**Research Article**
Volume 5:11, 2022

Journal of Biomedical and Pharmaceutical Sciences

ISSN: 2952-8100 Open Access

# The Effect of Dietary Co-enzyme Q10 Supplementation on Haematological Parameters and Splenic Morphology in Mouse Model of 5-Fluorouracil-Induced Toxicity

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## Abstract

Coenzyme Q10 (CoQ10) antioxidant strength has been seen in many studies. Most chemotherapy have been seen to cause side effect through oxidative stress. This work seeks to study the ability of CoQ10 to counter the oxidative stress and thus remediate the chemotherapy induced toxicities of chemotherapeutic agents.

Fifty male mice were used for this study. They were divided into 5 groups (of 10 rats each) which include normal control (standard diet plus intraperitoneal (i.p) normal saline), 5 Fluorouracil (5FU) control (negative control) (standard diet plus i,p 5 fluorouracil at 200 mg/kg body weight) and three treatment groups of CoQ10 incorporated into standard diet at 100 mg/kg, 200 mg/kg and 400 mg/kg of feed of feed. The weight of the rats was measured every 3 days and recorded. The experiment lasted for two weeks after which the rats were euthanized by cervical dislocation. Blood was collected for red blood cell indices (PCV, MCV, MCH, Hemoglobin, MCHC) and white blood cell parameters (WBC, NC, LC and Platelet count) hematological study and the spleen fixed in 10% formalin for histological study.

The weight did not show any significant difference all groups compared to the control while the red blood cell indices showed a significant decrease ($p<0.001$) in the negative control group when compared to the positive control group except MCHC which showed no significant difference ($p>0.001$). The treatment groups showed an increase in all parameters when compared to the negative control except MCHC. The white blood cell parameters all showed increase except for the Lymphocyte Count (LC). The histological slide showed the damage by 5-FU as seen in the negative control group while the treatments ameliorated it in the treatment group. The result showed the cytotoxic effect of 5-FU on the cells and the amelioration by the COQ10.

**Keywords:** White blood cell • 5-Fluorouracil • Coenzyme Q10 • Red blood cell

## Introduction

As a result of the healthy living trend, dietary supplements category is growing fast, leading to an urgent need for dietitians, physicians, and policy makers to broaden the scientific evidence on the efficacy and safety of a wide range of active ingredients. Coenzyme Q10 (CoQ10), as the third most consumed dietary supplement, and as a potential candidate for the treatment of various non communicable diseases that are among the global top 10 causes of death, has gained interest over years.

Coenzyme Q10, which is not an FDA approved drug, but yet sold as a food supplement, it is currently the third most consumed nutritional supplement after fish oil and multivitamins [1]. In addition, thanks to its strong antioxidant activity, and physiological key role in mitochondrial bioenergetics, it has also been considered as a potential candidate for the treatment of various diseases where oxidative stress plays a significant role such as cardiovascular diseases, neurodegenerative disorders, cancer, and diabetes, which are among the top 10 global causes of death [2]. For this reason, considering that CoQ10 supplementation has gained interest over years and has an expanding consumer base.

CoQ10, also known as Ubiquinone, is a fat soluble, vitamin like benzoquinone compound that is endogenously synthesized from tyrosine in the human body [3]. It comprises a quinone group and a side chain of 10 isoprenoid units [4]. Ubiquinol, the fully reduced form of CoQ10 is a good lipophilic antioxidant, capable of free radical neutralization and regeneration of the reduced form of vitamin E [5]. It can also inhibit lipid peroxidation in biological membranes and protect

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**Received:** 27 July, 2022, Manuscript No. JBPS-22-70446; **Editor assigned:** 01 August, 2022, PreQC No. JBPS-22-70446 (PQ); **Reviewed:** 16 August, 2022, QC No. JBPS-22-70446; **Revised:** 26 September, 2022, Manuscript No. JBPS-22-70446 (R); **Published:** 04 October, 2022, DOI: 10.37421/2952-8100.2022.5.385.

mitochondrial proteins and DNA from oxidative damage. In fact, it is the only lipophilic antioxidant that can be de novo synthesized by cells and that has enzymatic mechanisms to regenerate its reduced form [6].

Being strongly bound to the inner mitochondrial membrane and participating in the electron transport chain and oxidative phosphorylation, it plays an essential role in the synthesis of cellular energy in the form of ATP [7]. For this reason, it is found at higher concentrations in tissues with a high metabolic activity, such as heart, kidney, liver, and muscle [8]. However, different factors such as genetics, aging, and statins treatment can lower its physiological concentrations [9]. CoQ10 deficiencies have also been reported for conditions where oxidative stress plays a significant role, such as neurodegenerative disorders, diabetes, cancer, and cardiovascular diseases [10].

CoQ10 has been considered as a potential candidate for the treatment of various diseases [11]. CoQ10 supplementation supports oxidative phosphorylation, cell signaling, and protects certain cell types [12]. In addition, thanks to its strong antioxidant activity, it is also gaining popularity in the cosmetic industry [13]. These findings on the potential health benefits of CoQ10 supplementation have led to an increased consumer demand. In fact, it is currently the third most consumed nutritional supplement after fish oil and multivitamins [14]. It is highly safe, with an observed adverse effect level (NOAEL) of 1,200 mg/kg/day [15]. In fact, the minor side effects after taking CoQ10 mega doses, such as indigestion, are more associated with the copious amounts of oil used to solvate it [16].

Considering CoQ10 physicochemical characteristics, as a strongly hydrophobic compound with a high molecular weight (863 g/mol),It is extremely insoluble in aqueous phase, and it is absorbed slowly and incompletely from the small intestine resulting in low oral bioavailability in humans [17]. In addition, it is vulnerable to heat, light, and oxygen [18].

CoQ10 has a wide distribution in plant and animal tissues that are part of our diet. Though it can be found in vegetables, fruits and cereals (1 mg/kg to 10 mg/kg range), the richest dietary sources of CoQ10 are meat, fish, nuts, and some oils, which contain 10 mg/kg to 50 mg/kg. Since CoQ10 is mainly distributed in high energy-demanding tissues, animal hearts and livers represent the richest source of this bioactive molecule, with a content between 30 mg/kg and 200 mg/kg [19]. Although there is no established nutritional reference value for CoQ10, its daily average intake is around 5.4 mg and 3.8 mg for men and women, respectively [20]. However, as a non-essential nutrient, endogenous synthesis is believed to be its main source. While an average healthy adult's body contains 0.5 g to 1.5 g of CoQ10, its levels may be compromised by different factors.

Deficiency can occur as a result of physiopathologic conditions such as acquired or genetic alterations in metabolism or biosynthesis, an inadequate intake of CoQ10 or its dietary precursors, aging and oxidative stress that leads to an excessive utilization of the molecule, or due to a combination of these factors. It has to be noted that CoQ10 endogenous synthesis is a complex process that requires the participation of tyrosine and eight vitamins, which results in a high vulnerability of the process (Figure 1).

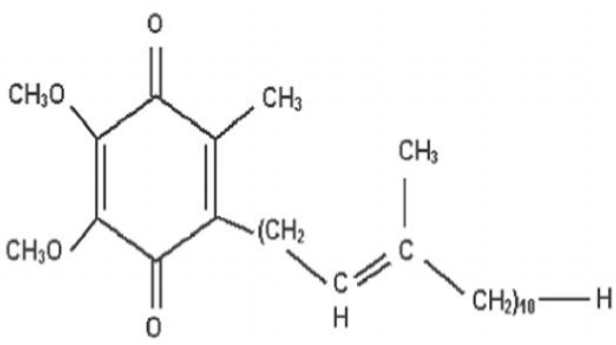


**Figure 1.** Chemical structure of CoQ10, Source: PubChem (2019).

5-Fluorouracil (5-FU) is a commonly used chemotherapeutic agent that blocks DNA synthesis and replication *via* inhibition of thymidylate synthase and incorporation of 5-FU metabolites into RNA and DNA. Presently, 5-FU is still a component of most of the currently applied regimens to treat solid cancers of the gastrointestinal tract, breast, head and neck, and pancreas. Derivatives of 5-FU, including carmofur, tegafur, and capecitabine (prodrugs which are metabolized to 5-FU, their only active product *in vivo*), are also often used clinically as anticancer drugs.

Most anticancer agents that kill cancer cells also have a low therapeutic index, thus affecting a diverse range of normal cell types. This leads to a myriad of adverse side effects on multiple organ systems and may severely limit their activity. Such effects on normal tissues have been observed for almost all classes of anticancer drugs, including alkylating agents, antimetabolites, and even anti hormonal agents. Typical side effects of 5-FU include myelosuppression, nausea, vomiting, diarrhea, and stomatitis. Previous studies have shown that 5-FU has the potential to induce toxicity in various tissues.

Cardiotoxicity is a well defined side effect of 5-FU, which often occurs as myocardial ischemia, and to a lesser extent, as cardiac arrhythmias, hyper and hypotension, left ventricular dysfunction, and cardiac arrest mediated by multifactorial pathophysiological mechanisms. Additionally, 5-FU was demonstrated to be genotoxic, as indicated by chromosomal damage in animals treated with 5-FU. Reported that 5-FU induced gastrointestinal dysfunction and enteric neurotoxicity *in vivo*. Furthermore, 5-FU was also found to cause hepatic damage from overproduction of free radicals and inflammatory mediators.

5-Fluorouracil not only causes adverse effects in primary organs where it accumulates, but also in secondary locations such as the Central Nervous System (CNS) upon systemic circulation. Mustafa, et al,. have shown earlier that 5-FU readily crosses the Blood-Brain Barrier (BBB) and affects spatial working memory and newborn neurons in the adult rat hippocampus (Figure 2).


**Figure 2.** 5-fluorouracil vials.

## Rationale of the study

CoQ10 provides antioxidant protection to cell membranes and plasma lipoproteins. By lowering lipid peroxidation of Low Density Lipoprotein (LDL) particles that contributes to atherosclerosis, CoQ treatment confers health benefits against cardiovascular diseases. The antioxidant function of CoQ is especially important in the plasma membrane by reducing vitamins C and E, and in preventing ceramide-mediated apoptosis.

## Hypothsis

The null hypothesis is co-enzyme Q10 has no effect on 5-fluorouracil induced toxicity in mice. While the alternate hypothesis is that co-enzyme Q10 does have effect on 5-fluorouracil induced toxicity in mice.

## Aim of the study

The aim of this study is to evaluate the effect of dietary co-enzyme Q10 supplementation on haematological parameters and splenic morphology in a mouse model of 5-fluorouracil-induced toxicity.

## Objectives

- To determine the effect of Co-enzyme Q10 on body weight and food intake in mice.
- To evaluate the effect of Co-enzyme Q10 supplementation on haematological parameters.
- To examine the effect of Co-enzyme Q10 on splenic morphology in a mouse model of 5-florouracil induced haematotoxicity.

## Co-enzyme Q10

Coenzyme Q10 (CoQ10) is a benzoquine (2,3-dimethoxy-5 methy l6-decaprenyl-benzoquinone) chemically similar to a liposoluble vitamin consisting of a crystalline powder in its pure form. This molecule can be found in many aerobic organisms ranging from bacteria to mammals and is present in almost all the cells of the human body. In the human organism, this enzyme plays an important role in the respiratory chain, acting as an electron transporter to produce Adenosine Triphosphate (ATP) inside the mitochondria. In its reduced form, CoQ10 acts as an antioxidant, protecting the biological membranes against oxidation, inhibiting lipid peroxidation, indirectly stabilizing the calcium channels to prevent calcium overload, and participating in the recycling of α-tocopherol.

As a food supplement, CoQ10 is mainly found in mono or multicomponent soft gels, capsules, and tablets. However, while it used to be incorporated as a simple crystalline powder or dispersed in oil, different novel delivery systems have been recently tested to improve its bioavailability. Thus, different formulation approaches, such as self-emulsified drug delivery systems, nano-emulsions, or cyclodextrin complexes, have been used and combined to improve CoQ10 bioavailability when incorporated into different pharmaceutical dosage forms.

The suggested daily dose varies depending on the indication but is usually around 30 mg to 100 mg for healthy people, reaching up to 60 mg to 1,200 mg when used in some medical conditions.

CoQ10 provides antioxidant protection to cell membranes and plasma lipoproteins. By lowering lipid peroxidation of Low Density Lipoprotein (LDL) particles that contributes to atherosclerosis, CoQ treatment confers health benefits against cardiovascular diseases. The anti-oxidant function of CoQ10 is especially important in the plasma membrane by reducing vitamins C and E, and in preventing ceramide mediated apoptosis, an important regulator of lifespan in the context of normal aging. It has been proposed that NAD(P)H: Quinone oxidoreductase 1 (NQO1) acts as a redox-sensitive switch to regulate the response of cells to changes in the redox environment. The pharmacokinetics variability of the different compositions of CoQ10 may result in fairly different plasma concentration time profiles after CoQ10 administration. CoQ appears suitable for use in the treatment of different diseases.

