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Effect of Vitamin C Supplementation on Serum Ascorbic Acid Level and Liver Function Profile in Healthy Individuals

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

Article Information

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Original Research Article

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ABSTRACT

This study was conducted to explore the effects of ascorbic acid supplementation on serum liver function tests in healthy individuals. A total of 200 subjects were selected randomly. 100 were given ascorbic acid supplementation for 30 days. The other 100 were not given ascorbic acid supplementation, and serum ascorbic acid level and liver function profile was observed before and after intake of ascorbic acid in group A and without intake in group B. The liver function parameters determined were aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), serum total bilirubin, direct bilirubin, indirect bilirubin and serum protein (total protein, albumin and globulin). These parameters along with serum ascorbic acid were measured before and 30 days after vitamin C supplementation. Various parameters of liver function profile were improved swiftly when compared to other group which was not given ascorbic acid supplementation. While comparing the two treatment groups for 30 days, statistically significant improvement was seen in serum ascorbic acid levels (p<0.001) along with improvement in some components of liver function profile such as serum ALT (p<0.01), AST (p<0.01), Total Bilirubin (p<0.01) and Direct bilirubin (p<0.001), Total Proteins (p<0.01) and Albumin (p<0.001) in group A as compared to Group B (without vitamin C supplementation intake). Conclusively, Liver Functions were significantly improved with vitamin C supplementation, giving the supportive evidence of the use of vitamin C as an antioxidant.

aminotransferases: serum bilirubin.

ABBREVIATIONS

ALT	:	Alanine aminotransferase
AST	:	Aspartate aminotransferase
ALP	:	Alkaline phosphatase
ATP	:	Adenosine tri phosphate
ug /ml	:	Microgram/milliliter
mm	:	Millimeter
mmol/l	:	Millimoles/liter
Vit C	:	Vitamin C
W.H.O	:	World Health Organization
gamma-G	Т:	Gamma-glutamyl transferase

1. INTRODUCTION

1.1 Overview of Liver Function Profile

The physiology of the liver contains basic contents such as its metabolism, excretion, and body defense. In terms of cellular functioning, the liver is the elementary place of multiple biochemical responses which are very essential to the human metabolic functions such as synthesis, degradation, transformation and biotransformation of certain substances or other biomolecules. Gowda et al.[1] The relationship between structure and functions of specific hepatic processes which control normal liver activities under normal conditions is essential for the understanding of liver immune responses to observe clinical diseases distressing the liver normal physiology. The overview of hepatic physiology underline some of the common facts of regular hepatic anatomy and functions of different liver function tests in relation to liver abnormalities and its diverse extents necessary for treating liver abnormalities in various diseases for patient's benefit. Corless and Middleton [2].

1.2 Liver Function Profile and Their Importance

Liver function tests are particular biochemical markers which reveal various liver diseases. The assessment of different abnormalities related to liver functions as observed commonly are aminotransferases (diagnostic liver function enzyme) as isolated due to abnormal enzymatic activity in conjugation with other parameters: the proportion alteration in liver function tests helps in the diagnosis of unpredictable disease. An isolated liver function test is of little role in selection of liver disease because many harmful

Keywords: Liver function tests; ascorbic acid; supplementation; serum proteins; serum

liver diseases may be correlated with normal levels of liver function tests. The outline of enzyme abnormalities in the perspective of patient's commonly observed symptoms and laboratory data might be helpful in directing the subsequent diagnosis of liver diseases. (Gowda et al).[1] Liver Function Tests are most generally used screening blood tests for assessment of different liver diseases and these tests provide a lot of evidence for disease processes, whether for the purpose of investigation of supposed liver disease or help in observing the progress of disease action or simply by blood investigation. The evaluation of different liver enzymes simply gives diagnostic information on basic level whether patient's principal disorder is actually hepatitis or cholestasis in source. However, it is necessary in various cases to evaluate LFTs with knowledge of liver functioning enzyme fractions-(Hall and Cash,).[3]

