Hepatoprotective Effect of Vitamin C (Ascorbic Acid)

Elias Adikwu1, Oputiri Deo2

1Department of Pharmacology and Toxicology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Choba, Nigeria; 2College of Health Technology, Otuogidi-Ogbia, Nigeria.

Email: adikwu_elias@gmail.com

Received October 25th, 2012; revised November 27th, 2012; accepted December 17th, 2012

ABSTRACT

Human and animal studies have shown that some drugs and chemical agents have potential hepatotoxic effects. The hepatotoxic effect of drugs and some chemical agents is reported to be associated with the generation of reactive oxygen species (ROS). These ROS are reported to be associated with lipid peroxidation in the liver. This mechanism has led to continuous evaluation of the hepatoprotective effect of antioxidants in humans and animals. Among the antioxidants been evaluated is vitamin C which is a water soluble antioxidant. Reports have linked vitamin C with hepatoprotective property in animals and humans. It synergistic hepatoprotective effect with other antioxidants was also reported. Due to these reports a comprehensive literature review on the hepatoprotective property of vitamin C in humans and animals was performed. It was observed that vitamin C exhibited a reputable hepatoprotective effect in humans and animals. Research showed that vitamin C inhibited hepatotoxicity induced by drugs, heavy metals, organophosphate insecticides and some chemical agents. Vitamin C was reported to normalized levels of serum alanine aminotransferase, aspartate aminotransferase, gamma glutamine, alkaline phosphatase, lactate dehydrogenase and malondialdehyde and serum bilirubin in intoxicated animals. It potentiates the activities of free radical scavengers, superoxide dimutase, and catalase glutathione peroxidase thereby preventing microsomal lipid peroxidation, liver fibrosis, liver necrosis and hepatic inflammation. In humans vitamin C was reported to be beneficial in non alcoholic steatohepatitis and in patients with fatty liver disease. Hepatoprotective property of vitamin C is attributed to it antioxidant property. Vitamin C (ascorbic acid) which is a major water-soluble antioxidant is believed to decrease lipid peroxidation either directly or indirectly by regenerating vitamin E. Vitamin C is an important free radical scavenger in extracellular fluids, trapping radicals and protecting biomembranes from peroxide damage. Vitamin C effectively scavenges singlet oxygen, superoxide, hydroxyl, water soluble peroxyl radical and hypochlorous acid. It is also reported to be an excellent source of electrons and therefore can donate electrons to free radicals such as hydroxyl and super oxide radicals and quench their activity. Vitamin C is an essential co-factor involved in many biochemical functions and acts as an electron donor or reducing agent. In this review it is observe that vitamin C has hepatoprotective effect which increases when co administered with other agents precisely antioxidants.