Following intraperitoneal administration of CoQ10 in rat, only small amount of the supplement reaches the kidney, muscle, and brain. Likewise, only a fraction of the orally administered CoQ10 reaches the blood while the major amount is eliminated *via* feces. The absorption of CoQ10 is slow and limited due to its hydrophobicity and large molecular weight and, therefore, high doses are needed to reach a number of rat tissues (e.g., muscle and brain) and we can only assume that this also happens in humans (Figure 3).


**Figure 3.** Coenzyme Q10.

## Characteristics of Co-enzyme Q10

**Chemical form:** CoQ10 is synthesized from the mevalonate cycle, obtained from acetyl-CoA, which goes on to produce cholesterol, dolichol and CoQ10 as the final product. CoQ10 is also known as ubiquinone in its oxidized form and ubiquinol in its reduced form. In humans, ubiquinone (2,3-dimethoxy-5-methyl-6-decaprenyl-benzoquinone) has a chain with isoprene units and derives from the conjunction of the benzoquinone ring with a chain of hydrophobic isoprenoids, all of them with a double bond and trans configuration.

**Function in the organism:** In the respiratory chain, CoQ10 is responsible for electron transport from the protein I complex (NADH dehydrogenase) to the protein II complex (succinate dehydrogenase), and from complex II to complex III (bc1 complex). When receiving the electrons from both complex I and complex II, it remains in its reduced form as ubiquinol and, after transferring the electrons to complex III it returns to its oxidized form as ubiquinone. The organs that require higher energy concentrations such as the brain, heart, kidneys and liver show higher CoQ10 rates.

**Antioxidant function:** By being vital for ATP synthesis, CoQ10 plays a crucial role in mitochondrial bioenergy, acting on all cells of the organism and thus being essential for health. Due to its redox property, it is useful for the neutralization of reactive oxygen species, *i.e.*, free radicals. CoQ10 is the only endogenously synthesized liposoluble antioxidant that can participate in redox reactions, acting on the prevention of damage to DNA and proteins and on lipid peroxidation, and indirectly stabilizing the calcium channels by preventing calcium overload. The enzyme acts on lipid peroxidation by either sequestering free radicals or reducing the α-tocopheryl radical to α-tocopherol. Its role is closely similar to that of vitamin E, although vitamin E depends exclusively on the diet and on hepatic reserves, with no endogenous synthesis, in contrast to CoQ10.

## Sources of coenzyme Q10

In healthy individuals, normal CoQ10 levels are maintained through two pathways, *i.e.*, the exogenous pathway by food ingestion and endogenous synthesis by the mevalonate cycle. In the endogenous production, the mevalonate cycle involves acetyl-CoA as the initial substrate and cholesterol, CoQ10 and dolichol as the final products, the last being crucial for protein glycosylation. In this pathway, the enzyme prenyl-transferase is responsible for the synthesis of the isoprenoid side chain of CoQ10, with the later occurrence of another condensation of this chain formed with 4-hydroxybenzoate.

In the exogenous pathway, CoQ10 is ingested in its oxidized form, being later transformed to its reduced form at the erythrocyte level. It is found naturally in small amounts in different foods, but it occurs in significant amounts in dark vegetables such as spinach and in legumes such as broccoli, grains such as soy and peanuts, oleaginous fruits such as nuts and almonds, and mainly in red meats such as heart and liver and in some fish like mackerel and sardines. However, the dose of CoQ10 that can be obtained from food is 2 mg/day-5 mg/day and only about 10% of what is ingested is absorbed by the gastrointestinal tract due to the low water solubility and high molecular weight of the enzyme, an insufficient amount to meet the demands of the organism in the presence of redox imbalance.

## Pharmacology of co-enzyme Q10

**Absorption:** In healthy individuals, about 95% of the CoQ10 circulating in plasma is in the reduced ubiquinol form. Because it is hydrophobic and has a high molecular weight, CoQ10 is absorbed from the diet in a slow and limited manner, as is the case for lipids. Plasma CoQ10 levels start to increase 1 h-2 h after oral intake, with maximum concentration occurring within 6 h-8 h and with a half-life that may reach 34 h.

CoQ10 is mainly absorbed in the small bowel and is then transported to the liver, forming the lipoprotein complex. For transport, CoQ10 is coupled to the chylomicrons, being taken up by the liver and being then incorporated into LDL, which transports 58% of it, and into HDL, which transports 26% of it. CoQ10 is then distributed to various tissues such as the spleen, adrenals, lungs, kidneys, and myocardium. The main pathways of CoQ10 elimination are the bile ducts and the feces, and a small fraction of what is absorbed ends up by being eliminated in urine.

**Supplementation:** Several brands of commercial products containing CoQ10 are available on the market as powders, capsules or oil, in the reduced or oxidized form and in different doses, representing different forms of bioavailability. Solubilized CoQ10 formulations have greater bioavailability and are absorbed at faster rates than powders, tablets, capsules or oil powder suspensions. Comparison of the solubilized forms of ubiquinol and ubiquinone has shown that ubiquinol is better absorbed. Several clinical trials involving the most diverse diseases have administered a variety of CoQ10 doses and have reported that adverse effects were more common at doses above 1200 mg/day, with doses of 22 mg/day-400 mg/day being considered safe.

## Contraindications and adverse effects

CoQ10 supplementation is quite safe. Several clinical trials using high doses did not show adverse effects significant enough to compromise the therapy. The enzyme should be administered with caution to pregnant or breastfeeding women or to small children since its effects during these periods have not been fully clarified. Gastrointestinal effects such as abdominal discomfort, diarrhea, vomiting, and nauseas, as well as headache and allergic skin rashes have been reported to occur in less than 1% of patients in clinical trials. Due to the antiplatelet and hypotensive potential of this medication, patients who use it should be monitored. Several studies have reported reduced CoQ10 values after its use in combination with HMG-CoA reductase inhibitors (statins) due to the fact that both CoQ10 and cholesterol are synthesized through the mevalonate pathway. The reduction of serum CoQ10 concentrations may be as high as 54%. The magnitude of the reduction of CoQ10 in combination with statins has been shown to be dose related and reversible with the cessation of treatment. It has been hypothesized that this reduction may be the cause of the adverse effects of statins, and CoQ10 supplementation during treatment with statins could be a possible mediator treatment as long as it is properly monitored (Figures 4 and 5).

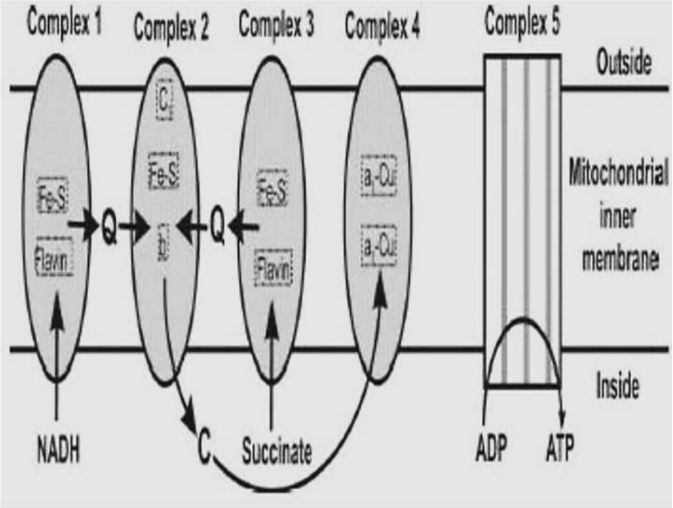


**Figure 4.** Mitochondrial electron transport chain.

NADH=Nicotinamide adenine dinucleotide,Q=CoQ10, C=Cytochrome C, Fe-S=Iron-Sulfur agglomerates, $C_1$=Cytochrome $C_1$, b=Cytochrome b, $a_1$-Cu=Copper-containing cytochrome $a_1$, ADP=Adenosine Diphosphate, ATP=Adenosine triphosphate. The arrows indicate the electron flow through the pathway.

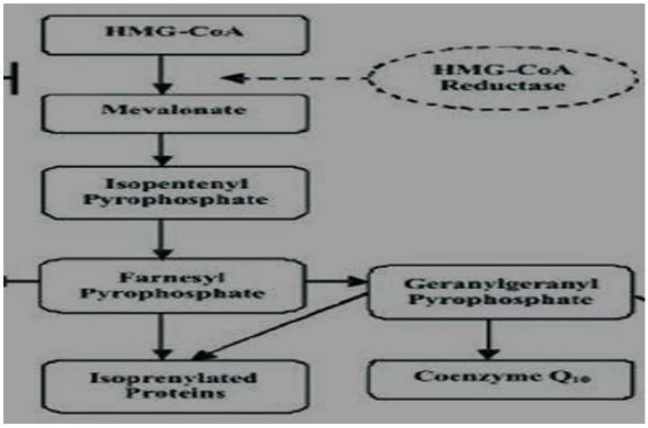


**Figure 5.** Mevalonate cycle.

Inhibition of HMG-CoA reductase by statins, including CoQ10.

## Measurement in plasma and analytical methods

In human beings, CoQ10 concentration peaks at about 20 years of age. Its plasma concentration in adults may range from 0.5 μg/ml to 1.7 μg/ml, with approximately 75% of it being the reduced form, and the highest concentrations are found in the muscles. However, several factors may interfere with CoQ10 levels, both in terms of an increase and a decrease.

The presence of free radicals and of other reactive oxygen and nitrogen species related to certain diseases such as diabetes mellitus, hypercholesterolemia, hypertriglyceridemia, Alzheimer's disease and vitamin A deficiency in some organs cause an increase in CoQ10 in order to increase the antioxidant defenses against the production of free radicals due to these diseases. With aging, CoQ10 levels in the organs may decrease by 30%-60%. Persons with heart or thyroid diseases and certain genetic deficiencies such as primary and secondary mutations affecting the biosynthesis of CoQ10, and even high-performance athletes appear to suffer the influence of CoQ10 levels.

The quantity, structure and size ore have been reports that the mitochondria may also be an additional factor inducing an increase or a decrease in CoQ10 levels in the organism. HPLC is the technique most frequently used to determine CoQ10 levels after extraction from plasma or tissue. Since CoQ10 is highly hydrophobic, its analysis is carried out in two phases. In the first, separation is carried out using a highly hydrophobic inverse phase with a C18 column with a high carbon load, and in the second a mobile phase is used based on inferior alcohols such as hexane or included heptane.

## Effects of CoQ10 on inflammation and fat metabolism

Inflammation is the response to injury caused by endogenous or exogenous factors to tissues or organs and is of help in the restoration of impaired homeostasis. During this process, various

inflammatory cytokines are generated, such as TNF-α and $IL^{-1}$ and $IL^{-6}$. In local inflammation, macrophage infiltration and fibroblast activation are two responses that, on a chronic basis, will trigger inflammation of adipose tissue. The mitochondria play a fundamental role in adipocyte differentiation and maturation, in addition to generating suffcient ATP to support the lipogenic processes that consume energy during preadipocyte differentiation. Mitochondrial dysfunction and the consequent reduction of CoQ10 levels can occur in obese individuals who exhibit increased fat deposition in the organism. Among the factors contributing to mitochondrial dysfunction is an excessive nutrient supply which will later contribute to the formation of reactive oxygen species and to the production of toxic lipid species, stress of the endoplasmic reticulum, aging and/or pro inflammatory processes and mitochondrial fission. Individually or in combination these events contribute to the development of insulin resistance and obesity in the organism as a whole. Fat deposition in the organism is related to high levels of hyperglycemia, hypertriglyceridemia and arterial hypertension, common characteristics of MS. Among the effects of CoQ10 is its ability to prevent the adipogenesis induced by rosiglitazone in obese rats and to inhibit adipocyte differentiation, and treatment with CoQ10 has been reported to increase fat oxidation and energy expenditure in inguinal white adipose tissue.

## Pharmacokinetics and bioavailability of the dietary supplement COQ10

The chemical structure of CoQ10 consists of a quinone group and a side chain of 10 isoprenoid units. It has a high molecular weight (863.34 g/mol) and is strongly hydrophobic. Therefore, it is extremely insoluble in aqueous phase, and is absorbed slowly and incompletely from the small intestine resulting in low oral bioavailability in humans. CoQ10 absorption follows a zero-order kinetics and is to follow the same pathway as other lipophilic substances. It is a complex process undergone by a combination of passive and active transport mechanisms that takes place in the small intestine, being, according to murine models, duodenum, colon, ileum, and jejunum regions from higher to lower permeability to CoQ10. Due to its insolubility in water, limited solubility in lipids, and its relatively high molecular weight, CoQ10 has a poor and slow rate absorption with a Tmax value of about 6 hours. At 24 hours following oral administration, a second plasmatic peak is observed, which could be attributed to enterohepatic recirculation and a redistribution from liver to circulation by lipoproteins. VLDL, LDL, and HDL carry a 16%, 58%, and 26% of serum CoQ10, respectively, and it is believed that CoQ10 is capable to prevent them from oxidation. In fact, many studies have linked total cholesterol or LDL levels and plasmatic CoQ10.

