ORIGINAL ARTICLE

Effects of Ascorbic Acid on Aspartame Induced Nephrotoxicity: An Experimental Rat Model

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ABSTRACT

Objective: To assess the nephroprotective role of Ascorbic Acid against Aspartame induced nephrotoxicity in Albino Wistar rats.

Study Design: Quasi-experimental study.

Place and Duration of Study: Postgraduate research laboratory at ISRA University, Hyderabad from August 2018 until November 2018.

Materials and Methods: Thirty albino Wistar rats were divided into three groups: Group I (Control group), Group II (Aspartame only), and Group III (aspartame and ascorbic acid combination). Pre and post-experiment body weight, the biochemical analysis was done through ANOVA. While Fisher's exact test was used for histological analysis in SPSS version 22.

Results: Statistically significant difference in mean post-experimental body weight was observed in all three groups (P-value<0.05). Marked reduction in mean body weight was observed in group II (171.4±17.5) as compared to group III (191.80±15.1). A statistically significant difference in mean serum levels of serum biomarkers was also observed in all three groups (P-value<0.05). Marked elevation in serum levels of urea, creatinine, C-reactive protein while the decline in serum levels of glutathione peroxidase was seen in group II as compared to group III. Histological alterations (mean diameter of proximal and distal renal tubules) were also more pronounced in group II (110.3±7.4 and 185.98±5.9) respectively as compared with group III (89.59±6.1 and 95±6.8).

Conclusion: Aspartame consumption causes significant nephrotoxicity and disturbs normal renal functions. Ascorbic acid used as a potent antioxidant can limit and/or decrease the toxic effects caused by aspartame.

Key Words: *Ascorbic Acid, Aspartame, Biochemical, Histological, Nephrotoxicity.*

Introduction

Aspartame (APM) is amongst the most widely existing artificially sweetening compounds, consumed by over 100 million people globally.[1] It is one of the constituent ingredients present in diet carbonated cola beverages, tabletop sweeteners and a large number of pharmaceutical products (cough syrups, lozenges, multivitamins etc.).[2] It is commercially available in markets with different names like; Diet sweetener, Nutra sweet, Candril etc. Constituents of APM include aspartic acid and phenylalanine.[3] With the positive uses of aspartame, several negative effects make it one of the most controversial artificial sweeteners.[4] Each year, it claims numerous health problems like headaches, dizziness, nephrotoxicity etc.[5] Consumption of aspartame poses some serious effects on neuronal tissues and leads to neurodegenerative disorders.[6,7] It is confirmed as a multipotent carcinogenic agent.[1] Once consumed, APM metabolized in the gastrointestinal tract (GIT) into aspartic acid, phenylalanine and menthol.[8,9] This menthol then oxidized into cytotoxic formaldehyde and formic acid.[6] Formic acid is considered as the chief metabolite responsible for the detrimental effects of acute intoxication by menthol in humans and animal trials whereas, formaldehyde is a known potent carcinogen.[10] They are mainly responsible for oxidative stress at the cellular level that may impair renal functions.[9,11] Consumption of aspartame for longer duration leads to the production of reactive oxygen species (ROS) include free radicals that cause

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renal tissue injury.[6,11,12]

These free radicals attack the cell membranes causing peroxidation of fats.[13] Consequently, damage to the cellular components resulting in oxidative stress (OS).[7] The elevation of menthol level soon after administration of aspartame has demonstrated by studies on humans and animals.[8]

Ascorbic Acid (AA) is one of the most significant water-soluble antioxidants needed in the body for several processes.[14] It is an effective antioxidant that showed a protective role against ischemic conditions, toxicity and injurious effects induced by OS in animal models as well as human studies.[15] It reduces OS thus avoiding several damaging processes within cells and has the potential to reverse the negative or adverse effects of carcinogenic substances like APM.[16] It can affect the endothelial functions in a positive way and exert anti-inflammatory activities.[17] Due to the protective roles of AA, it is frequently used in the field of medicine.[14,17]

Histological alterations in liver architecture resulting from APM consumption is reported by some researchers.[12] Whereas, the hepatoprotective effect of AA is reported by different studies.[18,19] However, a very limited number of studies have demonstrated the role of AA against the nephrotoxicity by APM.[20]

To the best of our knowledge, no study has been conducted in Pakistan that has demonstrated the protective role of AA in APM induced nephrotoxicity. The current study, therefore, was designed to highlight the nephroprotective effects of AA against APM related nephrotoxicity. This will not only provide the baseline for future human studies but also help in designing community-based programs to educate masses to raise awareness related to the harmful effects of APM containing products on their kidneys. Moreover, any significant findings of the present study will also be helpful in providing guidelines for the stakeholders to include AA as an ingredient in APM containing products to prevent and reduce the morbidity and mortality rate.