Keywords: Vitamin C; Hepatoprotection; Antioxidant

1. Introduction

Vitamin C was discovered by Szent-Gyorgyi (1928) [1]. It is a six-carbon compound structurally related to glucose, consisting of two inter-convertible compounds: L- ascorbic acid, which is a strong reducing agent, and its oxidized derivative, L dehydroascorbic acid. Vitamin C is found in citrus, soft fruits and leafy green vegetables. Kidney and liver are good animal-derived sources of vitamin C [2]. Vitamin C can be administered orally or intravenously [3]. It is well absorbed efficiently in the small intestine via a saturable active transport mechanism. Absorption efficiency of low oral doses of vitamin C (4 - 64 mg) may be as high as 98%, but decreases with increasing doses of the vitamin C. Vitamin C is widely distributed in all tissues of the body, with higher levels found in the adrenal glands, pituitary and retina. Kidney and muscle tissues have lower level of vitamin C [4]. Vitamin C is oxidized to dehydroascorbic acid, which is hydrolysed to diketogulonic acid and then oxidized to oxalic and threonic acid [5]. Oxidation to carbon dioxide occurs at high doses of vitamin C. Unmetabolized vitamin C and vitamin C metabolites, such as oxalate are largely excreted in the urine. Approximately 3% of a 60 mg oral dose is excreted in feces. More of the vitamin is excreted unchanged at higher levels of vitamin C intake [6,7]. Available data suggested that vitamin C is not associated with significant adverse effects and there are no obvious specific key toxic endpoints for vitamin C dose given orally to healthy subjects. But high oral doses of
vitamin C were reported to be associated with gastrointestinal effects like abdominal distention, flatulence, diarrhea and transient colic [8]. Vitamin C supplementation may be associated with an increased risk of calcium oxalate renal stones [9]. Vitamin C is hydrophilic and is an important free radical scavenger in extracellular fluids, trapping radicals and protecting biomembranes from peroxide damage. Vitamin C effectively scavenges singlet oxygen, superoxide, hydroxyl, water soluble peroxyl radical and hypochlorous acid [10]. It is also reported to be an excellent source of electrons and therefore can donate electrons to free radicals such as hydroxyl and super oxide radicals and quench their activity [11]. Vitamin C is an essential co-factor involved in many biochemical functions and acts as an electron donor or reducing agent. Vitamin C has been reported by researchers to have hepatoprotective property. This is said to be associated with its antioxidant property [12-15].

2. Hepatoprotective Effect of Vitamin C (Ascorbic Acid)

Reported researches showed that vitamin C has hepatoprotective property. This is linked to its antioxidative property. Vitamin C was reported to attenuate hepatic damage induced by some chemical agents especially in animals. This is supported by the work of Bashandy and Alwasel, (2011) [16]. They reported that vitamin C normalized levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, blood hydroperoxide and malondialdehyde in liver of carbon tetrachloride intoxicated rats. The ability of vitamin C to prevent Carbon tetrachloride induced hepatotoxicity in rats was also reported by some authors [17,18]. Ascorbic acid reduced cypermethrin induced Cytotoxicity in rat hepatocytes by recovering 60% of glutathione and 54% decrease in gamma glutamyl transpeptidase. Ascorbic acid was also able to preserved 100% of cell integrity and modulated alanine aminotransferase and aspartate aminotransferase [19]. Similar observation was reported when deltamethrin (1.28 mg/kg) was administered to wistar rats. Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and gamma glutamyl transpeptidase were significantly increased. Pretreatment with vitamin C (200 mg/kg) normalized the above mentioned parameters [20]. The hepatoprotective effect of vitamin C against organophosphate induced liver damage can be further buttress by the findings of Mossa et al. (2011) [21]. He and his co-researchers exposed rats to a mixture of organophosphate insecticides for 28 days. This induced liver toxicity via elevated levels of serum aspartate aminotransferase, Alanine aminotransferase, alkaline phosphatase and disruption of liver structure. Vitamin C supplementation was reported to attenuate the hepatotoxic effects induced by the mixture of organophosphate insecticides.

Similar observation was reported by Amballi et al. (2010) [22]. He and his co-workers evaluated the effect of vitamin C on short-term hematological and biochemical alterations induced by acute chlorpyrifos in wistar rats. They reported that vitamin C mitigated alterations in serum biochemical parameters via the normalization of alanine amino transferase, aspartate aminotransferase and alkaline phosphatase. Ozturk and his co-workers also reported the protective effect of ascorbic acid against hepatotoxic and oxidative stress caused by carbon tetra-chloride in the liver of wistar rats [23]. These observations are contrary to the work of Kamel et al. (2012) [24]. They reported that Ascorbic acid was ineffective against the elevation of enzymes leakage (alanine aminotransferase and aspartate aminotransferase) induced by Carbon tetrachloride in liver cells from BRL3A cell line. Al-Attar (2011) [25], also evaluated the hepatoprotective effect of vitamin C on theoacetamide (organosulphur) induced liver cirrhosis in wistar rats. Chronic administration of theoacetamide for 10 weeks increased liver transaminases. Liver histopathology showed centrilobular necrosis, hepatic cell degeneration and necrosis with loss of nucleus. The administration of vitamin C attenuated theoacetamide induced hepatotoxicity via normalization of biochemical and histopathological changes induced by theoacetamide. Fenvalerate an insecticide (20 mg/kg) was reported to induce liver toxicity in rats. Pretreatment with vitamin C 20 mg/kg for 30 days restored to near normal fenvaterate induced liver toxicity [26]. Similar effect was observed with Pretreatment using vitamin C against imidacloprid (systemic insecticide) induced oxidative stress in mice liver. It was observed that vitamin C pretreatment gave a better protection than post-treatment [27]. This agreed with the report of Omiami (2004) [28], who observed mild recovery in the liver of Japanese quail treated with vitamin C and imidacloprid. Supplementation with vitamin C, 25 mg/100gram body weight for 3 days in male guinea pigs ameliorated ethanol induced hepatotoxicity [29].