The liver performs several functions and collectively it is called the body's manufacturing center and filtering plant. Different blood tests used to evaluate abnormal liver conditions can be divided into those which represent liver cell damage or cholestasis. In various cases, the damage to the hepatocytes or liver cell causes elevation in these enzymes as compared to their normal values and the degree of elevation is very important in diagnosis of liver damage in acute disease but is not of much importance in chronic diseased condition (Suthar, and Harries).[4] The reasons for elevated levels of amino transferases are basically fatty liver, autoimmune hepatitis, medication induced hepatitis, viral hepatitis, liver necrosis and alcoholic liver disease, therefore, an abnormality or modification in these liver function tests does not mean that liver is not functioning properly. (Ji et al.).[5]

In fact, most patients with elevated levels of amino transaminases, have normal liver function tests and have no alterations seen in them as compared to values in healthy peoples. Most cases abnormality seen in chronic hepatitis C infection is often an elevated level of enzyme termed as alanine aminotransferase but it was also observed that 60% of patients infected with hepatitis C have a normal transaminase level in them at any stage of hepatitis. The elevation in level of serum ALT does not associate with specified disease condition and it might be normal in any stage of chronic hepatitis C. Innovative researches on hepatitis C indicates that an increase in alkaline phosphatase and total bilirubin as well as thrombocytopenia (low platelets) are also observed along with other abnormalities seen due to elevated levels of liver function enzymes and other parameters (Alter, and Hutin et al.).[6].

1.3 Role of Vitamins and Supplements for Human Health

Some decades ago, various experimental researches recommend that vitamin have a beneficial role to minimize abnormal enzymatic concentration in liver dysfunctions. The logic behind this strategy about use of vitamins is that liver is the major storage organ for vitamins, but this activity is minimized by disease conditions that distress the organ (Rocco et al.,).[7] The researchers aimed to further establish that regular or daily use of vitamin C would aid liver functions and probably prevent disease conditions in normal subjects. Additional studies and further investigations are needed before a final conclusion that addition of vitamin C to standard treatment would aid quick and better recovery in diseased conditions.

1.4 Effect of Ascorbic Acid (Vitamin C) on Human Health

In addition to its metabolic functions it was suggested that vitamin C plays a major role in advancement of immune functions and improves the absorption of ferrous form of iron in RBC's, (the form of iron present in plant-based foods. (Lewin.).[8] It was observed in many animal models that vitamin C has proven to be defensive against toxic substances and provides antioxidant and cyto-protective activity to hepatocytes. (AI Shamsi,).[9]

Ascorbic Acid is a well-known water-soluble antioxidant vitamin that is present in various fruits and vegetables. This is also present in human and animal cells and body fluids. Vitamin C is a powerful antioxidant which is proficient in foraging free radicals and taking part in multiple enzymatic reactions as a reducing agent. Vitamin C is protective against toxic free radical and ROS induced cellular damage as it neutralizes ROS and limits lipid peroxidation (Ray et al.,)[10], also suggested to be involved in the instruction of both circulating and hepatic lipid homeostasis (Ipsen et al.,).[11] It also has been shown to protect against liver injury in animal studies (Ozdil et al., [12] Ipsen et al.).[11].Vitamin C levels were shown to be negatively connected with aminotransferases levels suggesting that vitamin C could be an additional indicator of hepatitis C severity in Chronic Hepatitis C patients (Souza dos Santos et al.,).[13] Inadequate intake of vitamin C causes vitamin C deficiency disease known as scurvy which is characterized by mouth and gum lesions, bleeding through lips, lethargy or lassitude, gums inflammation, severe connective tissue weakness, deferment in wound healing and capillary fragility (Carr and Frei,).[14] The total body content of vitamin C (serum ascorbic acid level) ranges from about 300 mg to 2 g. High levels of ascorbic acid concentrations observed in various cells and maximum value observed in leukocytes, thyroid gland, brain and eyes. Low levels of vitamin C (micro molar concentrations) are also occurring in extracellular fluids and in various body organs, such as plasma, tissues, blood cells, and saliva. (Jacob, and Sotoudeh,).[15]

