Hepato-protective role of Vitamin Cagainst aspartame induced toxicity- An experimental rat study.

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ABSTRACT

Objective: To observe the hepato-protective effect of vitamin C supplementation through assessment of serum markers of liver function and histological alteration in liver caused by aspartame in Albino Wistar Rats **Methods:** Quasi-experimental study was carried out at Isra University, Hyderabad from March to August 2019. Thirty healthy adult male Albino Rats with body weight ranging from 250 to 300 grams were selected and were equally categorized in three groups as: Group A (received normal chow diet ad libitum), Group B (Aspartame150mg/kg with normal diet) and Group C (Aspartame150mg/kg and Vitamin C supplementation 100mg/kg with normal diet). After completion of experiment of 6 weeks, Biochemical (liver function tests) and histopathological analysis was performed in all groups. Statistical analysis of data was performed in SPSS version 24.Level of significance was set at p-value ≤ 0.05. **Results:** Difference in mean pre- and post-experimental body weight was observed in all three groups. There was a statistically significant decline in the bodyweight, and an incline in relative liver weight of group B in comparison with groups A and C(p-value<0.05). Liver function markers were also significantly elevated in B in comparison with groups A and C(p-value<0.05).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<0.05). **Conclusion:** Ascorbic acid therapy is a potent hepato-protective regime showing promising results in aspartame induced hepatotoxicity. **Keywords:** Aspartame, Histopathology, Liver,Vitamin-C.

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INTRODUCTION

The liver is a primary organ for metabolic activities of various xenobiotics and therapeutic agents which accumulates in various tissues, where as the liver cells take them towards bile formation for elimination.[1, 2] Measuring level of various liver-related markers e.g., serum Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), total protein, and total bilirubin had occurred in conformity with the level of hepatic impairment.[2] Aspartame can cause several histological variations within the 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 like sugar substitute, cough drops & multivitamins.[4] Several reviews on lab animals have been performed to make sure its toxicity. Aspartame was confirmed to

be a multi-potent carcinogenic agent. Aspartame consumption showed some neurodegenerative disorders, responsible for oxidative stress that induces 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 that don't need baking or cooking. It is frequently destroyed 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.[7] As well as it is consumed in drugs, for instance, cough therapy, and hygiene products.[8] It is proposed that degenerative variations noticed within the hepatic treatment with aspartame could be inflammatory such as hepatitis-like disorder, while other researchers also confirm edit as they noticed that interruption in the formation & secretion of coagulation factors VII & fibrinogen stimulated by aspartame resulted in long-term hepatitis.[9] Aspartame could disturb the sensitive balance amid negatively & positively charged residues of amino acids within humans, resulting in the development of a salt bridge amid these residues of amino acids and enable auto antigen presentation aswellasCD4helper-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 a weight basis, aspartame metabolism produces around 10% methanol, 40% aspartic acids, and 50% phenylalanine.[7] 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.[10]

Ascorbic Acid (Vitamin C), a significant water-soluble antioxidant needed in several bodily processes, reduces oxidative stress thus avoiding several damaging processes within cells.[11] Vitamin C is a well-known antioxidant that is needed in several bodily processes. Ascorbic Acid reduces oxidative stress thus avoiding several damaging processes within cells.[12] 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 antioxidants which could avoid overproduction of toxic radicals as well as the impairment due to them. Therefore, the objective of this study is to observe the hepato-protective effect of vitamin C supplementation through assessment of serum markers of liver function and histological alteration in liver caused by aspartame in Albino Wistar Rats.

METHODOLOGY

After being approved by the Isra university ethical review committee, this quasi-experimental study was carried out in the department of anatomy and postgraduate research laboratory at the Isra University, Hyderabad, Sindh from March 2019 to August 2019. The sample size was calculated using the standard method of power analysis for animal studies.[13] Thirty male albino Wistar rats were procured from Agriculture University of Tando Jam, Sindh by non-random purposive sampling technique. Male albino Wistar rats between the age of 8-10 weeks weighing between 250 to 300 grams without any disease or deformity were included in the study while rats that did not fulfill the above mentioned inclusion criteria were excluded from the study.