Some metals and heavy metals like copper, lead, chromium and cadmium are known to induce hepatic damage in animals and humans [30,31]. Some researchers have shown that vitamin C attenuated hepatic damage induced by these heavy metals. One of these researches was published by Banerjee and co-researchers. They reported that ascorbic acid combated arsenic induced oxidative stress in mice liver [32]. El-Demedash and his co-workers also posited that vitamin C ameliorated stannous chloride induced toxicity in the liver of male rabbits. Among other effects vitamin C decreased levels of free radicals and improved liver architecture in stannous chloride treated rats [33]. This agreed with the work of Yousef et al. (2007) [34]. He and his friends reported the protective effect of ascorbic acid against stannous chloride induced
toxicity in rabbits. It was also reported that prophylactic use of vitamin C gave hepatoprotection against lead induced liver toxicity [35].

Furthermore administration of sodium fluoride 10 mg/kg body weight and Aluminum chloride 200 mg/kg for 30 days decreased levels of glutathione, ascorbic acid and glutathione peroxidase in the liver of mice. Complete recovery occurred in all parameters after pretreatment with ascorbic acid [36]. Similar observation was reported by other scholars [37,38]. The report of Krishnamoorthy and Sangeetha gave credence to the hepatoprotective effect of vitamin C against metallic compounds induced liver toxicity. They found out that the co-administration of 300 mg/kg of sodium nitrate and 300 mg/kg of vitamin C ameliorated sodium nitrate induced lipid peroxidation in the liver of albino rats [39].

Drugs are chemical agents used for the treatment of ailments. Some drugs are known to have hepatotoxic effects in human and animals. Vitamin C has shown tremendous protective effect against drugs and chemical agents induced hepatotoxicity [40,41]. One of these reports was published by Awodele and Co-researchers. They observed that administration of vitamin C 8 mg/kg to rats for 90 days gave relative protection against rifampicin induced hepatotoxicity [42]. Also in the evaluation of the protective role of vitamin C on acetaminophen induced hepatotoxicity in mice, it was observed that ascobate esters showed protective effect against acetaminophen induced hepatotoxicity in mice [43]. Similar finding was reported by Peterson and Knodell in (1984) [44]. In their work they observed that ascorbic acid ameliorated acetaminophen and cocaine induced hepatic damage in mice. This result raised the possibility that ascorbic acid may be useful in preventing hepatic injury caused by some hepatotoxic drugs.

The hepatoprotective effect of vitamin C against drug induce hepatic damage was also reported by Remiao et al. (2004) [45]. He and his co workers reported that preincubation of freshly isolated and prepared rat hepatocytes suspension with ascorbic acid reverted N-methly-alpha-methyladopamine induce hepatotoxicity. The protective effect of vitamin C against drug induce liver damage was further evaluated by Ergul and Co-researchers. They evaluated the effect of vitamin C on oxidative liver injury induced by isoniazid in rats. They found out that isoniazid induced liver injury is associated with oxidative stress and pretreatment with vitamin C reduced liver damaged induced by isoniazid in rats [46]. Pretreatment with vitamin C normalized aspartate aminotransferase and alkaline phosphatase in halothane induced hepatotoxicity [47].

