are given as mean and standard deviation of six determinations.
*Values differ significantly from control (P <.05).

Information from protein assessment in various biochemical assays and studies has been of great importance as they are useful index of the severity of cellular dysfunction in chronic diseases associated with oxidative damage. In this study, the total protein concentrations in the serum, liver and the kidney (table 1) were significantly ($p<0.05$) decreased in the cyclophosphamide treated rats (group B) compared with other treated groups. The decreased protein concentration may be due to protein oxidation caused by ROS resulting in protein fragmentation and protein-protein cross-linkages (Zhang et al., 2013), which may implicate cyclophosphamide's inhibitory role in protein synthesis. Oral administration of ascorbic acid (group D) and the combined treatment (group C) shows increases in protein concentrations and more significantly by ascorbic acid (group D) compared with group B, suggestive of ascorbic acid anti-oxidant capacity to attenuates ROS production and thus activating pathways responsible for protein synthesis.

Results from the hematological indices (table 2), shows that group treated with cyclophosphamide only (Group B), elicit marked increase ($p<0.05$) in white blood cell count (WBC) compared with control (Group A). However group C rats treated with both ascorbic acid and cyclophosphamide shows marked decreases ($p<0.05$) in WBC compared with group B, while similar trends were observed in those treated with ascorbic acid only (group D). Also significant decreases ($p<0.05$) in red blood cell count (RBC) and Packed cell volume (PCV) were observed in cyclophosphamide treated rats (group B) compared with controls (Group A). This is consistent with the report of Ahmed and Hombal (1984) and Fraiser *et al*. (1991), as studies have correlated high dose of cyclophosphamide to severe aplastic anaemia owing to bone marrow toxicity induced by cyclophosphamide. The combined treatment (group C) however showed significant elevation ($p<0.05$) in RBC and PCV compared with group B, while the normal animals which received ascorbic acid only (Group D) also showed significant increases ($p<0.05$) in RBC and PCV compared with other treatment groups suggestive of its antioxidant potential to ameliorate cyclophosphamide toxicity as well as reduces oxidative stress and inflammation associated with cancer (Reuter et al., 2010).

Abnormal serum lipids (dyslipidemia) has been reported as one of the major event of metabolic syndrome and has been linked with high incidence of cardiovascular diseases (Lehto *et al*., 1997; Sarwar *et al*., 2007). In this study, serum total cholesterol concentration (table 3), was significantly increased ($p<0.05$) in the cyclophosphamide treated rats (Group B) compared with control (Group A). The combined treatment (Group C) showed significant decrease ($p<0.05$) in the total cholesterol levels compared with group B, as similar trend were observed with the ascorbic acid only (Group D). The behavior of ascorbic acid in this study when used with cancer patients is consistence with the findings of Tarchalski et al., (2003) and Takahashi et al., (2012). The significant increase in serum triglycerides level as observed in cyclophosphamide treated rats (Group B)

is consistent with the reports of Yeh and Bickford (2009), that shows a positive association between triglyceride levels and blood pressure. The rise in the triglycerides level may contribute to the expression of hypertension phenotype. The combined treatment (group C) and ascorbic acid (group D) however significantly decreased ($p<0.05$) triglycerides level when compared with group A and B.

Kamezaki *et al*. (2002) showed that lowering serum LDL-cholesterol and increasing HDL-cholesterol lead to a regression in atherosclerotic lesion. Interestingly, a significant decrease ($p<0.05$) in the concentration of HDL-cholesterol of cyclophosphamide treated rats (Group B) was observed while significant increases ($p<0.05$) were observed in the HDL-cholesterol concentration of the combined treatment (group C) and group treated with ascorbic acid only (Group D) compared with group B. The ability of ascorbic acid to normalize the lipid panels may be linked to its antioxidant capacity, since proteins and enzymes responsible for the metabolism of blood lipids are well protected against oxidants induced by cyclophosphamide.