In general, CoQ10 concentration time curve fits a three compartmental pharmacokinetic model based upon the assumption that it is taken up by the liver and then transferred to lipoproteins and redistributed from the liver to systemic blood. This could explain the prolonged elimination phase of CoQ10, with a half-life of about 33 hr. Regarding metabolism, CoQ10 is metabolized by cytochrome P450 enzymes.

Absorption and bioavailability studies show that, as high complexity processes, the individual response to CoQ10 supplementation is highly variable, and may be affected by different factors such as age, gender, diet, microbiota, and intestinal absorption capacity of fats among others. It has to be noted that while its slow absorption and low bioavailability have been associated with its large molecular weight, high lipophilicity and poor aqueous solubility, as with all water insoluble compounds, CoQ10 absorption is enhanced by the presence of a lipidic medium, and thus, it is recommended to take it with meals containing fat, or encapsulated within a suitable delivery system.

Due to its involvement in the cellular energy synthesis, CoQ10 is not uniformly distributed across the body, but concentrated in those tissues with a higher energy requirement. At a cellular level, because of its liposoluble character, is mainly located in cell membranes, with only a 10% of total CoQ10 located in the cytosol, and around 40% to 50% located in the internal mitochondrial membrane, where it plays its essential role in energy synthesis.

As for its oxidative states CoQ10 is mainly found in the reduced form, except in the brain and lungs, where oxidative stress is higher. In fact, 95% of circulating CoQ10 in humans is found in the reduced form, and no significant change in the reduced: Oxidized ratio occurs after its ingestion. This could be due to CoQ10 reduction during the absorption process, as reported in a study with human Caco-2 cells.

## Therapeutic indications

Despite CoQ10 has been proposed as a potential candidate for the treatment of different diseases, it is not an FDA approved drug. Instead, it is sold as a food supplement, and therefore, is not meant to treat, cure, or prevent any disease. However, a number of clinical trials have observed that CoQ10 oral administration provided beneficial effects on a range of different disorders that have been associated with low CoQ10 levels and high oxidative stress, such as mitochondrial, cardiovascular, and neurodegenerative diseases. These beneficial effects are associated with CoQ10 antioxidant activity and physiological key role in mitochondrial bioenergetics. Since CoQ10 is the only lipid-soluble antioxidant synthesized endogenously, and it plays an essential role in ATP synthesis, it is indispensable for the proper functioning of all tissues and organs, especially those with a high-energy demand.

In addition, CoQ10 has also been reported to exert beneficial effects on energy-yielding metabolism, maintenance of normal blood pressure and cholesterol concentrations, maintenance of normal cognitive function, protection of DNA, proteins and lipids from oxidative damage, and increase in endurance performance. However, since clinical evidence is still limited, the scientific panel of the EFSA could not establish a cause-effect relationship between the consumption of CoQ10 and these claimed effects. Thus, it does not have any of these food supplement health claims approved.

## Formulation

CoQ10 is a fine yellow to orange crystalline powder that decomposes and darkens when exposed to light. It is tasteless with a slight odor, practically water insoluble, slightly soluble in ethanol, and soluble in acetone and ether, and with a melting point around 48°C. Due to its high molecular weight (863.34 g/mol) and strong hydrophobicity, it has a low oral bioavailability in humans. In addition, CoQ10 is unstable and vulnerable to heat, light, and oxygen, which also limits its applications in medicine and functional food formulations. Its stability when stored in the original container,

protected from light, in a dry place at low temperature (below 25°C) is around 24 months. Concerning its formulation in food supplements, high dose and stable CoQ10 formulations are difficult to achieve due to its physicochemical properties. As a fine powder with poor rheology and low melting point, CoQ10 is difficult to be dosed accurately and pressed into tablets, especially when temperature rises beyond its melting point, leading to stickiness and adherence to machinery surfaces. In addition, since CoQ10 is affected by light, heat, and oxidation, it should be stored in a cool and dark place, preferably in an airtight container. For this reason and taking into consideration that CoQ10 has gained increasing interest over the years, research has been focused in overcoming the issues that limit its formulation into food supplements and medicinal products. Thus, research efforts have been made to improve its solubility, oral bioavailability, and stability. As a food supplement, CoQ10 suggested daily dose varies depending on the indication, but is usually around 30 mg to 100 mg for healthy people, reaching up to 60 mg to 1,200 mg when used in certain medical conditions. Since the efficiency of absorption decreases as the dose increases, and reaching certain CoQ10 plasma concentrations is necessary to promote uptake by peripheral tissues and to achieve clinical effects, formulations allowing a higher bioavailability should be developed, improved, and prioritized.

CoQ10 is mainly found in mono or multicomponent softgels, capsules, and tablets. However, while it is used to be incorporated as a simple crystalline powder, it was found that the amount and characteristics of co-ingested lipids may benefit CoQ10 absorption and bioavailability, and therefore it is usually dissolved in a lipid phase.

Carrier oil selection is extremely important, since it may result in different bioavailability. Solubilized formulas have shown higher absorption and bioavailability, and thus plasmatic levels were significantly higher when compared to non-solubilized COQ10 powder. For this reason, different formulation approaches including novel delivery systems such as Self-Emulsified Drug Delivery Systems (SEDDS), nano-emulsions, or cyclodextrin complexes have been tested to improve CoQ10 bioavailability when it is incorporated into soft gels, capsules, and tablets. By way of example, different formulation approaches will be briefly reviewed below. As for cyclodextrin inclusion, it is widely used in the pharmaceutical and food supplement industry since it provides improved stability against heat, oxidation, and UV. In addition, cyclodextrins can form inclusion complexes with lipophilic substances such as CoQ10, leading to improved stability, water solubility, and bioavailability. SEDDS, which are composed of an oil, surfactant, and cosurfactant, as well as the vehiculized active ingredient, are also used to enhance solubility and bioavailability of poorly water-soluble substances like CoQ10. In addition, certain formulations may take advantage of CoQ10's low melting point to form an amorphous solid dispersion, which is also effective at enhancing solubility, stability, and dissolution, and may also improve flowability. Besides, spray-drying microencapsulation with previous emulsification has also been successfully used to improve water dispersion, stability, and bio accessibility of CoQ10. Finally, despite further research is needed regarding oral supplementation with CoQ10 containing liposomes in humans, improved bioavailability was reported in oral and topical application of liposomal CoQ10 in rats.

## 5-Fluorouracil

Antimetabolite drugs work by inhibiting essential biosynthetic processes, or by being incorporated into macromolecules, such as DNA and RNA, and inhibiting their normal function. The fluoropyrimidine 5-fluorouracil (5-FU) does both. Fluoropyrimidines were developed in the 1950 s following the observation that rat hepatomas used the pyrimidine uracil-one of the four bases found in RNA more rapidly than normal tissues, indicating that uracil metabolism was a potential target for antimetabolite chemotherapy. The mechanism of cytotoxicity of 5-FU has been ascribed to the misincorporation of fluoronucleotides into RNA and DNA and to the inhibition of the nucleotide synthetic enzyme Thymidylate Synthase (TS). 5-FU is widely used in the treatment of a range of cancers, including colorectaland breast cancers, and cancers of the aerodigestive tract. 5-FU in combination with other chemotherapeutic agents improves response rates and survival in breast and head and neck cancers, it is in colorectal cancer that 5-FU has had the greatest impact.

5-FU based chemotherapy improves overall and disease-free survival of patients with resected stage III colorectal cancer. Nonetheless, response rates for 5-FU based chemotherapy as a first-line treatment for advanced colorectal cancer are only 10%-15%. The combination of 5-FU with newer chemotherapies such as irinotecan and oxaliplatin has improved the response rates for advanced colorectal cancer to 40%-50%. However, despite these improvements, new therapeutic strategies are urgently needed.

Understanding the mechanisms by which 5-FU causes cell death and by which tumours become resistant to 5-FU is an essential step towards predicting or overcoming that resistance.

## Mechanism of action of 5-FU

5-FU is an analogue of uracil with a fluorine atom at the C-5 position in place of hydrogen. It rapidly enters the cell using the same facilitated transport mechanism as uracil6. 5-FU is converted intracellularly to several active metabolites: fluorodeoxyuridine Monophosphate (FdUMP), Fluorodeoxyuridine Triphosphate (FdUTP) and Fluorouridine Triphosphate (FUTP)- these active metabolites disrupt RNA synthesis and the action of TS. The rate-limiting enzyme in 5-FU catabolism is Dihydropyrimidine Dehydrogenase (DPD), which converts 5-FU to Dihydrofluorouracil (DHFU). More than 80% of administered 5-FU is normally catabolized primarily in the liver, where DPD is abundantly expressed.

## TS inhibition

TS catalyses the reductive methylation of Deoxyuridine Monophosphate (dUMP) to Deoxythymidine Monophosphate (dTMP), with the reduced folate 5,10-Methylenetetrahydrofolate ($CH_2THF$) as the methyl donor.This reaction provides the sole de novo source of thymidylate, which is necessary for DNA replication and repair. The 36 kDa TS protein functions as a dimer, both subunits of which contain a nucleotide-binding site and a binding site for $CH_2THF$.

The 5-FU metabolite FdUMP binds to the nucleotide-binding site of TS, forming a stable ternary complex with the enzyme and $CH_2THF$, thereby blocking binding of the normal substrate dUMP and inhibiting dTMP synthesis. The exact molecular mechanisms that mediate events downstream of TS inhibition have not been fully elucidated.

Depletion of dTMP results in subsequent depletion of Deoxythymidine Triphosphate (dTTP), which induces perturbations in the levels of the other Deoxynucleotides (dATP,dGTP and dCTP) through various feedback mechanisms. Deoxynucleotide pool imbalances (in particular,the dATP/dTTP ratio) are thought to severely disrupt DNA synthesis and repair, resulting in lethal DNA damage. In addition, TS inhibition results in accumulation of dUMP,which might subsequently lead to increased levels of Deoxyuridine Triphosphate (dUTP). Both dUTP and the 5-FU metabolite FdUTP can be misincorporated into DNA. Repair of uracil and 5-FU-containing DNA by the nucleotide excision repair enzyme Uracil-DNA-Glycosylase (UDG) is futile in the presence of high (F) dUTP/dTTP ratios and only results in further falsenucleotide incorporation.These futile cycles of misincorporation,excision and repair eventually lead to DNA strand breaks and cell death. DNA damage due to dUTP misincorporation is highly dependent on the levels of the pyrophosphatase dUTPase,which limits intracellular accumulation of Dutp. Thymidylate can be salvaged from thymidine through the action of thymidine kinase,thereby alleviating the effects of TS deficiency. This salvage pathway represents a potential mechanism of resistance to 5-FU.

## RNA misincorporation

The 5-FU metabolite FUTP is extensively incorporated into RNA,disrupting normal RNA processing and function. Significant correlations between 5-FU misincorporation into RNA and loss of clonogenic potential have been shown in human colon and breast cancer cell lines. 5-FU misincorporation can result in toxicity to RNA at several levels. It not only inhibits the processing of pre-rRNA into mature rRNA, but also disrupts post-transcriptional modification of tRNAs and the assembly and activity of snRNA/protein complexes, thereby inhibiting splicing of pre-mRNA. In addition, rRNA, tRNA and snRNA all contain the modified base pseudouridine,and 5-FU has been shown to inhibit the post-transcriptional conversion of uridine to pseudouridine in these RNA species. polyadenylation OF mRNA is inhibited at relatively low 5-FU concentrations. These *in vitro* studies indicate that 5-FU misincorporation can potentially disrupt many aspects of RNA processing, leading to profound effects on cellular metabolism and viability.

## Modulation of 5-FU

5-FU has been used for more than 40 years in the treatment of colorectal cancer. 5-FU is given intravenously and has been used in a variety of different schedules to determine the optimum dose and mode of administration. The overall response rate for 5-FU as a single agent in advanced colorectal cancer is quite limited (approximately 10%-15%) however,over the past 20 years, important modulation strategies have been developed to increase the anticancer activity of 5-FU and to overcome clinical resistance. As a result, 5-FU has remained the main agent for the treatment of both advanced and early-stage colorectal cancer. Strategies that have been explored to modulate the anticancer activity of 5-FU include decreasing 5-FU degradation, increasing 5-FU activation and increasing the TS binding activity of FdUMP.