Animals were housed in a well-equipped and hygienic environment at the postgraduate laboratory in Isra University, Hyderabad at the optimum temperature of 24-26 centigrades in a day-night cycle of 12/12 hours. Before the initiation of the experiment, animals were kept for ten days of acclimatization. To avoid any harm, animals were placed in plastic cages with water drinkers having stainless steel nozzles and feed containers. Rats were provided free access to chow diet and clean water ad libitum. Their bedding consisted of sawdust and was renewed daily. All rats were equally (n=10) divided into, Group A (Control group and given a normal Chow diet, clean water ad libitum), Group B (Aspartame 150 mg/kg mixed with normal chow diet along with clean water for 6 weeks)[14] and Group C (Aspartame 150 mg/kg[(14)]and Vitamin C supplementation (100mg/kg)[15] mixed with normal chow diet along with clean water ad libitum for 6 weeks). Soon after the acclimatization period, the bodyweight of all rats was measured twice that is before initiation of the experiment and after completion of two weeks of the experiment using an electronic precision balance. On completion of experiment,

all rats were given anesthesia (inj. Sodium pentobarbital at 40mg/kg intra peritoneally) and sacrificed by cervical dislocation. For analysis of oxidative and liver function markers, blood was collected by cardiac puncture. The liver was removed after dissection and weighed using the same balance then washed with normal saline. Hepatic tissue was fixed in 10% formalin for at least 24 hours and passes in ascending grades of ethyl alcohol (70%, 80%, and 95%). After that, it was passed in xylene for clearing and embedded in paraffin wax. These paraffin sections were then cut into slices of 4-µm-thickness by the manual method using Rotary Microtome, 290 measures like body and liver weights, oxidative and liver function markers were expressed as mean and standard deviation while their comparison was analyzed by one way ANOVA and Post Hoc Tuckey analysis. The level of significance was set at p-value ≤ 0.05.

RESULTS Table I. below shows the changes in the pre and post-experimental body weights and relative liver weights of albino Wistar rats. There was a statistically significant decline in the bodyweight of group B in comparison with groups A and C. Moreover, a significant rise in relative liver weight was observed in group B in comparison with groups A and C. There was a significant derangement in the liver function markers post aspartame therapy. Post hoc analysis revealed that serum markers of hepatic function (ALT, AST ALP and LDH) were significantly raised in aspartame group (group B) in comparison with control(group A) and ascorbic acid group(group C,) as shown in Table II. In this study on observing histopathology, lymphocytic infiltrations in liver were found significantly higher in all experimental groups, as compare to control group. Fibrotic changes in liver were found in both experimental groups B and C 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. Necrotic changes and congested sinusoidal changes of liver were mostly seen in group B 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. Moreover only 3 rats in group B 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-value0.26.Table III. Table IV. Demonstrating the grade-wise comparison summary of all the histopathological changes in hepatic tissues observed in each group under light microscopy. (Table IV)

and stained with Hematoxylin and Eosin (H&E) for examination under the light microscope (Olympus BX51, Tokyo, Japan). Histopathological analysis of hepatic tissue was done by evaluating; the degree of sinusoidal dilation, infiltration of inflammatory cells, vascular hemorrhages, necrosis, etc. The changes in severity were observed using a graded scale adopted from a previous study.[16] The grading scale consists of rankings according to the tissue damage; (0) none, (I) mild, (II) moderate and (III) severe. Statistical analysis of data was performed in SPSS version 24. Findings of

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.[12] 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.[7] 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 and El Haliem et al. [3, 17]

In the current study, aspartame therapy was followed by derangement of liver function markers, indicating aspartame induced hepatic toxicity. These findings are similar to those reported by Hamza at al. who found that

Table I: Difference InMean Body Weight and Mean Relative Liver Weight Between ExperimentalGroups By Post-Hoc Tukey Test

	Group A	Group B	Group C	p-value
Initial body weight (gm)	262.7±3.8	259.3±2.4	261.1±3.1	0.38
Final body weight (gm)	270.2±6.4^{b,c}	221.9±4.1^{a,c}	238.1±2.7^{a,b}	0.00*
Relative weight of Liver (gm/100 gm)	3.24±0.25^{b,c}	5.20±0.39^{a,c}	6.19±0.43^{a,b}	0.00*