1.5 Ascorbic Acid and Liver Function Profile

The regular intake of antioxidant vitamin C may decrease the oxidative stress concomitant with different diseased condition and then restore the antioxidant protection system. As it was investigated the influence of different dosages of vitamin C on various biological factors of normal individuals and hepatotoxic subjects which have elevated relevant liver and kidney enzymes. The influence of vitamin C on biochemical and clinical parameters was determined. Liver enzymes were raised more commonly after the beginning of diseases. Adequate doses of vitamin C (P < 0.0008) decrease plasma gamma-glutamyl transferase level and ALT level. Vitamin C significantly (P < 0.04) reduced ALT and blood urea nitrogen levels. The plasma level of various electrolytes for example magnesium, calcium and sodium also changed after significant oral intake of vitamin C. (Al-shamsi et al.,).[9] Antioxidants commonly used may decrease clinical parameters due to construction of certain free radicals and toxic metabolites in experimental animals and also decreases elevated liver enzymes. (Levine et al.,).[16]

1.6 Aim of Study

The aim of this study is to determine the effect of ascorbic acid supplementation on various parameters related to liver function profile in healthy individuals and to predict whether ascorbic acid is beneficial to improve liver functions and manage deranged enzymes values.

2. MATERIALS AND METHODS

To investigate the effect of ascorbic acid supplementation, 200 healthy individuals were recruited by suitability purposive sampling for serum ascorbic acid and liver function profile. Exclusion criteria included the patients with any history of Peg-interferon treatment, presence of any other form of liver disease (including viral hepatitis A and B), decompensated cirrhosis or concomitant disease such as Diabetes and other hormonal diseases. It also included the use of hepato-tonic drugs, silymarin, ginkgo biloba, garlic oil (usage within 4 weeks period) pregnancy or lactation and refusal to contribute in the study. The study design was Parallel observational study. The recruited patients were divided in two groups. Group A: Healthy individuals without any disease, who were supplemented with ascorbic acid of 1000mg per day along with the intake of their regular diet for 04-weeks. Group B: Healthy individuals who were not receivina vitamin C supplementation. Values before the start of the treatment (at 0 weeks) are called pre-values whereas the results after 30 days are labelled as post-values.

2.1 Sample Handling and Collection

Blood samples were collected and Serum level of ALT and AST concentration was assayed based on a coupling spectrophotometric method using appropriate technique and determination of their absorbance using analytical grade laboratory reagents kits.

Serum level of ALP was also determined by spectrophotometric method using alkaline phosphatase kit. (Tietz,)[17]. Serum bilirubin (total, direct and indirect bilirubin), total serum protein, albumin, globulin and other parameters were measured by appropriate analytical methods using spectrophotometer method for accurate results. (Kaplan,).[18]

Serum Ascorbic Acid analysis for the determination of plasma ascorbic acid by 2,4dinitrophenyl hydrazine using the modified method of Keuther, and Roe., [19]as described by Nino and Shah (1986) was employed (Dogar et al.,)[20]. Ascorbic acid was converted to dehydro-ascorbic acid by cupric sulphate solution and then coupled with 2,4- dinitrophenyl hydrazine in the presence of thiourea as a mild reducing agent. Sulphuric acid then converted dinitrophenyl hydrazine into a red colored compound, which was assayed calorimetrically.

Biochemical Tests: All biochemical tests were performed at Biochemistry laboratory of Sargodha Medical College, Sargodha. These tests include direct bilirubin (DB), total bilirubin (TB), serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), serum proteins (total, albumin and globulin). Appropriate techniques were used for handling the samples, chemicals and laboratory instruments. All materials and quality controls were provided by Roche (Pakistan), Merck (France) and Ecoline (Germany).

Statistical analysis: Descriptive directories like mean, standard deviation and standard error were employed in this study. The 'independent sample t test' was used for analysis of independent variables and 'paired t test' were used to relate means of two groups. P-value of less than 0.05 was set as statistically significant. All statistical analyses were executed using SPSS 23 (SPSS Inc., Chicago, Illinois, USA).