Research showed that pretreatment with 50, 100 and 200 mg/kg of vitamin C attenuated carbamazepine (50 mg/kg) provoked hepatotoxicity in rats [48]. Vitamin C was also reported to prevent oxidative stress in rats’ liver induced by cisplatin [49]. This is supported by the evaluation of the prophylactic effect of vitamin C on cyclosporine-A induced liver toxicity in rats [50].

Cold ischemia and reperfusion of the liver may cause hepatic damage. Reports showed that vitamin C can mitigate cold ischemia and reperfusion induced liver damage. This can be seen in the findings of Park and Lee. They reported that cold ischemia/reperfusion of the liver elevated portal pressure, lactate dehydrogenase and purine nucleoside phosphorylase activities. These changes were attenuated at ascorbic acid concentration of 0.25 and 0.5 mm. Increase in lipid peroxidation and mitochondrial swelling were prevented by 0.5 mm of ascorbic acid. But these changes were augmented at higher dose (2 mm) of ascorbic acid [51].

Similar observation was reported by Seo and Lee, (2002) [52]. They posited that ascorbic acid acts primarily as an antioxidant in hepatic warm ischemia/reperfusion at low doses but as a prooxidant at high doses. It was also reported that ascorbic acid 2-glucoside prevented sinusoidal endothelial cell apoptosis in supercooled preserved grafts in rat liver [53]. Some scholars reported similar observations [54,55].

Dietary vitamin C supplement may have protective effect on the liver and also improve hepatic function as reported by some researchers. This is supported by findings from the evaluation of vitamin C on lipid peroxidation and glutathione system in the normal guinea pig heart. It was observed that dietary vitamin C supplementation is able to increase global antioxidant capacity of guinea pig heart tissues [56]. Dietary vitamin C supplement was also reported to confer protective effect against endotoxin induced oxidative damage to protein in guinea pig liver. This seems mainly due to a direct increase in hepatic ascorbate levels in vitamin C exposed animals [57]. Dietary vitamin C supplement was reported to decrease endogenous protein oxidative damage and lipid peroxidation in the guinea pig liver [58]. Further study showed that administration of monosodium glutamate (food additive) at a dose level of 0.6, 6 and 60 mg/kg for 14 days increased serum alanine aminotransferase and aspartate aminotransferase dose dependently. These elevated parameters were reduced after pretreatment with vitamin C. Histopathological changes induced by monosodium glutamate were also ameliorated [59].

In 1960, Calleja and Brooks reported that acute hepatitis was treated with high doses of vitamin C. They further explained that the beneficial effects of vitamin C were observed in 63 cases of hepatitis treated with high doses of vitamin C (10 grams) daily for 5 days administered rectally or intravenously. The appearance of urobilinogen in the urine was reduced by 50% [60].

The protective effect of vitamin C on the liver was
also studied by Willis in 1957. He found that vitamin C reversed fatty degeneration and acute non-fatty hepatocellular degeneration and promoted the laying of reticulum and collagen in the liver of scorbutic guinea pig [61].

3. Synergistic Hepatoprotective Effect of Vitamin C with Other Agents

Vitamin C was reported to form synergy with other agents. One of the visible synergies is between vitamin C and vitamin E. This is supported by the co-administration of vitamin C 200 mg and E 200 mg as food supplement administered for 7 days to ethanol intoxicated rats. This ameliorated ethanol induced hepatotoxicity via normalization of transaminases and inhibition of lipid peroxidation. Ethanol induced histopathological changes were also reversed [62]. This agreed with the report of Ozdil (2004) [63]. He discovered that co-administration of ascorbic acid, alpha tocopherol acetate and sodium selenate ameliorated ethanol induced liver damage in rats. Co-administration of vitamin C, vitamin E and sodium selenate 250, 250 and 0.5 mg/kg respectively for 30 days also prevented ethanol induced liver injury. This manifested through normalization of the functions of parameters like alanine aminotransferase, aspartate aminotransferase and some endogenous antioxidants [64].