* Statistically significant difference between the groups on ANOVA
$^{a, b, c}$ denote the statistically significant difference between control and treated groups, respectively through post hoc Tukey. (P-value < 0.05)

Table II: Difference In Mean Liver Function Markers Between Experimental Groups By Post-Hoc Tukey Test

	Group A	Group B	Group C	p-value
ALT (U/L)	15.17±1.35^{b,c}	131.21±21.36^{a,c}	54.17±6.34^{a,b}	0.00*
AST (U/L)	21.39±1.94^{b,c}	148.89±12.19^{a,c}	97.76±5.61^{a,b}	0.00*
ALP (U/L)	94.88±4.87^{b,c}	141.68±14.08^{a,c}	118.83±7.06^{a,b}	0.00*
LDH (U/L)	249.74±14.69^{b,c}	487.57±33.52^{a,c}	315.74±23.58^{a,b}	0.00*

* Statistically significant difference between the groups on ANOVA
$^{a, b, c}$ denote the statistically significant difference between control and treated groups, respectively through post hoc Tukey. (P-value < 0.05)

Table III: Histological hepatic changes comparison among study groups

Histological Changes		GROUPS A	GROUPS B	GROUPS C	Total	p- value
Infiltrative changes	Yes	0	8	3	11	0.003
	No	10	2	7	19	
	Total	10	10	10	30	
Necrotic changes	Yes	0	9	1	10	0.001
	No	10	1	9	20	
	Total	10	10	10	30	
Fibrotic changes	Yes	0	9	1	10	0.001
	No	10	1	9	20	
	Total	10	10	10	30	
Congested sinusoid changes	Yes	0	8	2	10	0.001
	No	10	2	8	20	
	Total	10	10	10	30	
Fatty changes	Yes	0	2	0	2	0.265
	No	10	8	10	28	
	Total	10	10	10	30	

* Statistically significant difference between the groups on Fisher's exact test

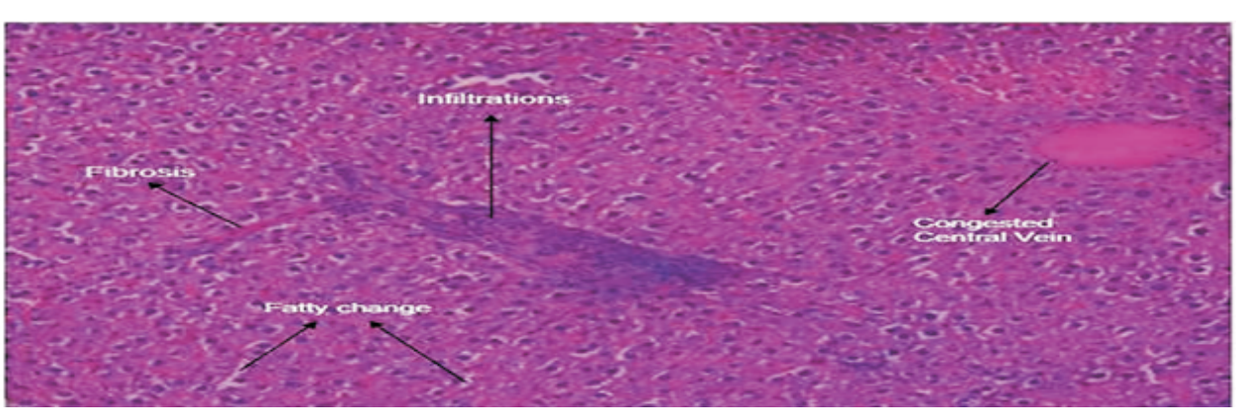

Figure 1: Photomicrograph of rat liver from experimental group b showing lymphocytic infiltrations, fibrosis, fatty changes and congested vessels