3. RESULTS

3.1 Effect of Ascorbic Acid Supplementation on Serum Ascorbic Acid Levels

The Ascorbic Acid levels at the start and after the 30 days of study were estimated. Levels of ascorbic acid were 0.78±0.276 (Mean ± SD) mg/dl before the start and 1.26±0.34 mg/dl after the end of the study in the group with intake of supplementation of VC. The mean values of ascorbic acid in group having no any intake of vitamin supplementation were 0.63±0.26 mg/dl before and 0.74±0.277 mg/dl after 30 days. Statistically substantial improvement in the ascorbic acid levels (p-value<0.001) in after observed intake serum were of supplementation.

3.2 Effect of Ascorbic Acid Supplementation on Serum Hepatic Enzymes Levels

In the group with vitamin C supplementation, pretreatment mean value of ALT was 135 ± 5 (Mean \pm SEM) IU/L and post-treatment it was 124 ± 4.8 (Mean \pm SEM) IU/L. Similarly, in the other group

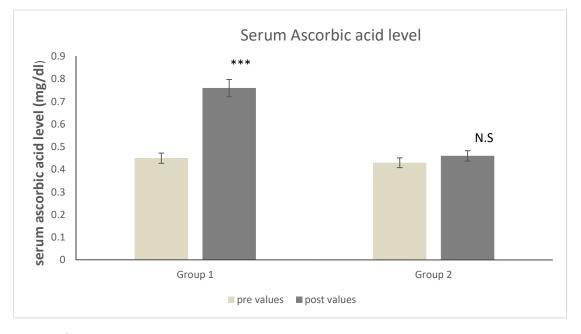


Fig. 1. Serum Ascorbic Acid levels: The ascorbic acid levels in healthy individuals with or without ascorbic acid supplementation

Significance: ***p <0.001, **p <0.01, *P<0.05 and p > 0.05 non-significant (N.S)

without vitamin C supplementation. ALT levels were observed to be 132±6.1 IU/L before intake of vitamin C and 134±6.1 IU/L post treatment, with p-value<0.05. This better trend towards the normalization of ALT is more obvious (pvalue<0.001) in group 1 as compared to group 2 who haven't taken vitamin C supplements comparing the post-treatment values for both groups. Similarly, AST was measured in both groups. In group A, the mean AST level was 101±7 (Mean ± SEM) IU/L at the start while posttreatment it was 86±5 IU/L. In those without VC supplementation for 30 days, AST was 102±6 IU/L before and 100±6 IU/L after 30 days. The AST levels shows better trend towards its normalization (p-value<0.01) as compared to those who haven't taken vitamin C supplements when comparing the post- values of both groups. The mean ALP levels in group A were 178±8 (Mean ± SEM) IU/L before and 169±7 (Mean ± SEM) IU/L post-treatment value was pvalue<0.05. The ALP levels in group B were not significantly improved with 170±7.4 IU/L at the start and 170±7.34 IU/L (with p-value=0.05) after 30 days without vitamin supplementation, but the post-treatment comparison of both groups was non-significant (p-value=0.05) (as shown in Fig. 2).

It was observed that deranged values of Total Bilirubin (p-value<0.01) and Direct (p value<

0.001) & Indirect (p-value<0.05) Bilirubin significantly shifted towards better level in the group with VC supplementation (Group A), whereas only Indirect Bilirubin levels (p-value= 0.04) improved in the patient without intake of ascorbic acid (Group B), when the results were compared pre-treatment and post-treatment. Moreover, assessing the data after 30 days of treatments, VC supplementation is found to improve the Serum Direct Bilirubin (p-value<0.001) and there was less improvement seen in serum Indirect Bilirubin (p-value<0.05) whereas it has no effect on Total Bilirubin (p-value=0.51), as shown in Fig. 3.

The normal range of Total Bilirubin is 0.1 to 1.2 mg/dL and Direct Bilirubin is less than 0.3 mg/dL (Pincus et al.,) [21]. Ascorbic acid works actively by loading the shift of the redox potential toward negative side. It also inhibits the breaking of porphyrin ring of bilirubin chain molecule, and then interferes with bilirubin formation (Koch et al.)[22].

