Original Article (APMC – 444)

DOI: 10.29054/APMC/19.444

Effects of Vitamin-C (Ascorbic Acid) On Histomorphometric Changes in Liver Induced by Aspartame in Albino Wistar Rats

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ABSTRACT

Objective: To observe histological changes in Liver induced by Aspartame and effects of Vitamin C on Aspartame induced histological alterations in Liver. **Study design:** Quasi experimental study. **Settings:** Department of Anatomy and Postgraduate Laboratory of Isra University Hyderabad Sindh-Pakistan. **Duration:** 6 months from May 2017 to September 2017. **Methodology:** Animal protocols were followed at Animal House Department of Animal Husbandry and Veterinary Sciences, Sindh Agricultural University Tandojam. Forty healthy adult Albino Rats with body weight ranging from 150 to 250 grams were selected and were categorized in four groups as: Group A (Control) 10 Rats received normal chow diet ad libitum. Group B (Experimental 1) 10 rats received Aspartame (200mg/kg/day) with normal diet. Group C (Experimental 2) 10 rats received Aspartame (200mg/kg/day) and Vitamin C supplementation (100mg/kg-bw) with normal diet. Group D (Experimental 3) 10 rats received Aspartame for 3 weeks than Vitamin C supplementation for 3 weeks with normal diet. After completion of experiment of 6 weeks, via cervical dislocation, all rats were sacrificed and livers of rats were removed following rinse with normal saline, and were fixed in 10% formaline, the tissues were processed to prepare paraffin blocks. 4-6 micrometer sections were obtained for slides and stained with eosin and haematoxylin to observe under light microscope. **Results:** Gross, histological changes in Liver were observed induced by Aspartame, and also effects of Vitamin C on Aspartame induced variations in Liver were evaluated. On histological examination Aspartame is significantly associated with lymphocytic infiltrative changes, fibrotic changes, necrotic changes, congested sinusoids and fatty changes, p-values were quite significant. Additionally, consumption of vitamin C significantly prevented these changes, p-value 0.001. **Conclusion:** Present study concluded that Aspartame causes the histological alterations in Liver and Vitamin C supplementation with Aspartame can prevent the hepatic alteration due to Aspartame.

Keywords: Liver, Histology, Aspartame, Vitamin-C.

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Submitted for Publication: 18-02-2018 *Accepted for Publication: 07-12-2018*

Citation: Kashif S, Memon ZA, Samo RP, Abbasi P, Parveen S, Hanif S. Effects of Vitamin-C (Ascorbic Acid) On Histomorphometric Changes in Liver Induced by Aspartame in Albino Wistar Rats. APMC 2019;13(2):166-70.

INTRODUCTION

Liver is a primary organ for metabolic activities of various xenobiotics and therapeutic agent which accumulates in various tissues, whereas the liver cells take them towards bile formation in order to eliminate.[1,2] Measuring level of various liver-related markers e.g., serum ALT, AST, ALP, total protein and total bilirubin had occurred in conformity with level of hepatic impairment.2 Aspartame can cause several histological variations within liver.[3] An artificial sweetener; aspartame, is extensively used by millions of individuals world around. Marketable names of freshly presented Aspartame include canderel, diet sweet, Nutra Sweet, and others. Contrasted to sucrose it is nearly 200 times sweeter. It is present in above 6000 products, such as tabletop sweeteners, candies, soft drinks, and certain pharmaceuticals for instance sugar substitute, cough drops & multivitamins.[4] Several reviews on lab animals have been performed to make sure its toxicity. Aspartame was confirmed to be multi-potent carcinogenic agent. Aspartame consumption showed some neurodegenerative disorders, responsible for oxidative stress that induce disturbance of liver and kidney functions and also caused oxidative stress and structural damages in cardiac tissue.[5,6] ASP is often consumed in diets which don't need baking or cooking. It is frequently destroys on being heated and most of its sweetness is lost, therefore it is consumed in toppings, yogurt, frozen desserts, gelatins, puddings and filling within prepared bakery products & cookies, instant coffee and tea, chewing gums, breath mints, and granulated sugar-free products. As well as it is consumed in drugs for instance cough therapy, and hygiene products.[7]

It was proposed that degenerative variations noticed within the hepatic treatment with aspartame could be inflammatory such as hepatitis-like disorder. Other researchers also confirmed it as they noticed that interruption in formation & secretion of coagulation factors VII & fibrinogen stimulated by aspartame resulted in long-term hepatitis.[8] Though, aspartame could possibly disturb the sensitive balance amid a negatively & positively charged residues of amino acids within humans. This could result in the development of a salt bridge amid these residues of amino acids and enable auto antigen presentation as well as CD4 helper-T cells activation in addition to a reduction in GH serum concentration. This leads to a reduction in

functions of several cytochromes P-450 as well as further enzymes that metabolize the drug. Ultimately, the patients acquire lupoid hepatitis.[3] On weight basis, aspartame metabolism produces around 10% methanol, 40% aspartic acids and 50% phenylalanine. A rather small quantity of aspartame can substantially increase levels of plasma methanol. Methanol is being progressively accepted as a hepatocytes damaging substance, where it oxidizes to formaldehyde.[9] By definition Oxidative stress is a severe imbalance amid production of ROS and RNS, as well as antioxidant protection.[9]