## Leucovorin

High intracellular levels of the reduced folate $CH_2THF$ are necessary for optimal binding of FdUMP to TS. Leucovorin (LV, 5-formyltetrahydrofolate) has been used to expand the intracellular concentration of $CH_2THF$ and has been shown to increase the *in vitro* and *in vivo* toxicity of 5-FU in many cancer cell lines. LV enters the cell *via* the reduced folate carrier and is anabolized to $CH_2THF$,which is then polyglutamated by folylpolyglutamate synthetase. Polyglutamation not only increases the cellular retention of $CH_2THF$, but also enhances the stabilization of its ternary complex with TS and FdUMP. In cell-free systems the pentaglutamate form was found to be 40-fold more potent in promoting ternary complex formation than the monoglutamate. The Advanced Colorectal Cancer Meta-Analysis Project (ACCMP) showed that 5-FU/LV generated significantly superior response rates compared with bolus singleagent 5-FU (23% versus 11%); however, this did not result in improved overall survival.

Inhibitors of dihydropyrimidine dehydrogenase.5-FU shows poor bioavailability due to its rapid degradation to DHFU by DPD 7. Several strategies have been developed to inhibit DPD-mediated degradation of 5-FU. The UFT (uracil/Ftorafur) formulation uses a 4:1 combination of uracil with the 5-FU pro-drug Ftorafur, which improves 5-FU bioavailability by saturating DPD with its natural substrate. Douillard and colleagues reported that UFT/LV produced equivalent response rates to 5-FU/LV and was a safer, more convenient treatment. In addition, DPD inhibitors, such as eniluracil. and 5-chlorodihydropyrimidine (CDHP), have been investigated. An interesting study by Spector et al. found that eniluracil improved the tumour response rate to 5-FU from 13% to 94% in a rat model (Furthermore, they showed that co-administration of DHFU with 5-FU and eniluracil reduced the response rate to 38%. These findings indicate that 5-FU catabolites might interfere with the antitumour efficacy of 5-FU and provide a further rationale for designing formulations that inhibit DPD. Another approach has been to design 5-FU prodrugs that avoid DPD-mediated degradation in the liver. Capecitabine is an oral fluoropyrimidine that is absorbed unchanged through the gastrointestinal wall and is converted to 5′-deoxy-5-fluorouridine (5′ DFUR) in the liver by the sequential action of carboxylesterase and ′cytidine deaminase 3, DFUR is then converted to 5-FU by Thymidine Phosphorylase (TP) and/or Uridine Phosphorylase (UP) both of which have been reported to be significantly more active in tumour tissue than in normal tissue.This might account for the observed tumour-selective activation of capecitabine to 5-FU. In clinical trials,capecitabine showed a significantly higher response rate than 5-FU/LV (24.8% versus 15.5%), although time to disease progression and survival were similar for the two treatment arms. Furthermore,the toxicity profile of capecitabine was more favourable with fewer treatment-related serious adverse events and hospitalizations.

## Methotrexate

Methotrexate (MTX) is an antifolate inhibitor of Dihydrofolate Reductase (DHFR), which catalyses the conversion of Dihydrofolate (DHF) to Tetrahydrofolate (THF). THF is required for purine biosynthesis and,as the precursor of $CH_2THF$ is also necessary for dTMP synthesis, MTX inhibits both purine and thymidine biosynthesis. I*n vitro*, and *in vivo*, studies have shown that MTX can synergize with 5-FU when administered before 5-FU.Inhibition of

purine biosynthesis by MTX increases the levels of phosphoribosyl pyrophosphate (PRPP), which is the cofactor required for the conversion of 5-FU to Fluorouridine Monophosphate (FUMP) by Orotate Phosphoribosyl Transferase (OPRT). So, the increased PRPP levels induced by MTX would promote conversion of 5-FU to FUMP. Several investigators have found that the antitumour activity of 5-FU was enhanced by pre-treatment with MTX,and this correlated with increased formation of 5-FU ribonucleotides and increased 5-FU incorporation into RNA. Clinically, the combination of MTX and 5-FU was found to be significantly superior to bolus single-agent 5-FU for the treatment of colorectal cancer,both in terms of response rate (19% versus 10%) and overall survival (10.7 months versus 9.1 months).

## Interferon

Interferons (IFNs) are pleiotropic cytokines that exert negative regulatory effects on the growth of normal and malignant cells *in vitro* and *in vivo*. Numerous *in vitro* studies have reported that 5-FU interacts with IFNs to produce greater than additive cytotoxicity in various cancer cell lines. Houghton et al. found that IFN-œenhanced 5-FU-mediated single and double-strand DNA breaks in colon carcinoma cells. IFN-γ has been reported to upregulate the activities of the 5-FU anabolic enzymes TP and UP, resulting in enhanced 5-FU activation. A study by Chu, et al. found that acute translational upregulation of TS expression by 5-FU (see below) was abrogated by IFN-γ in the H630 colon cancer cell line, resulting in enhanced TS inhibition. Several early clinical Phase II studies reported response rates of 42%-54% for the combination of 5-FU and IFN-α.

**Chemotherapy:** Chemotherapy is used primarily to treat systemic disease rather than localized lesions that are amenable to surgery or radiation. It uses antineoplastic agents in an attempt to destroy tumor cells by interfering with cellular function including replication. These drugs result in causing lethal injury to DNA which further leads to malignant cell death *via* apoptosis. In cancer treatment, mode of action of certain chemotherapeutic agents involves generation of free radicals to cause cellular damage and necrosis of malignant cells.

Drugs with free radical mechanism include but are not limited to alkylating agent (alkylsulfonates, ethyleneamines and hydrazines), anthracyclines (doxorubicin and doxorubicin), platinum coordination complexes (cisplatin, carboplatin), podophyllin derivatives (etoposides) and camptothecins (irinotecan, topotecan). These ROS often are sources of atrocious side effects which remains as long as the duration of chemotherapy treatment.

**Chemotherapy induced systemic toxicity:** By its very nature, anti-cancer chemotherapy is cytotoxic that means it is designed to damage human cells. Because anti-cancer drugs are cytotoxic for normal as well as neoplastic cells, the range of unwanted effects that accompanies their use is broad. Many of the side-effects are potentially life-threatening or seriously debilitating. The precursor cells of the hemopoietic system, sited in the bone marrow, undergo cell division more rapidly than those of any other organ system and thus are particularly vulnerable to damage from cytotoxic drugs, since most chemotherapeutic agents act principally on dividing cells. Accordingly, bone marrow depression is a side-effects of nearly all such drugs and is the dose-limiting side effect of most. Red blood cell macrocytosis is a common effect of hydroxyurea, methotrexate, cytarabine, 5 fluorouracil and other antimetabolites.

Nausea and vomiting which usually occurs within 24 hrs of drug administration can be amongst the most disturbing and unpleasant side effects induced by chemotherapy. If persistent, vomiting may lead to dehydration, electrolyte disturbances, metabolic alkalosis, weakness, weight loss, cachexia, nutritional impairment and physical injury such as esophageal tears and fractures. Diarrhea and constipation in cancer patients may be due to many factors that include age, anticholinergics, narcotics, low fibre diet, decreased appetite and inability to eat and drink due to oral mucositis or esophagitis apart from the side-effects of cytotoxic drugs. Cardiac damage is the dose-limiting toxicity of the anthracycline group of antitumor antibiotics related anthraquinones and can cause cumulative cardiomyopathy. Damageto theliver is a complication of many drugs. Since patients receiving chemotherapy often are very ill and simultaneously receiving other medications that may impair liver function, it is often impossible to determine which of their treatments is responsible for the liver abnormality. Furthermore, septicemia, parenteral nutrition, viral and fungal infections and metastatic disease itself also commonly cause hepatic disturbance. Pulmonary complications and kidney toxicity are being increasingly recognized and may be dose-limiting. Lung toxicity induced by methotrexate is said to occur in 5%-8% of patients and includes pulmonary edema, pulmonary fibrosis, capillary leakage and hypersensitivity reaction. The kidneys are vulnerable to damage from chemotherapeutic agents as they are the elimination pathway for many drugs and their metabolites. Cisplatin primarily causes proximal and distal tubular damage, although a rare hemolytic-uremic syndrome has also been reported. Fertility problems can be an unfortunate delayed side effect of chemotherapy. Cytotoxic drugs damage the germinal epithelium resulting in reduced testicular volume and sperm count. The degree of dysfunction depends on the dose of drug as well as age and pubertal status of the patient at the time of treatment. Often chemotherapy mediated toxicities are related to generation of ROS leading to oxidative stress in cell.

### Chemotherapy induced ROS and their intracellular sources

Most of the oxygen taken up by the cells is converted to water by the action of cellular enzymes. However, some of these enzymes leak electron into oxygen molecules and lead to the formation of free radicals. They are also formed during normal biochemical reactions involving oxygen. ROS is a collective term used for a group of oxidants, which are either free radicals or molecular species capable of generating free radicals.

There are two important sources of free radical formation. First the internal factors *i.e.* normal cellular metabolism like mitochondrial Electron Transport Chain (ETC), endoplasmic reticulum oxidation and many enzymic activities. Other exogenous factors are radiation, chemotherapy, cigarette smoke and oxygen itself. Intracellular free radical mainly comprises superoxide radicals ($O_2$), Hydroxyl Radicals (OH), Nitric Oxide (NO), Nitrogen dixiode ($N_2O$) and Lipid Peroxyl (LP) radicals. Under normal physiological conditions, nearly 2% of the oxygen consumed by the body is converted into $O_2$ through mitochondrial respiration, phagocytosis, etc.

Auto oxidation of ubisemiquinone is the major source of superoxide anion. Non radical or enzymic generation involves almost all oxidase enzymes (glycolate oxidase, D-amino acid oxidase, urate oxidase, acetyl-CoA oxidase, NADH oxidase and monoamine oxidase) generating $H_2O_2$. NO is an endothelial relaxing factor and

neurotransmitter, produced through nitric oxide synthase enzymes. NO and $O_2$ radicals are converted to powerful oxidizing radicals like Hydroxyl Radical (OH), Alkoxy Radical (RO), peroxyl radical (ROO), Singlet Oxygen ($1(O_2)$) by complex transformation reactions. Some of the radical species are converted to molecular oxidants like Hydrogen Peroxide ($H_2O_2$), Peroxynitrite ($ONOO^-$) and Hypochlorous acid (HOCl). Sometimes these molecular species act as sources of ROS. HO radical formation requires a cellular steady state level of both superoxide anion and $H_2O_2$, precursors of hydroxyl radicals *via* Fenton reaction. $ONOO^-$ at physiological concentrations of carbon dioxide becomes a source of Carbonate radical ($CO_3$) anion. Thus, chemotherapy becomes a substantial but indirect source of generating free radicals resulting into oxidative damage.

**ROS induced oxidative damage:** Depending upon their nature, chemotherapy induced ROS reacts with biomolecules to produce different types of secondary radicals like lipid, sugar, nitrogenous base, amino acid derived radicals and thiyl radicals. These radicals in presence of oxygen are converted to peroxyl radicals that often induce chain reactions. The biological implications of such reactions depend on several factors like nature of the substrate, site of generation, activation of repair mechanisms, redox status etc. Cellular membranes are vulnerable to the oxidation by ROS due to the presence of high concentration of unsaturated fatty acids in their lipid components. ROS reactions with membrane lipids cause lipid peroxidation, resulting in formation of Lipid Hydroperoxide (LOOH) which can further decompose to an aldehyde such as malondialdehyde, 4-Hydroxy Nonenal (4-HNE) or cyclic endoperoxide, isoprotans and hydrocarbons. The consequences of lipid peroxidation are cross linking of membrane proteins, change in membrane fluidity and formation of lipid-protein, lipid-DNA adduct which may be detrimental to the functioning of cell. The side chains of all amino acid residues of proteins, in particular tryptophan, cysteine and methionine are susceptible to oxidation by ROS. Protein oxidation products are usually carbonyls such as aldehydes and ketones. Proteins can undergo direct and indirect damage following interaction with ROS resulting into peroxidation, changes in their tertiary structure, proteolytic degradation, protein-protein cross linkages and fragmentation. Although DNA is a stable and well-protected molecule, ROS can interact with it and cause several types of damage such as modification of DNA bases, single and double strand DNA breaks, loss of purines, damage to the deoxyribose sugar, DNA protein cross linkage and damage to the DNA repair systems. Free radicals can also attack the sugar moiety, which can produce sugar peroxyl radicals and subsequently inducing strand breakage. The consequence of DNA damage is the modification of genetic material resulting in cell death, mutagenesis and ageing.

**Redox state and oxidative stress:** All forms of life maintain a steady state concentration of ROS determined by the balance between their rates of production and removal by various antioxidants. Each cell is characterized by a particular concentration of reducing species like GSH, NADH, $FADH_2$ etc., stored in many cellular constituents, which determine the redox state of a cell.