The objective of the present research work was to assess the nephroprotective role (hematological, anti-oxidative and histological) of AA against APM induced nephrotoxicity in Albino Wistar rats.

Materials and Methods

Quasi-experimental study was conducted at the postgraduate research laboratory at ISRA University Hyderabad from August 2018 to November 2018. Thirty male and healthy Albino Wistar Rats, 8-12 weeks old, 150 to 250 grams were included in the present study through a non-random purposive sampling technique. The animals were handled according to the national research council guidelines for laboratory animal handling.[21] Ethical approval was sought from the ethical review committee of ISRA University.

After acclimatization period of 1 week, we randomly divided animals into three groups (n=10); group I (controls), group II (aspartame 200mg/kg/day orally)[22] and group III (aspartame 200mg/kg/day orally + ascorbic acid 100mg/kg/day orally).[23] Experimental drugs were crushed and mixed with a normal chow diet, which was fed to the animals for six weeks.

Blood samples for biochemical analysis (Serum Urea, Creatinine, C-reactive proteins (CRP) and serum glutathione peroxidase (GPX) were collected twice from each rat model (before and after APM induction) from retro-orbital plexus (before) and then through cardiac puncture (after) in the study to evaluate the renal changes. All tests were carried out by Roche/Hitachi diagnostic kit method on an automatic modular analyzer while GPX performed on the bioassay technology ELISA kit.

After six-week, all animals were sacrificed under anesthesia and kidneys of all groups were removed soon after sacrificing the animals. Kidneys were then washed with normal saline and gross abnormalities as well as morphological parameters like those that weight & size were recorded on electronic precision balance and measuring scale respectively.

Collected specimens (kidneys) from all groups were fixed in 10% formalin for histological analysis. Tissues were passed in ascending grades of ethyl alcohol (70%, 80%, 90% and 100%) then in xylene for clearing. The tissues were processed to prepare paraffin blocks by the paraffin embedding method. Four-micrometer sections were obtained using Rotary Microtome, 290 (by manual method), for slides preparation. All slides then stained with hematoxylin and eosin (H & E) to observe under a light microscope at 400 magnifications.

Data were analyzed by SPSS (Statistical packages for social sciences) version 22.0. ANOVA was applied to

compare the body weights and hematological findings. Whereas, Fisher Exact Test was used for histological findings. The significance level was set at P-value ≤ 0.05.

Results

Weight of all rats in groups I, II and III were observed prior to the experiment and were found to be 201.2±5.7 gm, 215.7±8.5 gm and 204.6±7.4 gm respectively. At the end of the experiment the rats have weighed again and statistically significant difference in mean post-experimental body weight was observed in all three groups (p-value <0.05) (Table I)

Table I: Mean Body Weight (In Grams) of Animal Groups (n=30)

Groups	Mean (SD ±)	F-Value	P-Value
Group I (Controls)	223.50 (15.7)	26.39	<0.05 (0.001)
Group II (Aspartame induced Group)	171.40 (17.5)		
Group III (Aspartame + Ascorbic acid Group)	191.80 (15.1)		

Pre experimental biochemical analysis of all three groups was performed in the present study. Pre-experimental mean level of serum urea, creatinine, CRP and GPX in group I was (19.05±2.61 mg/dl, 0.46±0.07 mg/dl, 0.117±0.05mg/L and 1.44±0.17 ng/ml respectively), in group II (19.72±2.52 mg/dl, 0.49±0.09 mg/dl, 0.117±0.07mg/L and 1.43±0.16 ng/ml respectively) while in group III these were found to be (19.33±2.51 mg/dl, 0.52±0.09 mg/dl, 0.12±0.08mg/L and 1.41±0.14 ng/ml respectively).

The mean ± SD of post-experimental biochemical analysis findings of all three-study groups of rats are shown in Table II. Aspartame induction resulted in a rise in serum levels of urea, creatinine and CRP whereas; significant decline serum level of GPX was observed in group II. There was a statistically significant difference (p<0.05) of mean levels of serum urea, creatinine, CRP and GPx in the post-experimental analysis of blood samples in group II comparison to the group I and III. (Table II)

Marked changes in the histology of mean Proximal Convoluted Tubules (PCT) and Distal Convoluted Tubules (DCT), as well as mean distance between visceral and parietal layers of Bowman's capsule (BC), observed in-group II in comparison with the group I and III. Changes were also observed in group III but less in comparison with group II. There was a statistically significant (p<0.05) difference in renal histology i.e. changes in the diameter of PCTs, DCTs and distance between visceral and parietal layers of Bowman's capsule (Table III).