*Shalan et al. (2007)* [65] reported that treatment of male rabbits with vitamin C (1 mg /100g body weight) and vitamin E (1 mg/100g body weight) for 2, 4, 6 and 8 weeks ameliorated ethanol induced hepatomegaly, apoptotic DNA fragmentation in hepatocytes, normalized alanine aminotransferase and aspartate aminotransferase activities.

Oral pretreatment of rats with vitamin C (0.02 g/100g bwt) and vitamin E 120 mg/kg 15 minutes prior to daily nolvadex administration was reported to ameliorate histopathological, histochemical and ultrastructural changes induced by nolvadex in the liver of rats [66].

This research is in agreement with the findings of *Soylu* and co-researchers who evaluated the antioxidants effect of vitamin E and C in hepatic fibrosis in biliary - obstructed rats. They reported that vitamin C and E at a dose level of 10 mg/kg and 15 mg/kg coadministered retarded hepatic fibrosis in biliary obstructed rats [67].

In 2003, *Olivera* and his colleagues studied the role of vitamin C and E in the prevention of non alcoholic fatty liver disease in choline deficient diet fed rats. They induced fatty liver disease in wistar rats using choline deficient diet. Administration of vitamin C 30 mg/kg/day orally reduced oxidative stress and inhibited the development of liver steatosis in these animals [68]. The report of Olivera and his co-workers agreed with the findings of some researchers [69].

More insights on the synergistic hepatoprotective effect of vitamin C and vitamin E was reported by *Tawfik and Al-badr* (2012) [70]. They evaluated the protective effect of vitamin C and E on the adverse effects of monosodium glutamate on liver and kidney functions in adult rats. They observed that 0.6 and 1.6 mg/kg of monosodium glutamate impaired hepatic function in treated rats which was ameliorated after pretreatment with vitamin C (0.3 mg/kg) and vitamin E (0.2 mg/kg). Combined antioxidant effect of vitamin C and E demonstrated cytoprotective effect against 4-ene valporic acid induced injury in cellular glutathione depleted hepatocytes [71].

Treatment with vitamin C and E also reduced methidathion (organophosphate insecticide) induced toxicity in rat liver. Elevated biomarkers were normalized by vitamin C and vitamin E [72]. In an histological examination of the protective effect of vitamin E and vitamin C in Cisplatin induced hepatotoxicity. Coadministered vitamin E 5 mg/kg and vitamin C 8 mg/kg to rats for 3 months reduced cisplatin induced hepatotoxicity [73].

*Uboh* and others investigated the effect of vitamin C and E against gasoline vapour-induced liver injury in rats. They reported that gasoline vapour caused a significant increase in serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and degenerative changes in structural architecture of the liver tissue. They further stated that administration of vitamin C and E normalized these changes [74].

Significant decrease in serum transaminases and serum bilirubin was observed in dimethoate intoxicated rats. Administration of combined dose of vitamin E, 200 mg kg$^{-1}$ day$^{-1}$ and vitamin C 200 mg kg$^{-1}$ day$^{-1}$ to the guinea pigs ameliorated dimethoate induced hepatotoxicity [75].

The synergistic effect of vitamin C and other antioxidants was further evaluated by Balasundaram (2010) [76]. He was able to show that administration of vitamin A (40,000 and 50,000 iu), L ascorbic acid (500 and 1000 mg) and vitamin E succinate (200 - 500 mg) synergistically reduced the amount of azo-dye binding protein in the liver of rats treated with p-dimethylaminobenzene.