3.3 Effect of Ascorbic Acid Supplementation on Serum Total Proteins, Albumin & Globulin Levels

Serum Total Proteins, Albumin and Globulin levels were estimated in both groups before the start (pre-treatment) and after 30 days

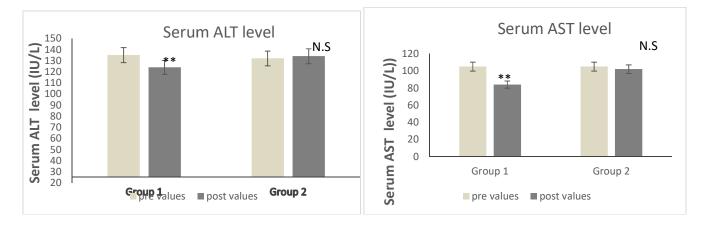
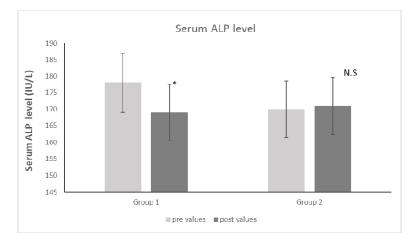


Fig. 2A. Serum ALT level in Group 1 and Group 2







The pre-treatment and post-treatment levels of serum ALT (2A) serum AST (2B) and serum ALP (2C) estimated in both groups: Significance;***p<0.001, **p<0.01, *P<0.05 and p>0.05 nonsignificant (N.s.)

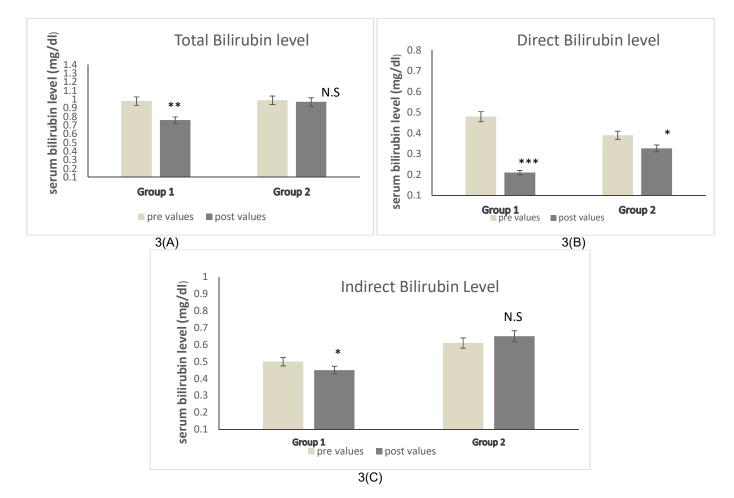
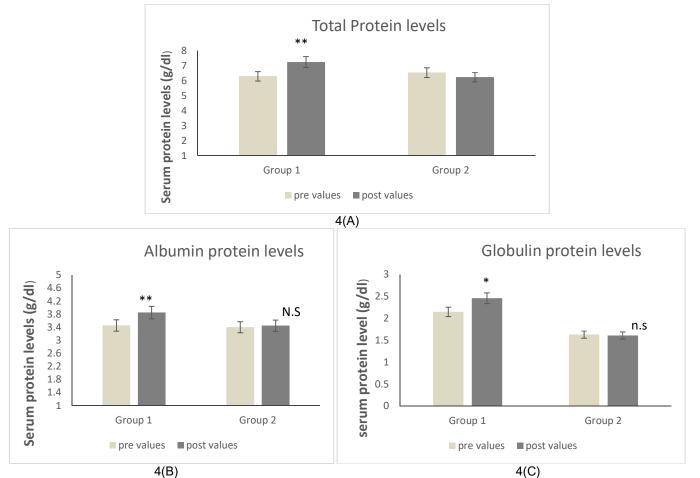
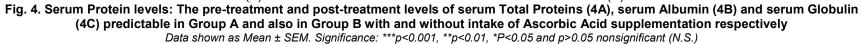


Fig. 3(3A,3B,3C). Serum Bilirubin levels: The pre-treatment and post-treatment levels of serum Total Bilirubin (3A) serum Direct Bilirubin (3B) and serum Indirect Bilirubin (3C) estimated in (Group A) or without (Group B) Data shown as Mean ± SEM. Significance: ***p<0.001, **p<0.01, *P<0.05 and p>0.05 nonsignificant (N.S.)