One of the major consequences of hepatotoxicity resulting from redox imbalance induced by cyclophosphamide is the leaking out of markers of liver damage (Alanine amino transferase (ALT), Alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT)) from the hepatocyte resulting in increased enzyme activity in the systemic circulation (Senthhilkumar *et al*., 2006). Various studies have demonstrated that increased in serum enzyme activity is a reflection of cellular damage and alteration of functional membrane (Kumar *et al*., 2005). In line with these studies (table 4), significant increases ($p<0.05$) in the serum ALT, ALP and GGT activities were observed in Cyclophosphamide treated rats (Group B) compared with control. The increased levels of these serum enzymes activities is due to oxidative liver damage caused by cyclophosphamide intoxication (Fraiser et al., 1991; Mousa and Ayman 2014). Increase in serum GGT activities showed that, the increase in ALP activities may be due to liver damage and not kidney since an isoform of ALP is also produced in the kidney while GGT is absent in the kidney; although, the increase may also indicate kidney damage. Group C and D treated rats however significantly decreased ($p<0.05$) ALT, ALP and GGT activities compared with group B rats with the ascorbic acid eliciting possible attenuating effects on cyclophosphamide toxicity in rats which may be responsible for its hepatoprotective potential.

Drug induced nephrotoxicity is common in specific clinical condition (Naughton, 2008), as mechanism behind drug induced nephrotoxicity has been reported to includes urine sediment abnormalities, electrolyte imbalances and most commonly a decline in the glomerular filtration rate (Nolin and Himmelfarb 2010). Results from table 5, shows that the serum concentrations of urea and creatinine were significantly increased ($p<0.05$) in the rats treated with cyclophosphamide only (Group B) compared with control. The marked increase is due to decrease in glomerular filtration rate and may culminate into electrolyte imbalance and kidney diseases (Nolin and Himmelfarb 2010). The group boosted with ascorbic acid before cyclophosphamide treatment (Group C) and those which received ascorbic acid only (Group D) were consistent with decreases in urea and creatinine concentrations with greater effect by ascorbic acid only (group D). The use of cyclophosphamide is associated with the induction of tubular necrosis, tubular fibrosis, glomerular congestion and inflammation, which ultimately causes renal dysfunction due to its toxic metabolites (Monika, 2014).

Evaluation of the anti-oxidant indices such as malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) in the liver and kidney in this study revealed that cyclophosphamide showed significant ($p<0.05$) decreases in GSH level, SOD and CAT activities with corresponding significant ($p<0.05$) increases in MDA concentration in both the liver and the kidney. However this trend was reversed by groups treated with ascorbic acid and cyclophosphamide (group C) as well as those treated with ascorbic acid only (group D). Toxic behavior exhibited on the antioxidant status by cyclophosphamide is associated with its bio activation in the liver which produces ROS that decreases cellular antioxidant capacity resulting in lipid peroxidation of membranes associated with imbalanced redox potential in cells (Senthilkumar et al.,2006; Ray et al.,2010), with the resultant effects leading to tissue toxicity (Oboh, 2006).

The active metabolites of cyclophosphamide are phosphoramide mustard and acrolein. Phosphoramide produces an antineoplastic effect and acrolein produces free radicals by interacting with the body's antioxidant defense system. These free radicals are highly reactive and produce oxidation of various enzymes (Senthilkumar *et al*., 2006). The acrolein binds to GSH leading to GSH depletion in the cells which in turn result to cellular damage (Ohno and Ormstad 1985). Acrolein impaired the glutathione dependent antioxidant system and increased free radical generation. These free radicals become elevated due to deterioration of the nitric oxide/nitric oxide synthase system in vasculature with corresponding decreases in endogenous SOD and CAT (Yousefipour *et al*., 2005; Rouissil et al., 2012). The reversed of this trends as shown by ascorbic acid

in the results (group C and group D), may be attributed to its ability to upset some of these mechanisms associated with cyclophosphamide induced toxicity via free radical production resulting in imbalanced cellular redox potential either by activating other optional pathways that may lead to increases in anti-oxidant defence system of the body or minimize or impede free radical production associated with cyclophosphamide action.

Conclusion

Results from this study shows that cyclophosphamide induce oxidative damage through redox imbalance in tissues by tilting the oxidant-antioxidant balance of the cells towards the former. It also caused bone marrow toxicity by marked decreases in RBC and PCV, marked elevation of liver enzymes in the serum and increased levels of serum total cholesterol and triglycerides levels with decreasing HDL-cholesterol level and decreased glomerular filtration by diminished clearance of urea and creatinine from the serum. However administration of ascorbic acid with cyclophosphamide significantly attenuates the cyclophosphamide-induced multiple organ dysfunctions via possible activation of anti-oxidant pathways, validating its use as complement in cancer chemotherapy.

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