Ascorbic Acid (Vitamin C) is a significant water-soluble antioxidant. It defends the human body against oxidative damage.[10] Vitamin C is a well-known antioxidant which is needed in several body processes10. Ascorbic Acid reduces oxidative stress thus avoiding several damaging processes within cells.[11] Keeping in view the above reports, it could be considered that one of the ways to deal with the aspartame intoxication could be the use of antioxidant which could avoid over production of toxic radicals as well as the impairment due to them. Vitamin C shows protective effects on hepatic health but we could not find any study on vitamin C supplementation effects on aspartame induced changes on the microscopic structure of Liver. Therefore, this study is an attempt to observe histological alteration in liver caused by aspartame and the protective effect of vitamin C supplementation in Albino Wistar Rats. Any significant findings from this study will be helpful to aware and educate the general public about the adverse effects of Aspartame consumption on liver and to take vitamin C along with aspartame in order to prevent and reduce the morbidity and mortality rate.

METHODOLOGY

Study Design: Quasi Experimental Study.

Settings: Department of Anatomy and Postgraduate Laboratory of Isra University Hyderabad Sindh-Pakistan.

Duration: 6 months from May 2017 to September 2017.

Methods: The animal protocol was followed at Animal House Department of Husbandry and Veterinary Sciences, Sindh Agricultural University Tandojam. Healthy Albino Rats 150-250 gm were taken from Animal House Department of AHVS, SAU Tandojam. The animals were kept in plastic cages and equipped with plastic drinkers with nozzles of stainless steel and feed containers of stainless steel. They were free to access standard chow diet and water prior to & following experiments. Saw dust was put as beddings and was renewed daily. The animals were kept under a well-ventilated & hygienic environment at 26◦C of room temperature and light/dark cycle of 12 hours.

Group A (Control) (n=10 rats) were given normal chow diet along with clean water ad libitum.

Group B (Experimental 1) (n=10 rats) received Aspartame (200mg/kg/day)[18] mixed with normal chow diet along with clean water for 6 weeks.

Group C (Experimental 2) (n=10 rats) received Aspartame (200mg/kg/day)[18] and Vitamin C supplementation (100mg/kg-bw)[20] mixed with normal chow diet along with clean water ad libitum.

Group D (Experimental 3) (n=10 rats) received Aspartame (200mg/kg/day)[18] for 3 weeks followed by Vitamin C supplementation (100mg/kg-bw)20 supplementation for 3 weeks mixed with normal chow diet.

After completion of experiment of 6 weeks the weights all animals were measured by electronic measuring balance. All the rats were sacrificed by cervical dislocation. Liver of rats were removed and following washing with normal saline, and were fixed in 10% formaline for histological analysis. Tissues were passed in ascending grades of ethyl alcohol (70%, 80%, 90% and 100%) then were passed in xylene for clearing. The tissues were processed to prepare paraffin blocks by paraffin embedding method. 4 micrometer sections were obtained, by cutting on microtome, for slides and stained with haematoxylin and eosin to observe under light microscope at 100 and 400 magnifications. Data was analysezed by spss version 16.

RESULTS

In this study gross histological changes in Liver were observed induced by Aspartame, and also effects of Vitamin C on Aspartame induced changes in Liver were evaluated. According to the mean body weight was 210.84 ± 23.47 grams of Group A, 196.30 ± 21.79 grams of group B, 207.15 ± 22.10 grams of group C and 180.51 ± 20.35 grams of group D. Table 1

Table 1: Mean weight of animals n=40

Groups	Mean ± SD (gms)
Group A	210.84 ± 23.47
Group B	196.30 ± 21.79
Group C	207.15 ± 22.10
Group D	180.51 ± 20.35