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(post-treatment). Interestingly, it was found that serum total proteins and Albumin levels (pvalue<0.01) were significantly improved in the group with vitamin C supplementation intake (Group A), whereas serum Globulin were altered insignificantly in both groups as shown in the Fig. 4. The pre-treatment serum total Protein levels in the group A were 6.3±0.12 g/dl (Mean ± SEM) pre-treatment, and 7.25±0.26 mg/dl (Mean ± SEM) post-treatment (p-value=0.01), whereas in group B values were 6.54±0.14 g/dl before 6.24±0.08 g/dl (psupplementation and value=0.13) after 30 days without use of supplementation (post-treatment). Serum Albumin levels in group A with use of VC supplementation were 3.45±0.04 (Mean ± SEM) g/dl at start and 3.85±0.06 (Mean ± SEM) g/dl (pvalue<0.01) after 30 days, whereas in group B the values were 3.40±0.04 g/dl (Mean ± SEM) pre-values, and post-values were 3.44±0.031 g/dl (Mean ± SEM) (p-value<0.05). Serum Globulin levels in group A were 2.15±0.17 g/dl at start and 2.46±0.19 g/dl (p-value<0.05) after 30 days, whereas in group B were 1.63±0.09g/dl before and 1.61±0.09g/dl (p-value=0.17) after 30 days of supplementation, so group B gave nonsignificant results, as shown in Fig. 4. Moreover, assessing the results at 04 weeks of treatments, supplementation of ascorbic acid is observed to improve the Serum Total Protein (p-value< 0.01) and serum Albumin (p-value=0.01) whereas it has minor improved effect on serum Globulin (pvalue<0.05), as shown in Fig. 4.

4. DISCUSSION

proposed In this study, we that the supplementation of Vitamin-C may lessen the oxidative pressure associated with elevated liver functions, and its addition in diet or as an adjuvant drug may be beneficial to restore the antioxidant defense system. Liver plays an important role in the instruction of various processes such as metabolism, storage, and the clearance of endogenous and exogenous substances. Once liver is hurt by pursuing a wrong diet and inflammation takes place, most of these physiological and biochemical functions get altered (Sila et al.) [23]. Studies recommended that anti-oxidative supplements may be helpful in improving liver functions due to reduction in oxidative stress, signifying that the antioxidants protect against liver damage and leads to decrease in the elevated LFT levels induced by hepatotoxicity (Prabu et al.) [24]. Vitamin C regulated levels of liver function enzymes and blood hydroperoxide in hepatic

cells. It might able to conserved cell integrity and potentiates activities of ALT and aspartate AST. Alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase were sensitively increased in hepatitis C patients. It was concluded that treatment with vitamin C (1000g/day) helps to normalize liver function enzymes (Mossa et al.).[25].

Due to this fact that ascorbic acid reduces hepatocytes inflammation and increase antioxidant capacity, present study evaluated the protective effects of ascorbic acid supplementation in normal individuals. In normal individuals, ascorbic acid supplementation produced certain healthy effects as well as improves serum ascorbic acid level which was decreased markedly in some individuals due to lower consumption of vitamin C or deficiency of serum ascorbic acid in them in normal daily diet (Ipsen et al.).[11].

In a study, investigations based upon effective use of vitamins proved that vitamin A, E and C can be found to protect primary liver functions against acute oxidative stress such as in hepatitis, liver necrosis, in various types of animals and human models and it was observed that combined effect of antioxidant vitamins act as a useful caring therapy to treat liver damage triggered by oxidative stress (Pearson et al.[26]. The results from previous studies related to effect of vitamin C against liver injury in male rats are characterized with increased activities of serum enzymatic levels of AST, gamma-GT, and ALP (Bashandy and Alwasel).[27] Vitamin C also have a restorative effect on liver cell necrosis and the safe administration of vitamin C can normalize histological damage associated. Thus, it potentially holds that this flavanone containing antioxidant vitamin might protect against liver damage produced due to various pathological conditions. Such types of results were observed after supplementation with other antioxidant nutrients alone or combined with vitamin A and E as detected in subjects exposed to cadmium induced hepatotoxicity (Murakami et al.).[28].