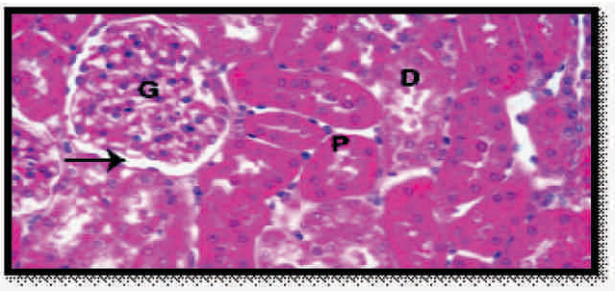
Table II: Mean Biochemical Analysis Findings of All Study Groups (n=30)

		Mean (SD±)	F-value	p-value
Serum Urea	Group I	21.12 (0.573)	2821.7	<0.05 (0.0001)
	Group II	39.83 (0.55)		
	Group III	26.71 (0.58)		
Serum Creatinine	Group I	0.48 (0.06)	176.09	<0.05 (0.0001)
	Group II	2.39 (0.28)		
	Group III	1.69 (0.266)		
C-Reactive protein	Group I	0.11 (0.0264)	375.5	<0.05 (0.0001)
	Group II	0.75 (0.061)		
	Group III	0.50 (0.062)		
Serum Glutathione peroxidase	Group I	1.51 (0.098)	264.9	<0.05 (0.0001)
	Group II	0.757 (0.079)		
	Group III	1.95 (0.160)		

Table III: Renal Histological Difference between the Study Groups (n=30)

Renal Changes		YES	NO	p-value*
Changes in diameter of the proximal convoluted tubule	Group I	0	10	<0.05 (0.001)
	Group II	8	2	
	Group III	3	7	
Changes in diameter of distal convoluted tubule lumen	Group I	1	9	<0.05 (0.018)
	Group II	7	3	
	Group III	3	7	
Changes in distance between visceral and parietal layers of the Bowman's capsule	Group I	0	10	<0.05 (0.003)
	Group II	7	3	
	Group III	6	4	

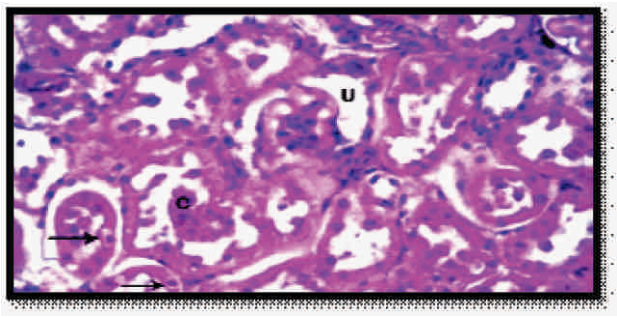
***Fisher Exact Test**



GROUP I

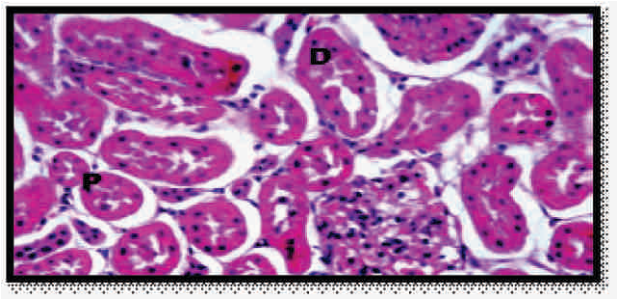
Normal Glomerular tuft (G) with urinary space (arrow)

and renal corpuscles seen in the renal cortex. Proximal convoluted tubules (P) and Distal convoluted tubules (D) lined by the cuboidal cells. Acidophilic cytoplasm and presence of apical brush border seen in PCT (P).
(H&E) X 400 (4µm)



GROUP II

Low cuboidal cells lining the renal tubules with a vacuolated cytoplasm (arrow) and pyknotic nuclei seen in this group. Shrinkage of renal corpuscle with widened urinary space (U). The dilated tubular lumens contain sloughing necrotic cells (C).
(H&E) X 400 (4µm)



GROUP III

Most of the glomeruli and tubules in the renal cortex seen. These are more or less similar to that of the control group. Brush borders in most of the proximal (P) and distal tubules (D) are preserved.
(H&E) X 400 (4µm)