In this study on observing histopathology, lymphocytic infiltrations in liver were found significantly higher in all experimental groups, as compare to control group. p-value 0.003. Fibrotic changes in liver were found in two experimental groups B and D as accumulation of collagen fibers were seen in interstitial spaces, around sinusoids and some fibrotic areas were observed in portal areas around bile duct, while in group C only one rat showed liver fibrotic changes. These phenomena suggested that Aspartame is significantly associated with causing fibrotic changes in liver of rats, and combination with vitamin C significantly reduced it, p-value 0.001. Necrotic changes and congested sinusoidal changes of liver were mostly seen in groups B and D as damaged hepatocytes as compare to group A and C. These findings show that consumption of Aspartame is significantly linked to cause necrotic changes in liver, and additional consumption of vitamin C significantly prevent from it, p-value 0.001. Moreover only 3 rats in group B and 2 in group D were observed with micro- vesicular fatty changes in liver as small intra-cytoplasmic triglyceride vacuoles were seen, while only 1 rat was found with fatty changes in group C and no rat showed fatty changes in group A (control group). These results showed that Aspartame consumption is

insignificantly linked to fatty changes in liver P- value 0.26. Table 2

Table 2: Histological hepatic changes comparison among study groups n=40

Histological Changes		GROUPS				Total	p-value
		Group A	Group B	Group C	Group D		
Infiltrative changes	Yes	0	8	3	4	15	0.003
	No	10	2	7	6	25	
	Total	10	10	10	10	40	
Necrotic changes	Yes	0	9	1	8	18	0.001
	No	10	1	9	2	22	
	Total	10	10	10	10	40	
Fibrotic changes	Yes	0	9	1	8	18	0.001
	No	10	1	9	2	22	
	Total	10	10	10	10	40	
Congested sinusoid changes	Yes	0	8	2	6	16	0.001
	No	10	2	8	4	24	
	Total	10	10	10	10	40	
Fatty changes	Yes	0	2	0	1	3	0.265
	No	10	8	10	9	37	
	Total	10	10	10	10	40	

Regarding Gender no significant difference was found histologically in liver considering fatty changes, congested sinusoidal changes, fibrotic, necrotic changes and lymphocytic infiltrative changes according to gender p-value 0.74, 0.52, 0.51 and 0.54 respectively. Table 3

Table 3: Histological hepatic changes according to Gender n=40

Hepatic changes	Gender		p-value
	Male	Female	
Lymphocytic Infiltrations	8	9	0.54
Necrotic changes	8	10	0.51
Fibrotic changes	10	8	0.51
Congested sinusoids	9	7	0.52
Fatty Changes	2	4	0.74

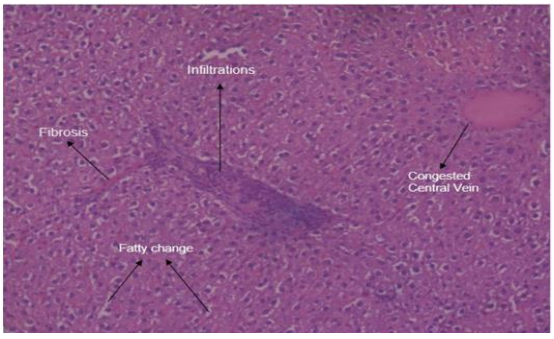

Figure 1: Photomicrograph of rat liver from experimental group b showing lymphocytic infiltrations, fibrosis, fatty changes and congested vessels

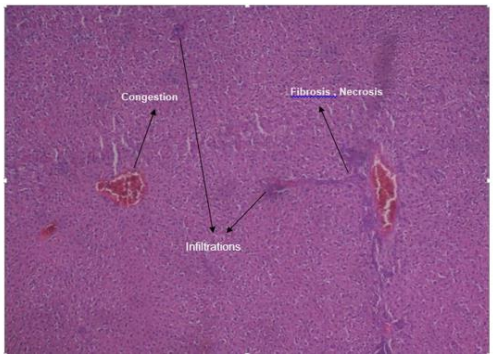

Figure 2: Photomicrograph of rat liver from experimental group B showing distorted liver architecture with lymphocytic infiltrations, fibrosis, necrosis and congested vessels

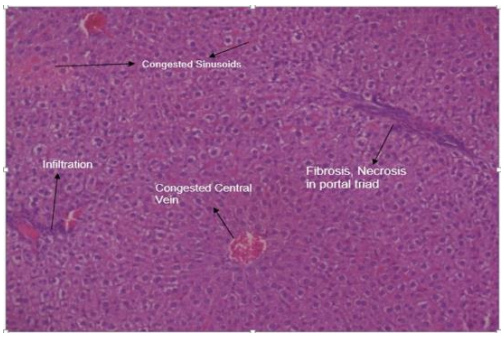

Figure 3: Photomicrograph of liver of group D showing distorted liver parenchyma congested central vein, fibrosis, necrosis and lymphocytic infiltrations. (H&E) 100 X

DISCUSSION

Aspartame is consumed frequently now a days to decrease sugar consumption and to reduce caloric intake in diabetic patients as well as in healthy persons.[11] Liver is a major metabolic organ accountable for disposal of up to 1/3rd of oral glucose burden & involved in regulation of metabolic activities of glucose. In this study histological changes in Liver were observed that were induced by Aspartame, and the effects of Ascorbic acid on Aspartame induced variations in Liver were evaluated. In some studies, it is reported that continual use of aspartame in rats causes injury of liver cells and variations in hepatic antioxidant status and several histological variations have been documented in liver sections from aspartame-treated albino rats, respectively, reported by Abhilash M et al[12] and El Haliem et al.[13]