Reports showed that vitamin C and some agents’ synergistically normalized diabetes induce hepatotoxicity. This can be seen from the work of Eze and others. They reported that Co-administered vitamin C and Zinc 100 and 50 mg/kg respectively restored alanine aminotransferase and aspartate aminotransferase function in diabetes induced hepatotoxicity [77]. This showed that vitamin C and Zinc may play an important role in the prevention of hepatocellular injury that may occur in diabetes. This is further supported by the findings of Hamden *et al.* 2009 [78]. He and co-researchers showed that vitamin C co-administration with vitamin E ameliorate oxidative stress, pancreatic and hepatic injury in alloxan diabetic rats.

The synergistic effect of vitamin C and Zinc was further evaluated in animals by Samir and his colleagues.
They clearly stated that co-administration of vitamin C and Zinc during nickel intoxication reversed nickel induced oxidative stress in the liver of rats [79]. Similar synergistic effect between vitamin C, vitamin E and L-methionine was observed against lead induced oxidative stress in the liver of rats [80,81].

Vitamin C, in combination with silymarin, has also been shown to effectively reduce the hepatotoxic effect of acute lead poisoning [82-84]. In an animal study using toxic amounts of lead (500 mg/kg diet), vitamin C (1 mg/100g body weight) and silymarin (1 mg/100g body weight) were supplemented in an attempt to inhibit genetic damage to hepatocytes and halt the onset of acute hepatitis. The combination of vitamin C with silymarin significantly normalized alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transpeptidase, alkaline phosphatase and mitigated histopathological liver damage [85].

This is in agreement with the findings of flora and other workers who observed that 250 mg/kg of vitamin C and dimercaptosuccinic acid conferred protection against lead impaired hepatic function [86]. Wang and coworker in 2007 also evaluated the protective effect of Ascorbic acid and thiamine supplementation at different concentrations against lead induced toxicity in mice. They reported that administration of vitamin C and thiamine inhibited lead acetate induced apoptosis in mice liver.

In humans it was also reported that vitamin E formed synergistic hepatoprotective effect with some chemical agents. This can be seen from a placebo controlled double blind study by Harrison and colleagues who randomized 49 patients with biopsy-proven non alcoholic steatohepatitis. These patients received vitamin E 1000 IU qd, vitamin C 100 mg qd and placebo for 6 months. Biopsy revealed statistical significant improvement in score although inflammation grade remained unchanged [87].

Furthermore twenty eight patients were treated with vitamin E plus vitamin C while 29 patients received Ursodeoxycholic acid. It was found that vitamin E and C combination is effective in patient with non alcoholic fatty liver disease and is as effective as Ursodeoxycholic acid. It was concluded that vitamin E and C combination is safe, inexpensive and an effective treatment option for patients with fatty liver disease [88]. In a retrospective single centre study of 68 patients with non alcoholic steatohepatitis 38 patients were administered vitamin E (400 - 800 IU/day) and vitamin C 500 - 1000 mg/day while 30 patients served as control. It was observed that vitamin E and C produced significant improvement in aminotransferase [89].

Treatment of homozygous beta-thalassemic children with combined dose of vitamin C, E and A for 6 and 12 months led to improvement in liver function status of these patients [90]. Co-administration of vitamin C 1 g, atorvastatin 20 mg and vitamin E 1000 IU in human subjects was reported to reduce the odds of having hepatic steatosis by 71% in healthy individuals with non-alcoholic liver disease at baseline of 4 years of active therapy [91]. The hepatoprotective effect of vitamin C is said to be associated with it oxidative property. Vitamin C is a water-soluble antioxidant which decreases lipid peroxidation either directly or indirectly by regeneration vitamin E, the major lipid-soluble antioxidant [92]. Vitamin C was also reported to scavenge aqueous reactive oxygen species (ROS) by rapid electron transfer that inhibits lipid peroxidation [93]. In this work it is observed that vitamin C may have hepatoprotective effect. This hepatoprotective effect tends to increase synergistically when co administered with other agents precisely antioxidants.

REFERENCES

Hepatoprotective Effect of Vitamin C (Ascorbic Acid)

89


Hepatoprotective Effect of Vitamin C (Ascorbic Acid)


M. G. Shalana, M. S. Mostafa and M. M. Hassoua,