Fig 1: Histological Slides of Renal Cortex

Discussion

APM is one of the most controversial artificial sweeteners used by people around the globe.[24] It is a Food and Drug Administration (FDA) approved sugar alternate but its use in different routine food and medicinal products is highly debated.[25] Although its consumption within normal and approved range is considered safe the findings of some experimental and epidemiological studies demonstrated its adverse effects like hyperglycemia, obesity, cardiovascular disease, metabolic syndrome, neurobehavioral disturbances, cancers etc.[8,9,11,13,26]

Several studies in recent years demonstrated the nephrotoxic effects of artificial sweeteners like APM and its close relationship with renal dysfunctions.[8,13,26] Similarly, studies also demonstrated that long-term consumption of APM could lead to an increase in the production of harmful free radicals that cause renal tissue damage.[11,13,27]

In the present study, we observed the nephroprotective effects of AA on the APM-induced nephrotoxicity on albino Wistar rats. We found that the majority of rat in-group II showed statistically significant ($P < 0.05$) loss in their body weights. Similar findings reported and supported by the studies conducted in the past few years.[8,12,26] This decrease in body weight may be attributed to the wasting of protein from the body secondary to the reduced availability of carbohydrates as a potential energy source. Another study found that consumption of APM triggers the secretion of a peptide in GIT (glucagon-like peptide) that may result in weight loss.[28] Furthermore, a statistically significant difference in mean weight-loss in-group III i.e. lesser decline weight loss) was observed compared with group II.

In our study, we found that levels of GPx, CRP, urea and creatinine were disturbed significantly after the induction of APM in experimental groups. A statistically significant decline in GPx level in group II and elevation in levels of CRP, urea and creatinine in the same group noticed in comparison with the other two groups. This change in levels may occur due to the toxic consumption of APM resulting in renal dysfunction linked with the damage to glomerular epithelium and filtration resulting from APM metabolite. The findings of other studies are consistent with the current study.[9,11,13,29] These studies demonstrated the negative impact of APM on GPx and other markers of renal dysfunction. These studies also demonstrated that consumption of APM for a longer duration or in high doses causes injuries to the renal tissues leads to the depletion of GPx and eventually disturb the biochemical markers like CRP, serum urea and creatinine. The findings of our study are consistent with the findings these studies confirmed that consumption of APM linked closely with the renal dysfunction.

Moreover, we also observed the levels of serum GPx

that declined due to APM induction but remained sustained with AA i.e. in-group III. While levels of CRP, urea and creatinine found lower in AA treated group (group III) in comparison to group II. These differences in serum values of mentioned markers attributed to the anti-inflammatory and vaso-protective role of AA against the APM. These findings are consistent with the other experimental studies that reported the protective role of AA in the presence of nephrotoxic, hepatotoxic or other substances or drugs.[15,16,21]

Renal histological changes were also observed in this study. In comparison to the control group, in the APM alone group II there is complete to a partial loss of the brush-bordered lining of tubular epithelium and diameter of lumens detected. Remnants of the cell (exfoliated cells) were also present in the lumen of some tubules. We also noticed the increase in urinary space and atrophic glomeruli in some renal corpuscles etc. These findings are consistent with the findings of similar studies.[12,13,27,29]

We observed that AA is effective in decreasing the toxic effects of APM on renal tissues. In-group III, glomeruli appeared normal and showed no significant changes in comparison to group II. Slight edema of tubular cells, the mean diameter of PCTs and DCTs as well as the mean distance between visceral and parietal layers of Bowman's capsule, increased less in comparison with group II. Similar findings were observed by conducted in Egypt.[20]

This study is one of its kind, because to the best of knowledge no other studies are available demonstrating the protective effects of AA in APM induced nephrotoxicity. However, the present study had certain limitations. We had limited time and availability of funds due to which other laboratory tests of renal function (urine analysis, plasma levels of albumin, serum electrolytes) and oxidative stress (malondialdehyde, Catalase) could not be performed. Therefore, further research is recommended to assess the various protective effects of AA on various blood and urine parameters as well as on other organ systems. Moreover, further studies are also recommended to explore the effects of AA in combination with other anti-nephrotoxic agents such as L-Arginine and Resveratrol etc.

Conclusion

The current study concluded that aspartame causes significant hazardous effects on the body, consequently affecting the normal renal tissue and ultimately resulting in severe nephrotoxicity. Histological changes also endorsed these findings and showed the serious damage and alterations in normal renal histology.

On the other hand, ascorbic acid use showed promising results and highly significant protective effects on renal functions and its histological reparations, when given with APM.

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