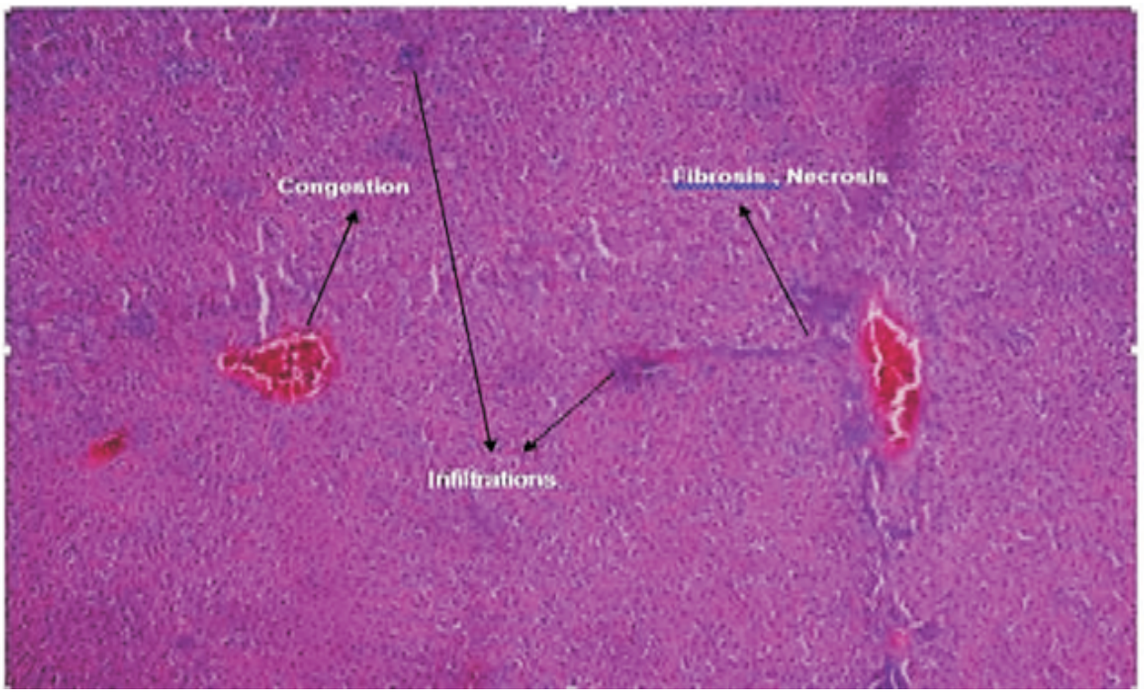

Figure 2: Photomicrograph of rat liver from experimental group B showing distorted liver architecture with lymphocytic infiltrations, fibrosis, necrosis and congested vessels