In another study, the intake of ascorbic acid supplementation was also reported to cause a decrease in serum levels of ALT and AST in patients who displayed increased levels prior to start of supplement intervention along with deficiency of serum ascorbic acid because previous studies reported that intake of ascorbic acid induced a significant reduction in some serum liver enzymatic values and the p-values

obtained for AST was p<0.0365, ALT was p<0.4672, and ALP was p<0.0273. In addition to reducing oxidative stress in lipopolysaccharide induced hepatotoxicity, it also showed that this flavanone prevents against liver damage (Ji et al.)[5]. Scorch et al. conducted a double-blind trial in 94 elderly individuals by administration of vitamin C. Primarily low levels of plasma ascorbic acid and leukocytes (mean values 0.17 mg/100 ml plasma and 10.1 µg leukocytes) were observed. After 2 months, plasma and leukocyte ascorbic acid levels had increased extensively in those receiving vitamin C supplements and in 2nd group, minor but significant increases in the mean values for body weight 0.41 kg, plasma albumin 0.46 g/l, and pre-albumin 25.4 mg/l paralleled was observed in those getting sample therapy with a decreases of 0.60 kg (Schorah et al.).[29].

It was also reported in another study that vitamin C minimized the liver damage caused by various chemical mediators. In the study, it was observed that it helped to normalize the abnormal levels of ALT, AST and ALP. Ascorbic acid was capable of conserving-cellular integrity and restrained the activities of alanine aminotransferase and aspartate aminotransferase. This observation was reported in Wister rats for estimation of hepatic functions and enzymes related to significant liver activities. AST, ALT, ALP and gamma-GT values were also significantly improved (Ergul et al.).[30]. Studies results also shown that pretreatment with ascorbic acid 200 mg/kg efficiently regulated liver related parameters and helped to minimize elevated liver enzyme values (Bashandy and Alwasel,)[27]. Dietary vitamin C supplements markedly decline endogenous level of protein related oxidative damage related to liver. Another assessment presented that administration of varying doses of monosodium glutamate (0.6mg and then 6, 12, 30 and 60 mg/kg) for 2 weeks increased serum ALT and AST. Observed abnormal parameters were improved after treating with vitamin C (Ibrahim et al.).[31]. In another study effects of vitamin C on various liver related enzymes and other LFT parameters in patients suffering from hepato-toxicity and elevated liver enzymes were observed. Activities of (ALT) SGPT, (AST) SGOT and ALP were expressively increased p < 0.05, p < 0.01, p < 0.05 in hepatitis C suffered humans but reduced by intake of vitamin C. Serum levels of triglycerides, cholesterol, serum protein, serum total bilirubin and creatinine were statistically exaggerated by intake of vitamin C at p < 0.05. (Karakilick et al.).[32]. These results

indicated that, vitamin C show a central role in the avoidance of hepatic cellular damage [33].

The-strong correlation of this study is that antioxidants are useful and beneficial agents that normalize elevated different liner enzymes. Secondly, the study examined the role of dietary VC intake with good sample size and exclusion of a considerable number of potential confounding factors. Moreover, it is the first study examining the aforementioned role of VC in healthy individuals specifically in the Pakistani population. However, the limitation of the present study include that this study is unable to explain the mechanism of action of VC and liver function profile in healthy subjects. We have compared the ascorbic acid levels in both groups of individuals in local Pakistani population, but the assessment of plasma Ascorbic Acid concentration between normal subjects and patients suffering from liver diseases especially hepatitis or liver necrosis still need more studies to elucidate this causal relationship.

5. CONCLUSION

In conclusion, ascorbic acid supplementation has a subsidiary positive role in the improvement of different parameters of liver. Ascorbic acid supplementation (1000 mg/dl) significantly reduced plasma transaminase levels. As it was observed that vitamin C supplementation have substantial effect on elevated LFT levels and there was clear difference observed in LFT values before supplementation and after use of vitamin C supplementation which indicate that ascorbic acid helps to improve liver function profile of the body. It is proposed that the consumption of vitamin C may lessen the oxidative pressure associated with abnormal liver functions and its addition in diet becomes beneficial to restore the antioxidant defense system.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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