By definition, redox state is the total reduction potential or the reducing capacity of all the redox couples such as GSSG/2 GSH, $NAD^+$/NADH found in biological fluids, organelles, cells or tissues. Redox state not only describes the state of a redox pair, but also the redox environment of a cell. Under normal conditions, the redox state of a biological system is maintained towards more negative redox potential values. However, this balance can be disturbed when level of ROS exceeds and/or levels of antioxidants are diminished. This state is called 'oxidative stress' and can result in serious cellular damage or apoptosis of normal cells if the stress is massive and prolonged. In contrast to oxidative stress-induced apoptosis, excessive oxidative stress inhibits caspase activity and drug-induced apoptosis, thereby interfering with the ability of antineoplastic agents to kill tumor cells. Electrophilic aldehydes, such as tetrapeptide aldehyde (acetyl-Tyr-Val-Ala-Asp-H) that are used to characterize caspase-1, covalently bind to the sulfhydryl group of the cysteine residue at the active site of caspases and inhibit their activity. Thus during oxidative stress, aldehyde generation resulting in caspase inhibition may account for the reduced efficacy of antineoplastic agents. If so, antioxidants may enhance the anticancer activity of cancer chemotherapy by reducing aldehyde generation during chemotherapy-induced oxidative stress.

**Antioxidant system:** In order to check the activities of ROS/RNS *in vivo* and maintain cellular redox homeostastis, antioxidant system has evolved. Antioxidants are substances that may protect cells from the damage caused by free radicals, and may play a role in heart disease, cancer and other diseases. Antioxidants neutralize free radicals by donating one of their own electrons and ending the electron"stealing" reaction. This helps to prevent ROS mediated cell and tissue damage. Antioxidants are often described as "mopping up" free radicals, meaning they neutralize the electrical charge and prevent the free radical from taking electrons from other molecules.

Endogenous compounds in cells can be classified as enzymatic antioxidants such as superoxide dismutase, catalase, glutathione dependent enzymes and non-enzymatic antioxidants, further divided into metabolic and nutrient antioxidants. Metabolic antioxidants belonging to endogenous antioxidants such as GSH, lipoic acid, L-arginine, coenzyme Q10, melatonin, uric acid, bilirubin etc. are produced by metabolism in the body. Nutrient antioxidant belonging to exogenous antioxidants which are taken through food supplements are vitamin E, vitamin C, carotenoids, trace elements, flavonoids, polyphenols etc.. A delicate balance exists between antioxidant repairing systems and pro-oxidant mechanism of tissue destruction, which if tipped in favour of cellular damage, could lead to significant tissue mutilation.

Antioxidants prevent cellular damage by reacting and eliminating oxidizing free radicals thereby finding relevance in adjuvant chemotherapy.

The use of antioxidant supplements by patients with cancer is estimated to be between 13 and 87%. Such broad range of percentage might be attributed to the difference in cancer types, age, education, complementary medicines and ethnicity in the group undertaken for the study. The use of supra-dietary doses of antioxidant has attracted increasing interest as a possible primary and secondary cancer deterrence strategy.

Higher levels of endogenous antioxidant may protect against chemotherapy induced oxidative stress especially in some cancer patients having impaired capacity to deal with oxidative insult. However, in cancer chemotherapy, a mode of action of certain antineoplastic agents involves generation of free radicals further leading to cellular damage and necrosis of malignant cells. Hence

use of antioxidant during chemotherapy is criticized due to fear of causing interference with efficacy of the drug.

**Chemotherapy, oxidative stress and antioxidants:** Chemotherapy drugs that cause high levels of oxidative stress are thought to rely, in part, on using this stress mechanism to kill cancer cells. But oxidative stress might actually reduce the overall effectiveness of chemotherapy. Oxidative stress slows the process of cell replication, but it is during cell replication that chemotherapy actually kills cancer cells, so slower cell replication can mean lower effectiveness of chemotherapy. One approach towards addressing this problem is the addition of certain antioxidants at specific dosages to lessen oxidative stress, thus making the chemotherapy treatment more effective. The interaction between chemotherapy and antioxidants is more complex than simply promoting and inhibiting oxidative stress. However, there are several mechanisms by which chemotherapy functions and antioxidants also have a number of different effects on the body. Each antioxidant has a different interaction in chemotherapy and this effect can even change based upon the dosage used. Some antioxidants have been found to be useful for restoring the natural antioxidants in the body, which are often depleted after the completion of chemotherapy, resulting in decreased side effects and increased the survival time in patients undergoing chemotherapy. Thus, targeted nutrient therapies using antioxidant or their precursors can prove to be beneficial in reducing the toxic effect of medications thereby improving the therapeutic efficacy.

**Antioxidants in chemotherapy:** Role of antioxidants are controversial in cancer therapy because of two very imperative features. First, there are two different kinds of antioxidants doses used based on which the data on the role of antioxidants in cancer therapy can be categorized as: A preventive dose, which is a low dose, and a therapeutic dose, which is a high dose. For the preventive dose, the data has shown protection of normal cells and tumor cells. For the therapeutic dose, the data shows that it inhibits the growth of cancer cells but not the normal cells. Therefore researchers are looking at data for preventive doses, which is perplexing. Numerous original research articles have focused on the topic of whether supplemental antioxidants administered during chemotherapy can protect normal tissue without adversely influencing tumor control. Due to variation in study design, intervention protocol, cancer type, timing of observation, inclusive criteria, statistical analysis, chemotherapy regime develops uncertainty to make definitive conclusion regarding the risk of decreased tumor control as a consequence of administering supplemental antioxidant during chemotherapy. On the contrary certain recent review definitely concludes that that antioxidant when given concurrently

- Do not interfere with chemotherapy.
- Enhance the cytotoxic effect of chemotherapy.
- Protects normal tissue.
- Increases patient survival and therapeutic response.

Moss, investigated articles and reviews to find out the use of a-tocopherol for the amelioration of radiation induced mucositis; pentoxifylline and vitamin E to correct the adverse effects of radiotherapy; melatonin alongside radiotherapy in the treatment of brain cancer; retinol palmitate as a treatment for radiation-induced proctopathy; a combination of antioxidants (and other naturopathic treatments) and the use of synthetic antioxidants like amifostine and dexrazoxane, as radioprotectants. With few exceptions, most of the studies draw positive conclusions about the interaction of antioxidants and radiotherapy. Currently, evidence is growing that antioxidants may provide some benefit when combined with certain types of chemotherapy. Because of the potential for positive benefits, a randomized controlled trial evaluating the safety and efficacy of adding antioxidants to chemotherapy in newly diagnosed ovarian cancer is underway at the University of Kansas Medical Center. In Long Island breast cancer patient study project, Greenlee and colleagues have reported that among 764 patients, 663 (86.8%) were found to be receiving adjuvant treatment for their breast cancer. Of those 663 women, 401 (60.5%) reported using antioxidants during adjuvant treatment. 210 of 310 women (38.7%) used antioxidants during chemotherapy, 196 of 464 women (42.2%) used them during radiation, and 286 of 462 women (61.9%) used them during tamoxifen therapy. In year 2012, same group published a data investigating the associations between antioxidant use after breast cancer diagnosis and breast cancer outcomes in 2264 women. Antioxidant supplement use after diagnosis was reported by 81% of women. Among antioxidant users, frequent use of vitamin C and vitamin E was associated with decreased risk of BC recurrence, vitamin E use was associated with decreased risk of all cause mortality but conversely, frequent use of combination carotenoids was associated with increased risk of death from breast cancer and all cause mortality. A report on population-based prospective cohort study of 4877 women was conducted in the first 6 months after breast cancer diagnosis and during cancer treatment with total mortality and recurrence. Vitamin use shortly after breast cancer diagnosis was found to be associated with reduced mortality and recurrence risk, adjusted for multiple lifestyle factors, sociodemographics, and known clinical prognostic factors. Researchers concluded that vitamin supplement use in the first 6 months after breast cancer diagnosis may be associated with reduced risk of mortality and recurrence. Cancer patients suffer from vitamin deficiencies, particularly of folic acid, vitamin C, pyridoxine and other nutrients because of poor nutrition and treatment. Chemotherapy reduces serum levels of antioxidant vitamins and minerals due to lipid peroxidation and thus produces higher level of oxidative stress. Therefore, supplementation of certain antioxidants and nutrients can help to enhance the health status of patients undergoing continous regime of chemotherapy. Vitamin E has been shown to decrease chemotherapy mediated toxicity and with omega-3 fatty acid increase survival time in terminal cancer patients. Other than suppressing free radical induced progression of lipid peroxidation in normal cells, vitamin E is also known to induce apoptosis in experimental tumor lines and increase the efficacy of chemotherapy. Kline, et al. have reported approximately 50 vitamin E analogues being synthesized and screened for their ability to induce human tumor cells to undergo apoptosis. Eleven vitamin E analogues exhibited to have potent anticancer properties. Liposome-formulated a-TEA administered to BALB/c mice by aerosol for 17 days significantly reduced subcutaneous injected mouse mammary tumor cells growth and lung metastasis. Tumor volume was reduced by 65% in comparison with the aerosol control. Schwenke concluded that dietary exposure to a-tocopherol may modestly protect women from breast cancer. Some reports have suggested that Vitamin E Succinate (VES) inhibits the growth of human breast cancer in culture by induction of DNA

synthesis arrest, cellular differentiation and apoptosis. The authors here wish to emphasize that combinations not studied *in vivo* risk potential adverse reactions and should be monitored closely.

**Effect of COQ10 in cancer chemotherapy:** CoQ10 is an important antioxidant capable of preventing lipid peroxidation and oxidative damage of DNA and proteins. Since oxidative DNA damage, including mutagenic and cytotoxic lesions, is implicated in the initiation phase of cancer, CoQ10 could reduce the susceptibility of cells to cancer development. In addition, low levels of CoQ10 were observed in breast tumour tissues, when compared to the corresponding noncancerous tissues. For this reason, its exogenous administration may help increase the protective effect of endogenous CoQ10 in breast tissue, especially in high-risk patients. CoQ10 levels were also significantly lower in melanoma patients and in those who developed metastasis, than in control subjects, and the disease-free interval was shorter in patients with lower levels of CoQ10. Moreover, there was a significant correlation between CoQ10 levels and the thickness of the primary tumour, with the highest CoQ10 levels being observed in patients with thinner tumors.

In contrast, concerning the cell response to chemotherapeutic agents, tumor cells tend to present higher antioxidant activities, that is the Warburg effect, which confers an increased protection to oxidative stress, and therefore resistance to pro-oxidant chemotherapeutic treatments. In addition, reported that chemotherapy induced an increase in CoQ10 levels in cancer cell lines, which is believed to be part of a cellular defence mechanism against chemotherapy treatment.

Therefore, by means of the same mechanism, the administration of CoQ10 could be translated into increased CoQ10 levels that may also protect the surrounding normal cells from toxicity and contribute to its survival, leading to an improved tolerability of anticancer drugs. For instance, cardiotoxicity associated to anthracyclines treatment, which is a commonly used chemotherapeutic agent, may be preventable by its concurrent administration with CoQ10. Roffe, Schmidt, and Ernst reviewed the evidence available for oral supplementation with CoQ10 to reduce the toxicity and improve the tolerability of cancer treatments and concluded that CoQ10 may provide improved tolerability as well as protection against toxicity.

However, due to weaknesses in the design and a poor overall methodologic quality of the trials reviewed, these results should be interpreted with caution. CoQ10 could also be useful to reduce the risk of developing metastases. While immune-modulators such as interferons (IFN)are used in patients with melanoma to prevent the development of residual micro-metastases after surgery, the immune response initiated by IFN seems to require large amounts of ATP. Thus, CoQ10 could be useful to enhance IFN action. In fact, reported that the risk of developing metastases was about 10 times lower in patients treated with IFN+CoQ10, compared with the IFN group. In addition, reported a reduction of serum tumor marker levels, and thus a reduced risk of cancer recurrence and metastases when breast cancer patients received a supplement of CoQ10, riboflavin, and niacin, along with tamoxifen. Overall, observational studies showed that lower CoQ10 plasmatic concentrations were associated with a higher risk of suffering cancer, and with a worse prognosis.

In contrast, those patients receiving CoQ10 treatment may have an increased life expectancy, probably due to antioxidant protection against heart and hepatic toxicity caused by free radicals generated by chemotherapy.