In this study infiltrative changes, fibrotic changes and Necrotic changes were higher in as partum consumed group B and D as compare to control group and those vitamin adnimisted as group. These results suggested that Aspartame is significantly associated with causing infiltrative changes, fibrotic changes and Necrotic changes. Similarly, Finamor I et al[14] reported that aspartame administration increased hepatocellular injury and infiltrative changes in the liver. Guven et al[15] didn't observe any necrosis or rise in collagen fibers or marked peri-sinusoidal fibrosis within the liver architecture in the streptozotocin-induced

diabetic rat model, this may be due to different dosage of aspartame i-e 40mg/kg-body weight, which is inconsistent with the present study.[15] Similarly in the study of Abd EA et al[16] reported that aspartame usage causes hepatic histopathological lesions and variations of the hepato-genetic system and bone marrow within albino rats.[16] Khidr BM et al[17] reported that Rats that received Aspartame exhibited severe histological variations, in the form of disorganized tissues of liver and necrotic regions.

Congested sinusoids and fatty changes of liver were mostly found in groups B and D, as compare to control and group C. Similarly the studies of Suez J et al and Nseir W et al reported that Aspartame was as well linked to fatty liver disease correlated to metabolic syndrome.[18,19] Similarly in the study of Ebraheim LL et al reported that congestion of the sinusoids found in rats treated with aspartame.[20] Inconsistently, Finamor I et al reported that after aspartame administration raised hepatic sinusoidal diameter within different hepatic regions in male swiss mice so no congestion of sinusoids were seen.

Lastly in this study we found liver safety in group C (Aspartame (200mg/kg-bw) and Vitamin C supplementation (100mg/kg-bw) with normal diet) because when vitamin C consumed with aspartame, we found no significant hazardous effects on liver from aspartame including histological changes, this showed that vitamin C can prevent the liver from histomophological hepatic alteration due to aspartame by inhibiting oxidative stress. Alkafafy ME et al[21] reported that biochemical results exhibit that, both saccharin & aspartame can possibly provoke oxidative stress on hepatocytes via decreasing the catalase activity as well as TAC in plasma. It is worth stating that, the effect of saccharin was further pronounced. Additionally, in a previous study of Abhilash M et al exhibited that the prolonged use of aspartame within rats causes an imbalance in pro-oxidant/antioxidant status in the liver tissues.[12] Moreover in a study of Naziroglu M et al[22] reported the effect of dietary supplementations of vitamins E and C on antioxidant redox systems and oxidative stress within rats treated with aspartame. Heistad et al[23] reported that the major natural antioxidant which are derived from the natural sources by dietary intake is vitamins C and furthermore Shireen et al[24] Budin et al[25] stated that interest has recently grown in the role of the natural antioxidant as a strategy to prevent oxidative damage as a factor in the pathophysiology and histopathology of various health disorders. Among antioxidants, vitamin C and vitamin E used as nutritional supplements, are the essential elements in almost all biological systems. In another study of Magdy BW,et al also reported that Vitamin C, antioxidant molecules that have been used to mitigate oxidative damage.[26]

CONCLUSION

In the present study, it is concluded that Aspartame can cause significant gross and histological alterations in hepatic tissue. Vitamin C (Ascorbic Acid) has highly significant protective effect on gross and histological damages in liver when given along with Aspartame. Whereas when vitamin C was given after the injury has been caused by aspartame, it did not show significant protective role.

RECOMMENDATIONS

More research is needed on this topic which should be conducted with large sample size and various doses in order to observe the damage and in order to develop the preventive strategies to reduce the hepatic alteration.

Protective effects with other vitamins on aspartame induced alterations and on other organs should also be studied.

Medical practitioners should advice the use of Vitamin C supplementation to patients using Aspartame.

Concerned health authorities should organize programs at community level to bring awareness among people to use Vitamin C supplementation so as to reduce the adverse effects of the food products containing Aspartame.

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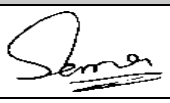
Dr. Sana Kashif Lecturer, Department of Anatomy Isra University Hospital, Hyderabad	Conceptualization, Data Collection	
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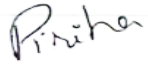
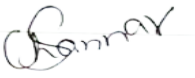
Dr. Piriha Abbasi Assistant Professor, Department of Anatomy Isra University Hospital Hyderabad	Statistical analysis	
Dr. Shazia Parveen Senior Lecturer, Department of Pharmacology Isra University Hospital, Hyderabad	Guided in Data Collection & helped in research process	
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