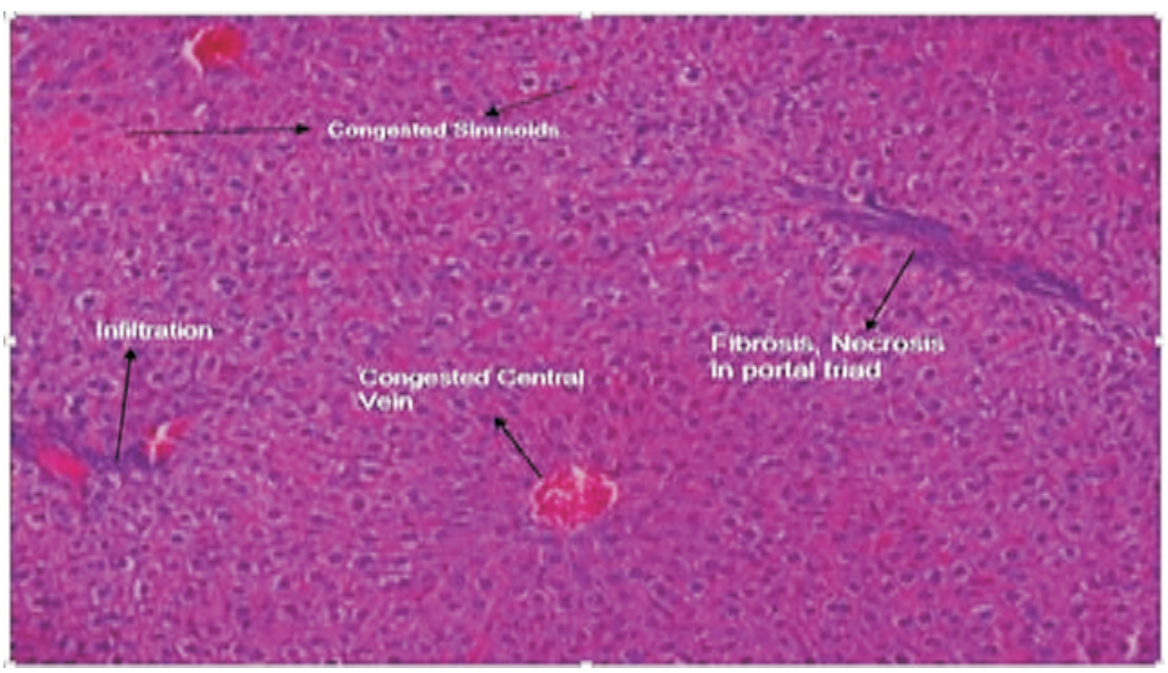

Figure 3: Photomicrograph of liver of group D showing distorted liver parenchyma congested central in, fibrosis, necrosis and lymphocytic infiltrations. (H&E) 100X

Table IV: Grading wise comparison of histopathological changes in hepatic tissues of rats

	Cellular infiltration	Necrotic Changes	Fibrotic Changes	Vascular congestion	Fatty Changes
Group A	0	0	0	0	0
Group B	III	III	III	II	I
Group C	I	II	II	I	0

Grading score follows: none (0), mild (I), moderate (II) and severe (III)

aspartame was directly related to elevation of serum markers of liver function in a dose dependent manner.[14] This increase in the activity of serum enzymes, attributed to loss of effective cell membrane integrity secondary to intracellular infiltration, is also an important indicator of aspartame induced hepatic damage.[14, 17] However, this rise in serum markers of liver function was less pronounced in animals that received co-treatment with ascorbic acid (group C). These findings are consistent with the findings of Bashandy et al. who reported that ascorbic acid attenuates hepatic damage owing to its antioxidant properties and prevents the harmful rise in liver function markers.[18] Similar findings have also been reported by Ajayi et al. which are consistent with the findings of the current study.[19] In this study, infiltrative changes, fibrotic changes and necrotic changes were higher in group B as compared to group A (control) and as group C (vitamin C administer). These results suggested that Aspartame is significantly associated with causing infiltrative changes, fibrotic changes and necrotic changes. Similarly, Finamor et al. reported that aspartame administration increased hepatocellular injury and infiltrative changes in the liver.[20] Guven etal. Did n'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.[21] Similarly in the study of Elfatah et al. reported that aspartame usage causes hepatic histopathological lesions and variations of the hepato-genetic system and bone marrow within albino rats.[22] Similarly, Khidret al. reported that rats that received aspartame exhibited severe histological variations, in the form of disorganized tissues of liver and necrotic regions.[23]

Lastly in this study we found liver safety in group C 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 histo morphological hepatic alteration due to aspartame by inhibiting oxidative stress. These findings are consistent with those reported by Kashif et al. who also found that ascorbic acid exerts a significant protective effect on hepatic tissue against aspartame induced toxicity.[(7)]In their study, Histological aberrations such as cellular infiltration, congestion and fibrotic changes were far less pronounced in those animals which received ascorbic acid therapy as compared to those that received aspartame alone, which is consistent with the findings of the current study. Alkafafy et al. 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.[24] Additionally, in a previous study of Abhilash et al. exhibited that the prolonged use of aspartame within rats causes an imbalance in pro- oxidant/antioxidant status in the liver tissues.[17] Moreover, a study by Naziroglu M et al. reported the positive effect of dietary supplementations of vitamins E and C on antioxidant redox systems and oxidative stress within rats treated with aspartame.[25] Similarly, Shireen et al.Budin et al. 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.[26, 27] The present study had certain limitations, of which the foremost were the time constraints and deficiency of resources. Due to these limitations, other parameters like hematological parameters, serum albumin, and oxidative stress markers have could not be observed and researched.

CONCLUSION

This study concludes that Aspartame can cause significant alterations in hepatic function and histological architecture. Vitamin C (Ascorbic Acid) has highly significant protective effect liver function and histological damages in liver when given along with Aspartame.

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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