## Histology of the spleen

Located in the abdomen, directly beneath the diaphragm, and connected to the stomach, the spleen is the body's largest filter of the blood. In essence, the spleen is organized as a 'tree' of branching arterial vessels, in which the smaller arterioles end in a venous sinusoidal system. The organ is surrounded by a fibrous capsule of connective tissue, stemming from which are trabeculae that support the larger vasculature. The smaller branches of the arterial supply are sheathed by lymphoid tissue, which forms the white pulp of the spleen. In humans, part of the bloodstream ends in the perifollicular zone. With its location in the circulatory system and with the unusual structure of its lymphoid compartments, the spleen is a unique lymphoid organ. This is also reflected in its embryological development, which differs from that of other lymphoid organs. The combination of highly adapted macrophages and specific anatomical features, of the marginal zone in particular, underlies the fact that the spleen is a crucial site of early exposure to encapsulated bacteria.

**The red pulp:** The efficient blood-filtering system of the spleen and its importance for iron recycling by splenic macrophages of the red pulp. In addition, we discuss that, in these macrophages, the processes that are involved in iron metabolism are also involved in the removal of bacteria from the blood (Figure 6).

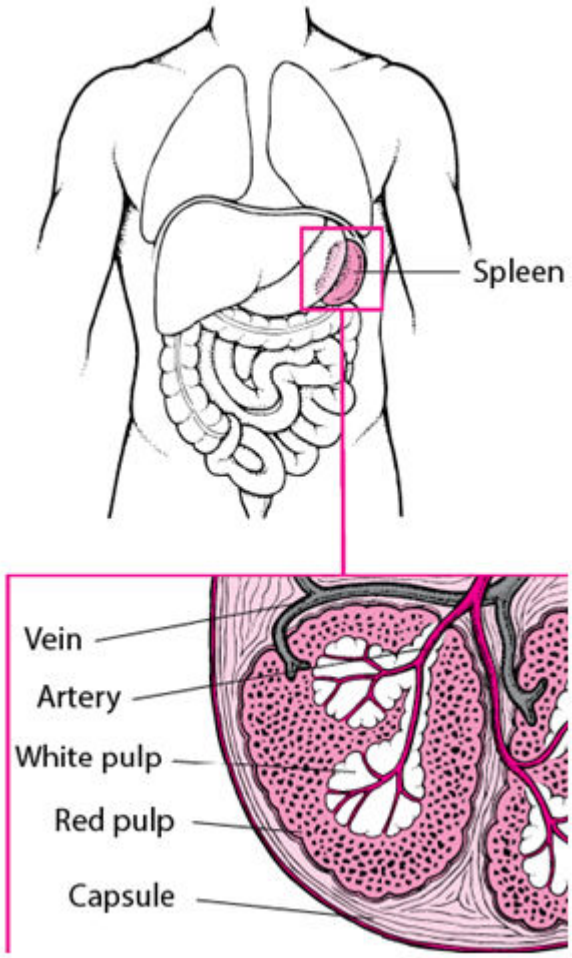


**Figure 6.** Histology of the blood.

**Filtering the blood:** The specialized structure of the venous system of the red pulp gives this area its unique capacity to filter the blood and remove old erythrocytes. Arterial blood arrives into cords in the red pulp, which consist of fibroblasts and reticular fibres and form an open blood system without an endothelial lining. In these cords, many macrophages are found.

From the cords, the blood passes into the venous sinuses of the red pulp, which collect into the efferent vena lienalis. These sinuses are lined by endothelium that has an unusual discontinuous structure, with stress fibres extending underneath the basal plasma membrane, running parallel to the cellular axis. The stress fibres connect the endothelial cells to components of the extracellular matrix and are composed of actin and myosin like filaments, indicating that there might be a sliding filament action by which the spaces between the endothelial cells are controlled. The arrangement of the stress fibres, together with the parallel arrangement of the endothelial cells of the sinuses, forces the blood from the cords into the sinuses, through the slits that are formed by the stress fibres. This passage becomes difficult for ageing erythrocytes, which have stiffening membranes, such that they stick in the cords and are phagocytosed by the red-pulp macrophages that are located in the cords. The contractility of the stress fibres might also aid in the retention of erythrocytes in the spleen (as has been observed in various mammals, such as dogs and horses), thereby forming a reservoir of erythrocytes and reducing stress on the heart by reducing the viscosity of the blood during rest.

**Recycling iron:** Erythrophagocytosis is important for the turnover of erythrocytes, and recycling of iron is an important task of splenic macrophages, in conjunction with those of the liver. Erythrocytes are hydrolysed in the phagolysosome of macrophages, from which haem is released after the proteolytic degradation of haemoglobin. Haem is then further catabolized into biliverdin, carbon monoxide and ferrous iron ($Fe^{2+}$), after which the iron is either released from cells or stored. Iron that is not used or released by a cell is stored as ferritin, which is a cytosolic protein. For the storage of larger amounts of iron in a cell, ferritin can aggregate into haemosiderin, which is an insoluble complex of partially degraded ferritin, deposits of which can easily be observed in red-pulp macrophages. Iron can be released from macrophages as ferritin or as low-molecular-weight species, and these rapidly bind plasma transferrin, which functions as a transporter protein. In addition to such phagocytosis of erythrocytes, a considerable proportion of erythrocytes are also destroyed intravascularly throughout the body, as a result of continuing damage to their plasma membrane. This leads to the release of haemoglobin 8, which is bound rapidly by haptoglobin. Receptor mediated endocytosis of CD 163, a haemoglobin specific receptor at the cell surface of macrophages, leads to scavenging of haemoglobin from the circulation in the spleen. The release of iron from its storage in splenic macrophages is regulated by the requirements of the bone marrow, but the underlying mechanisms are not well understood. Iron uptake by most cells is mediated by a pH-dependent transporter for divalent metals natural-resistance-associated macrophage protein 2 ($NRAMP_2$) which is found in transferrin receptor-positive recycling endosomes, where it mediates the transport of ferritin iron across the endosomal membrane into the cytoplasm 11. Interestingly, macrophages and monocytes express another NRAMP molecule, $NRAMP_1$. $NRAMP_1$ was originally found to be involved in the resistance of inbred mice to certain intracellular pathogens, and this turned out to result from the ability of this molecule to transport iron across the phagosomal membrane. Although there is some debate on the direction of this transport, the result is that there is interference with the iron metabolism of the bacterium, thereby limiting its growth. Interestingly, $NRAMP_1$ seems to result from a basic iron-transport mechanism being adapted to fight pathogens in specific cells that are already engaged in iron metabolism through erythrophagocytosis, thereby linking two important functions of the splenic red pulp (Figure 7).

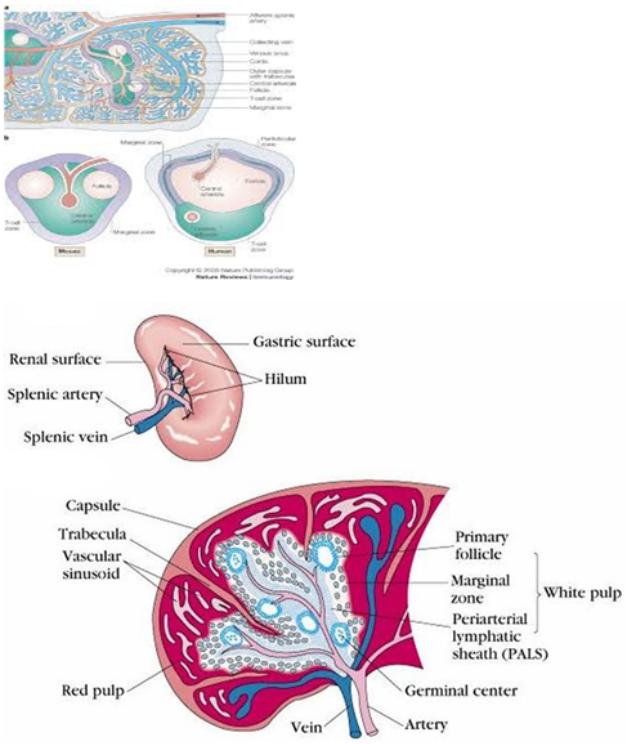


**Figure 7.** Red and white pulp of the spleen.

**Producing antibodies:** The red pulp is also known to be a site where plasmablasts and plasma cells lodge. After antigen specific differentiation in the follicles of the white pulp, plasmablasts migrate into the red pulp, initially just outside the marginal zone. However, the exact anatomical position and cellular interactions that are involved in the retention of these cells are not clear.

The position of plasmablasts in the red pulp resembles the localization of plasmablasts in the medullary cords of lymph nodes, and this extrafollicular antibody production leads to rapid entry of antibody into the bloodstream. Evidence indicates that plasmablasts are attracted to the red pulp after upregulating their expression of the chemokine receptor CXC chemokine receptor 4 ($CXCR_4$); this receptor binds the chemokine CXC-chemokine ligand 12 ($CXCL_{12}$), which is expressed in the red pulp. This coincides with downregulation of expression of the chemokine receptors $CXCR_5$ and CC-Chemokine Receptor 7 ($CCR_7$), which bind the homeostatic chemokines that are present in the B-cell follicles and T-cell zone of the white pulp. Interestingly, it has been found that plasmablasts require CD11 chi Dendritic Cells (DCs) to survive in the red pulp and to make the transition into plasma cells. The presence of CD11 chi DCs in the T-cell zone of the white pulp, as well as in the bridging channels that extend into the red pulp, might be of assistance in this transition. The bridging channels are where antibodyforming cells have been described to temporarily reside after antigenic challenge.

Organization of the lymphoid compartments In this section, we describe the structure of the lymphoid region of the spleen the white pulp with an emphasis on new insights into how the cells of the immune system migrate and lodge in the various compartments of the white pulp.

**White pulp:** The white pulp is organized as lymphoid sheaths, with T and B-cell compartments, around the branching arterial vessels, so it closely resembles the structure of a lymph node. The correct organization and maintenance of the white pulp is controlled by specific chemokines that attract T and B cells to their respective domains, thereby establishing specific zones within the white pulp. In the T-cell zone (also known as the periarteriolar lymphoid sheath, PALS), T cells interact with DCs and passing B cells, whereas in the B-cell follicles (also known as the B-cell zones), clonal expansion of activated B cells can take place, which leads to isotype switching and somatic hypermutation. $CXCL_{13}$ is required for B cells to migrate to the B-cell follicles 21, whereas CC-chemokine ligand 19 ($CCL_{19}$) and $CCL_{21}$ are involved in attracting T cells and DCs to the T-cell zones of the white pulp 22,23. Expression of these chemokines is controlled by lymphotoxin-$\alpha_1\beta_2$ (LT-$\alpha_1\beta_2$) and Tumour-Necrosis Factor (TNF). When signalling through the LT-β receptor (LT-βR) or TNF receptor 1 (TNFR1) is lacking, levels of the homeostatic chemokines $CXCL_{13}$, $CCL_{19}$ and $CCL_{21}$ are reduced in the spleen, which results in disorganization of white-pulp regions. Both LT-βR and $TNFR_1$ are expressed by radiation-resistant stromal cells, whereas their ligands are expressed by haematopoietic cells, most probably B cells, as occurs in RADIATION CHIMERAS. After engagement of these receptors, nuclear factor-κB becomes activated, resulting in induction of expression of the various chemokines (Figure 8).

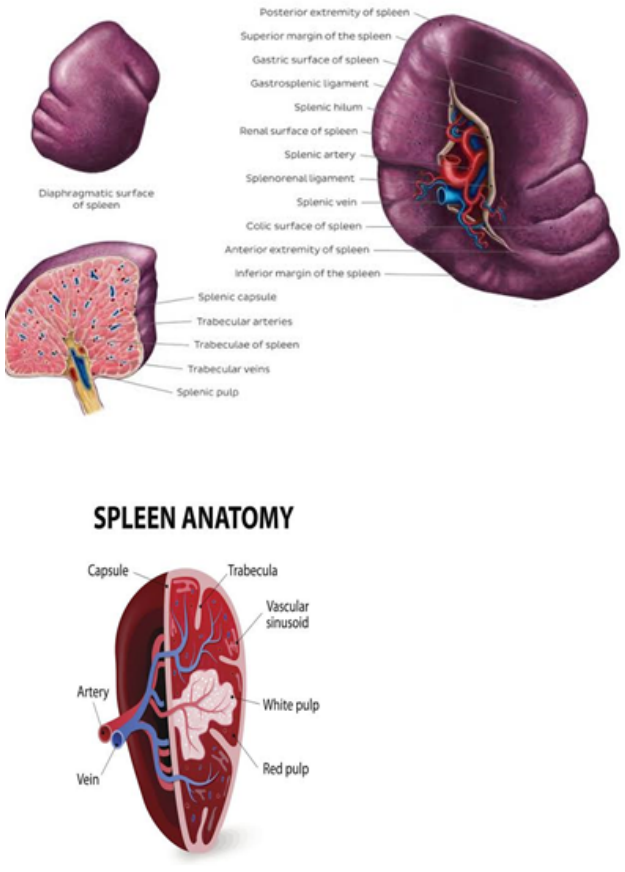


**Figure 8.** Spleen anatomy.

## Materials

Water trough, feeding trough, water bath, cover slip, cassette, glass slide, slide rack, slide box, microscope, electronic precision balance, mice feed, cages, needle and syringe, gloves, cotton wool, EDTA bottle, universal bottle.

## Drugs and reagents

Co-enzyme Q10 and 5 fluorouracil were procured from pharmacy stores, distilled water, haematoxylin and eosin stains.

## Animals

Healthy male swiss mice used in this study were obtained from the animal house of the Ladoke Akintola University of Technology Ogbomoso, Oyo State, Nigeria. Mice were housed in plastic cages measuring 12 × 9 × 6 inches (3 mice per cage) in a temperature-controlled (22.5°C ± 2.5°C) quarters with lights on at 7/00 am. Mice were allowed free access to food and water. All procedures were conducted in accordance with the approved protocols of the Ladoke Akintola University of Technology and within the provisions for animal care and use prescribed in the scientific procedures on living animals, European Council Directive (EU2010/63).

## Diet

All animals were fed the commercially available standard rodent chow (29% protein, 11% fat, 58% carbohydrate), At the beginning of the experimental period animals were either fed standard chow (29% protein, 11% fat, 58% carbohydrate) or co-enzyme Q10 incorporated into the standard diet at 100 mg/kg, 200 mg/kg and 400 mg/kg of feed which was administered ad libitum for a period of two weeks.

## Study site

This study was carried out in animal house of the department of medical laboratory science, ladoke akintola university of technology, Osogbo, Osun State. Haematology Laboratory of LAUTECH Teaching Hospital, Osogbo, Osun state. Histopathology Laboratory of LAUTECH Teaching Hospital, Osogbo, Osun state.

## Study design

The study is an experimental study design.

## Time frame

This project was completed within two (2) month.

## Experimental design

In this study, fifty (50) mice weighing 20 g-30 g was selected. The mice were randomly assigned into five (5) groups of 10 mice each. Mice were grouped as follows, normal control (standard diet plus intraperitoneal (i.p) normal saline), 5 fluorouracil (5FU) control (standard diet plus i.p 5 fluorouracil at 200 mg/kg body weight) and three groups of co-enzyme Q10 incorporated into standard diet at 100 mg/kg, 200 mg/kg and 400 mg/kg of feed. Weekly body weight change was assessed by weighing animals (7.00 am, before feeding) using a weighing balance (Mettler Toledo type BD6000, Greifensee, Switzerland). Animals received a single dose of 5FU while standard

diet, or CQ10 incorporated into standard diet was fed daily for a period of ten days. At the end of experimental period, mice were fasted overnight following which they were euthanized by cervical dislocation and blood collected *via* an intracardiac puncture for evaluation of haematological parameters. A midline abdominal incision was made, and the spleen dissected, observed grossly, weighed and fixed in 10% neutral buffered formalin. Sections of THE SPLEEN were processed for paraffin-embedding, cut at 5 µm and stained with haematoxylin and eosin for general histological study (Table 1).

| Group | Treatment | Inference |
|---|---|---|
| A | Mice+Normal feed. | Normal control |
| B | Mice+i.p of 5FU (200 mg/kg/day) single dose | 5FU control |
| C | Mice+i.p of 5FU (200 mg/kg/day) single dose+CoQ10 at 100 mg/kg of feed | Test |
| D | Mice+i.p of 5FU (200 mg/kg/day) single dose+CoQ10 at 200 mg/kg of feed | Test |
| E | Mice+i.p of 5FU (200 mg/kg/day) single dose+CoQ10 at 400 mg/kg of feed | Test |

I.p: intraperitoneal, 5FU 5 Fluorouracil, CoQ10: Coenzyme Q10.

**Table 1.** Experimental protocol.

### Determination of heamatological parameters

The automated haematological analyzer (SYSMEX) was used to analyze the haematological parameters such as red blood cell counts, packed cell volume, haemoglobin level, total and differential white blood cell count, platelet count, red cell indices (MCV, MCH, MCHC, RDW, PDW, PLCR). The analyses were carried out based on standard methods.

### Histopathological study

The spleen tissues from the experimental animals were fixed in 10% neutral buffered formalin. Representative samples were placed in cassettes and processed using the automatic tissue processor. After processing, the tissue were embedded in molten paraffin wax which formed a solid support for microtome after cooling. The tissue blocks were cut into thin sections of 4 micron using a rotary microtome, floated out and picked with the slides in a water bath. The slides were placed in an oven for drying where the sections adhered to the slides firmly. They were stained with Haematoxylin and Eosin (H and E) staining technique for general tissue morphology.

### Statistical analysis

Data was subjected to descriptive and inferential statistics and expressed as Mean ± SEM (standard error of mean), and between the test and control subjects, Tukey HSD was used to show significant difference using SPSS (statistical package for social sciences). Values of $p<0.05$ were considered significant (Figure 9).

## Results

### Effect of co-enzyme q10 on body weight in 5-fluorouracil induced haematoxicity

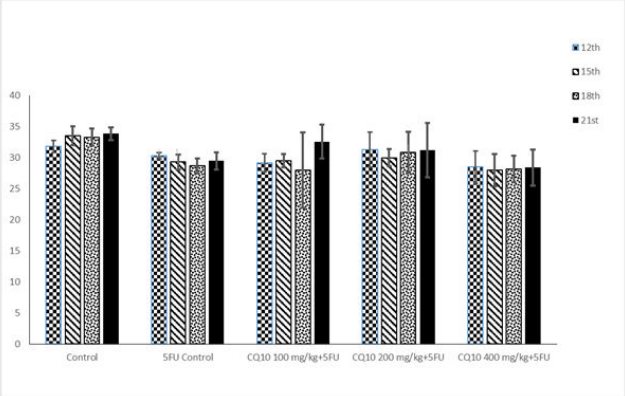


**Figure 9.** Effect of Coenzyme Q on bodyweight in 5 FU-induced haematoxicity.

Showed no significant difference in all groups.

### Effect of co-enzyme q10 on red cell parameters in 5-fluorouracil induced haematoxicity

The effect of coenzyme Q10 on erythrocyte indices including Packed Cell Volume (PCV), Red Blood Cell Count (RBC), Mean Corpuscular Volume (MCV) and Haemoglobin Concentration (HB). Total red blood cell count decreased significantly ($p<0.001$) with 5FU group and the groups fed with CQ10 diet at 100 mg/kg, 200 mg/kg and 400 mg/kg of feed compared to control. Compared to 5FU control there was a significant increase in RBC count with CQ10 at 100 mg/kg, 200 mg/kg and 400 mg/kg of feed. Haematocrit or Packed Cell Volume (PCV) decreased significantly ($p<0.001$) with 5FU group and the groups fed with CQ10 diet at 100 mg/kg, 200 mg/kg and 400 mg/kg of feed compared to control. Compared to 5FU control there was a significant increase in PCV with CQ10 at 100 mg/kg, 200 mg/kg and 400 mg/kg of feed. Mean Corpuscular Volume (MCV)

decreased significantly (p<0.001) with 5FU group and increased in the groups fed CQ10 diet at 200 and 400 mg/kg of feed compared to control. Compared to 5FU control there was a significant increase in MCV with CQ10 at 100, 200 and 400 mg/kg of feed. Mean corpuscular haemoglobin (MCH) decreased significantly (p<0.001) with 5FU group and the groups fed CQ10 diet at 100 mg/kg, 200 mg/kg and 400 mg/kg of feed compared to control. Compared to 5FU control there was a significant increase in PCV with CQ10 at 100 mg/kg, 200 mg/kg and 400 mg/kg of feed. Mean corpuscular haemoglobin concentration (MCHC) did not increase significantly in any of the groups fed CQ10 compared to normal or 5FU control. Haemoglobin concentration decreased significantly (p<0.001) with 5FU and the groups fed CQ10 diet at 100, 200 and 400 mg/kg of feed compared to control. Compared to 5FU control there was a significant decrease in HB with CQ10 at 100 mg/kg and an increase with CQ10 at 200 and 400 mg/kg of feed (Table 2).

| Groups | PCV (%) | RBC (x106/L) | MCV (fl/cell) | HB (g/dL) | MCH (pg) | MCHC (g/dl) |
|---|---|---|---|---|---|---|
| Control | 40.2 ± 0.07 | 7.12 ± 0.01 | 52.0 ± 0.04 | 11.10 ± 0.31 | 16.1 ± 0.01 | 30.11 ± 0.01 |
| 5FU Control | 23.3 ± 0.02* | 4.31 ± 0.01* | 49.0 ± 0.04* | 7.20 ± 0.11* | 15.2 ± 0.01* | 31.01 ± 0.01 |
| CQ10100 mg/kg +5FU | 27.1 ± 0.05*# | 4.60 ± 0.01*# | 52.04 ± 0.02# | 6.31 ± 0.22*# | 15.6 ± 0.01*# | 30.01 ± 1.01 |
| CQ10 200 mg/kg +5 FU | 26.30 ± 0.20*# | 4.50 ± 0.01*# | 54.64 ± 0.02*# | 7.70 ± 0.21*# | 15.8 ± 0.01*# | 30.11 ± 0.01 |
| CQ10400 mg/kg +5FU | 25.0 ± 0.01*# | 5.20 ± 0.01*# | 55.23 ± 0.03*# | 7.70 ± 0.20*# | 15.7 ± 0.01*# | 31.30 ± 0.01 |

Values are presented as mean ± SEM, *p<0.05 significant difference from control, number of animals per group=5, 5FU: 5Fluorouracil, CQ10: Co-enzyme Q10.

**Table 2.** Effect of coenzyme Q10 on erythrocyte indices in 5-fluorouracil induced toxicity.

## Effect of co-enzyme Q10 on white cell parameters in 5-fluorouracil induced haematoxicity

Table 3 shows the effect of coenzyme Q10 on white cell indices. Total white blood cell count increased significantly (p<0.001) with 5FU, and decreased with CQ10 diet at 100 mg/kg, 200 mg/kg and 400 mg/kg of feed compared to control. Compared to 5FU control there was a significant decrease in WBC count with CQ10 at 100 mg/kg, 200 mg/kg and 400 mg/kg of feed. Neutrophil Count (NC) increased significantly (p<0.001) with 5FU and CQ10 diet at 100 mg/kg, 200 mg/kg and 400 mg/kg of feed compared to control. Compared to 5FU control there was a significant decrease in NC with CQ10 at 100 mg/kg, 200 mg/kg and 400 mg/kg of feed. Lymphocyte Count (LC) decreased significantly (p<0.001) with 5FU and the groups fed CQ10 diet at 100, 200 and 400 mg/kg of feed compared to control. Compared to 5FU control there was a significant decrease in LC with CQ10 at 100 mg/kg, 200 mg/kg and 400 mg/kg of feed. Platelet count (PC) increased significantly (p<0.001) with 5FU and the groups fed CQ10 diet at 100 mg/kg, 200 mg/kg and 400 mg/kg of feed compared to control. Compared to 5FU control there was a significant increase in PC with CQ10 at 100 mg/kg and a decrease with CQ10 at 200 mg/kg and 400 mg/kg of feed (Table 3).

| Groups | WBC ($\times10^3$/L) | NC (%) | LC (%) | Platelet ($\times10^{12}$/L) |
|---|---|---|---|---|
| Control | 8.10 ± 0.10 | 28.0 ± 0.01 | 75.66 ± 0.32 | 728.11 ± 0.45 |
| 5FU Control | 9.33 ± 0.10* | 49.0 ± 0.01* | 52.23 ± 0.20* | 1520.00 ± 0.20* |
| CQ10 100 mg/kg+5FU | 7.43 ± 0.10*# | 45.0 ± 0.01*# | 56.10 ± 0.10*# | 1708.10 ± 0.13*# |
| CQ10 200 mg/kg+5FU | 7.30 ± 0.10*# | 44.0 ± 0.01*# | 57.05 ± 0.12*# | 1225.10 ± 0.40*# |
| CQ10 400 mg/kg+5FU | 7.12 ± 0.10*# | 41.0 ± 0.01*# | 59.10 ± 0.11*# | 1220.00 ± 0.45*# |

**Values are presented as mean ± SEM, *p<0.05 significant difference from control, number of animals per group=5, 5FU: 5-Fluorouracil, CQ10: Co-enzyme Q10.**

**Table 3.** Effect of coenzyme Q10 on leukocyte and platelet indices in 5-fluorouracil induced toxicity.

## Effect of co-enzyme Q10 on splenic morphology in 5-fluorouracil induced haematoxicity

Histological examination of haemtoxylin and eosin-stained sections of the spleen showed normal morphology for groups A. Slides for group B showed distortion of splenic architecture and fibrosis while slides for groups C, D and E showed varying degrees of amelioration of 5FU-induced myelofibrosis (Figures 10-14).

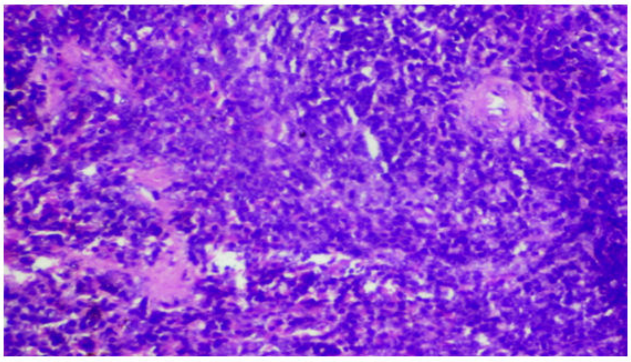

**Figure 10.** Normal control.

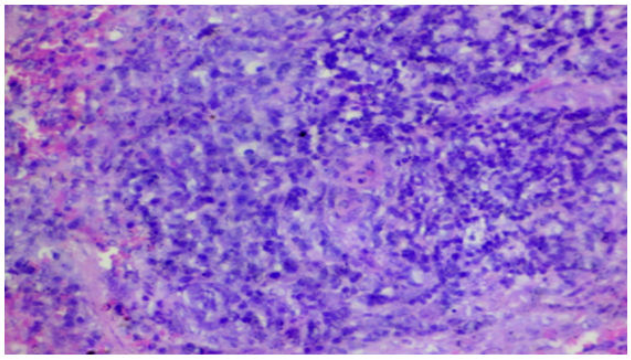

**Figure 11.** 5FU Control.

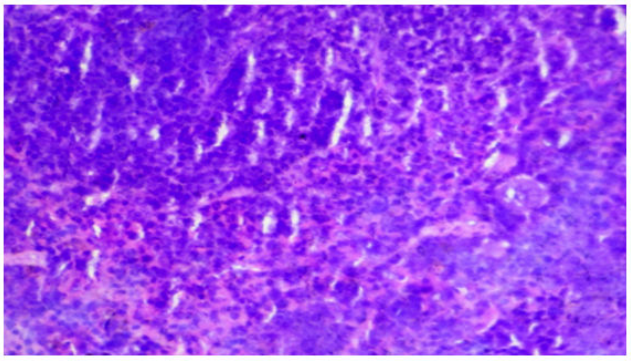

**Figure 12.** CQ10 100.

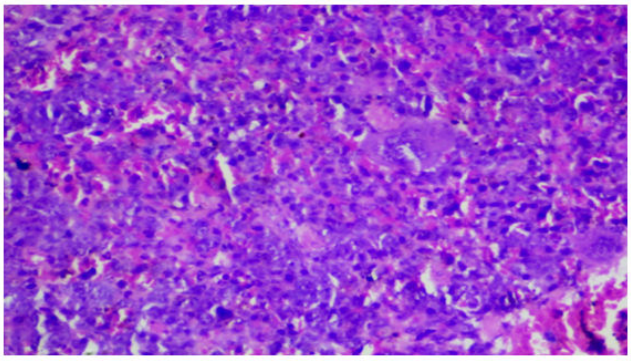

**Figure 13.** CQ10 200.

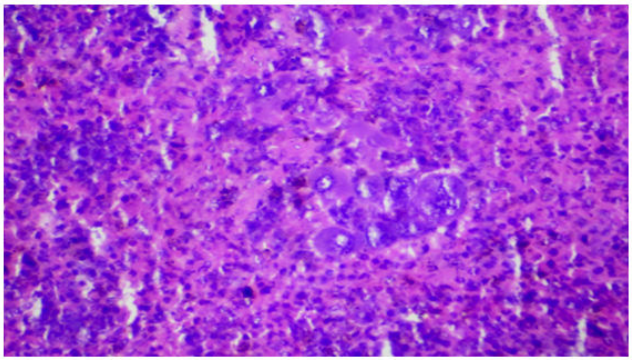

**Figure 14.** CQ10 400.

## Discussion

CoQ10, also known as Ubiquinone, is a fat-soluble, vitamin-like benzoquinone compound that is endogenously synthesized from tyrosine in the human body. It comprises a quinone group and a side chain of 10 isoprenoid units. Ubiquinol, the fully reduced form of CoQ10 is a good lipophilic antioxidant, capable of free radical neutralization and regeneration of the reduced form of vitamin E. It can also inhibit lipid peroxidation in biological membranes and protect mitochondrial proteins and DNA from oxidative damage. In fact, it is the only lipophilic antioxidant that can be de novo synthesized by cells and that has enzymatic mechanisms to regenerate its reduced form.

Being strongly bound to the inner mitochondrial membrane and participating in the electron transport chain and oxidative phosphorylation, it plays an essential role in the synthesis of cellular energy in the form of ATP. For this reason, it is found at higher concentrations in tissues with a high metabolic activity, such as heart, kidney, liver, and muscle. However, different factors such as genetics, aging, and statins treatment can lower its physiological concentrations. CoQ10 deficiencies have also been reported for conditions where oxidative stress plays a significant role, such as neurodegenerative disorders, diabetes, cancer, and cardiovascular diseases.

CoQ10 has been considered as a potential candidate for the treatment of various diseases. CoQ10 supplementation supports oxidative phosphorylation, cell signaling, and protects certain cell types. In addition, thanks to its strong antioxidant activity, it is also gaining popularity in the cosmetic industry.

One of the other important roles of CoQ10 is inhibit certain enzymes that plays in formation of free radicals and so attenuates oxidative stress.

5-FU is widely used in the treatment of a range of cancers, including colorectal and breast cancers, and cancers of the aerodigestive tract. 5-FU in combination with other chemotherapeutic agents improves response rates and survival in breast and head and neck cancers, it is in colorectal cancer that 5-FU has had the greatest impact.

5-FU-based chemotherapy improves overall and disease-free survival of patients with resected stage III colorectal cancer. Nonetheless, response rates for 5-FU-based chemotherapy as a first-line treatment for advanced colorectal cancer are only 10%-15%. The

combination of 5-FU with newer chemotherapies such as irinotecan and oxaliplatin has improved the response rates for advanced colorectal cancer to 40%-50%. However, despite these improvements, new therapeutic strategies are urgently needed.

### Effect of $C_OQ10$ supplementation on body weight in 5-Fluorouracil induced haematoxicity

Both the negative control and the treatments showed no significant difference with the positive control which shows that there was no effect on the weight of the rats.

### Effect of Coenzyme Q10 on haematological parameters in a mouse model of 5-Fluorouracil induced toxicity

The PCV and the RBC count showed a decrease in the negative control group when compared to the positive control while amelioration was seen in the treatments compared to the negative control. The cause of this decrease has been shown to be the pharmacological suppression of hematopietic stem cells thus decreasing the production of the erythrocytes. The 5-FU oxidative activity on bone marrow has been established in literature and the reduction in the blood cell production. COQ10 being a known strong antioxidant have the capacity to reverse this effect as it role in bone marrow has been well established in literature. The moderation of the effect was seen when the treatment was compared with negative control. On a long term study, the effect may be totally remediated. This imply that COQ10 may be a good agent in the treatment of chemotherapy induced anaemia.

Mean Corpuscular Volume (MCV), Haemoglobin (Hb) and mean corpuscular haemoglobin (MCH) showed a significant decrease ($p<0.001$), while mean corpuscular haemoglobin concentration (MCHC) showed no significant difference ($p>0.001$) in the negative control compared to the positive control. This shows the hematotoxic capacity of the chemotherapy in causing microcytic anaemia as observed in the work of Xu, et al., shortness of breath and fatigue.
The COQ10 amelioration was seen when compared to the negative control.

Summarily the effect of COQ10 on the blood parameters showed the potential in treatment of chemotherapy induced anaemias.

### Effect of Coenzyme Q10 on White Blood Cell parameters in a mouse model of 5-fluorouracil induced toxicity

There is a clear reduction in blood cells of patients treated with 5-fluorouracil, because the 5-FU drug is caused to suppress severe immunization caused by the reduction of white blood cells, in particular central and peripheral lymphocytes that are directly affected by the drug, which could lead to the result of the significance decrease of the number of white cells in the peripheral blood. Also, the drug affects in the lymphocytes type of T and B in the spleen and lymph glands. The results of the study were agreed with have shown that the cause of this decrease is due to the degree of suppression of bone marrow cells that resulting due to treatment with anti-cancer cells from the bone marrow stem cells which established the blood cells.

Platelet increase significantly ($p<0.001$) with 5FU group and also with group fed with CQ10 at 100 mg/kg, 200 mg/kg and 400 mg/kg. This is in contrary to the study as shown by Avraam, et al. Also the 5-FU drug have been shown to affect the Platelet Activating Factor (PAF), which has a role in the regulatory impact of the proliferation and differentiation the cells, as well as its presence in the spleen and thymus gland which has a regulatory role in the blood-forming organs so the low level of this factor by the drug 5-FU causes a decrease in the number of blood cells types.

### Histological findings

Histological examination of haemtoxylin and eosin-stained sections of the spleen showed normal morphology for groups A which is the normal control. The 5-fluorouracil control group showed distortion of splenic architecture and fibrosis while the group fed with CQ10 at 100 mg/kg, CQ10 at 200 mg/kg and group fed with CQ10 at 400 mg/kg showed varying degrees of amelioration of 5FU induced myelofibrosis.

Meanwhile, this research is subjected to further work in other to fully understand the effect of $C_OQ10$ on splenic morphology.

Numerous original research articles have focused on the topic of whether supplemental antioxidants administered during chemotherapy can protect normal tissue without adversely influencing tumor control. Due to variation in study design, intervention protocol, cancer type, timing of observation, inclusive criteria, statistical analysis, chemotherapy regime develops uncertainty to make definitive conclusion regarding the risk of decreased tumor control as a consequence of administering supplemental antioxidant during chemotherapy. On the contrary certain recent review definitely concludes that that antioxidant when given concurrently,

- Do not interfere with chemotherapy.
- Enhance the cytotoxic effect of chemotherapy.
- Protects normal tissue.
- Increases patient survival and therapeutic response.

## Conclusion

The most significant finding in this study revealed that COQ10 as an antioxidant was able to curate the effect of 5-fluorouracil which cause the release of free radicals on cells and ultimately bringing it to normal. CoQ10 has been considered as a potential candidate for the treatment of various diseases. CoQ10 supplementation supports oxidative phosphorylation, cell signaling, and protects certain cell types. In addition, thanks to its strong antioxidant activity, it is also gaining popularity in the cosmetic industry.

## Recommendation

Further experiments are needed to better understand the effect co-enzyme Q10 dietary supplement on haematological parameters and splenic morphology in mouse model of 5-fluorouracil induced toxicity.

## Dedication

I dedicate this project to almighty god, the alpha and omega, the one who makes everything beautiful in his own time, I thank him for his great grace and success over this work.

Also to my ever supportive parent Mr and Mrs Akanmu, I want to say a big thank you.

## Acknowledgement

I thank the Almighty GOD, the one who was, who is and is to come, I appreciate my maker for the enablement granted unto me to begin and finish this journey.

My sincere gratitude goes to my awesome supervisor, Dr. O.J Onaolapo for his love, time and support even amidst his very tight schedule, which led to the success of this project. Thank you Sir for all that GOD has done through you. May Almighty God bless and reward you abundantly.

My unreserved appreciation goes to my ever loving Parents, Mr. J.A Akanmu and Mrs. T.A Akanmu. Thank you for your encoursgement since the journey started, I am grateful, May all your labor over us not be in vain in Jesus name. (AMEN). I will also like to appreciate a father figure in this journey in person of Dr. O.S Bolaji, thank you for believing so much in me, I really appreciate all you do, and great is your reward. Also to my siblings and Uncle, thank you all for your prayers, financial gifts, moral support and encouragement.

My profound gratitude also goes to the head of department, Dr. S. A. Nassar, the entire teaching and non-teaching staff of this great department for their impact towards the successful completion of this project.

Special thanks to the scientists of ladoke akintola university of technology teaching hospital, Osogbo, you have been a great support and also helping us see what this profession entails, thank you for giving your best. Also to my project partners; timilehin, bukola and nnamdi i say thanks for your positive attitude towards the success of this work. Also my Bestfriends, Janet, opeyemi and others you all are definition of a true friend, thank so much, GOD bless you.

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**How to cite this article:** Akanmu oluwatosin, Ayomiposi. "The Effect of Dietary Co-enzyme Q10 Supplementation on Haematological Parameters and Splenic Morphology in Mouse Model of 5-Fluorouracil-Induced Toxicity." *J Biomed Pharm Sci* 5 (2022): 385.