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Review Article
Health Implications Associated with Aspartame Consumption: A Substantial Review

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Abstract

Aspartame, an artificial sweetening agent belongs to dipeptide chemical category with a very strong sweetening potential. Although research findings in humans and non-human primates have demonstrated numerous negative effects of aspartame (biochemical, histological, neurological, behavioral, genetic etc)., the status of aspartame is still debatable. Present manuscript is a critical review of the substantial research findings related to aspartame intake on different research models. Purpose of this review was to spread the awareness about adverse effect of aspartame intake to outline the occurrence of health issues among the population. The process of uptake, storage, compartmentalization and distribution of aspartame within the body is associated with metabolic disorders and various clinical conditions. Available research literature indicates that higher amount of aspartame ingestion should be monitored carefully to avoid health implication within society.

Key words: Aspartame, artificial sweeteners, dose dependent toxicity, oxidative stress, systemic toxicity

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INTRODUCTION

Over a century ago, non-nutritive (NNS) or artificial sweeteners have been introduced to society for providing a sweet taste to foods without the associated high energy content of caloric sugars. Aspartame (L-aspartyl-L-phenylalanine methyl ester), being one of them, is a dipeptide artificial sweetener containing aspartic acid and phenylalanine methyl ester[1]. Although they have not replaced sugar completely, they became the preferred choice of a large set of population[2]. Aspartame is commercially available under Nutrasweet, Equal, Sugar-free, Canderel, like popular brand names. Aspartame is widely consumed by the population via, cold drinks, diet soda, sugar-free sweet products, medications, low-calorie sweet products etc.[3].

Aspartame is almost 200 times more potent than sucrose with a respective low caloric value (0.5% of kilocalorie of the respective amount of sugar). This property promotes the use of commercially available aspartame as a substitute for routine sugar since many decades. Upon the hydrolysis, aspartame breaks down inside the gastrointestinal lumen and gives rise to three chemical moieties named, phenylalanine (50%), aspartic acid (40%) and methanol (10%)[4]. Apart from the controversy[5,6], it is still most widely used artificial sweetener. The Present review is an effort to summarize the possible recapitulation of facts available in the form of literature related to aspartame consumption. Aspartame gained much popularity owing to their reduced costs, low caloric intake, attractive advertisements and promise to contribute to weight reduction[7]. For these reasons, aspartame increasingly got introduced into commonly consumed foods such as diet sodas, cereals and sugar-free desserts and is being recommended for weight loss and for individuals suffering from glucose intolerance and type 2 diabetes mellitus[8-11].

Research papers including review papers, original research papers and scientific reports published during the last decade (2006-2017) were thoroughly studied. A literature survey was performed using Google, Research Gate, Google Scholar, Medline, PubMed, PubMed Central like search engines. More than two hundred papers were collected during the study. Some of the potential evidences were included to justify the health implications associated with aspartame consumption.

RESEARCH ON HUMAN MODELS

Growing evidence against sugar-containing beverages consumption, responsible for increased weight gain, obesity, metabolic syndrome etc. prompted many individuals to resort to artificial, non-nutritive sweetener (NNSs) substitutes to reduce sugar/calorie intake without lost of sweetness. However, controversies followed NNSs consumption since the time of approval for use in foods, regarding the biological consequences of NNSs consumption. Increased evidence against the use of NNSs, suggest that NNSs interfere with feeding and metabolism through various physiological mechanisms. As a matter of fact, NNSs are not physiologically inert compounds and considering the potential biological mechanisms, NNSs consumption may affect a number of systemic functions like, energy balance and metabolism, mode of action on sweet taste receptors, metabolic hormone secretion, besides affecting cognitive processes like, learning, memory and taste perception and gut microbiota[12-14]. Research studies in which biological consequences of aspartame consumption in human models at different doses were studied report numerous direct/indirect undesirable after-effects resulting from aspartame consumption[15-18]. However, studies on human subgroups are limited to a narrow range of alterations[19]. There are not many research studies, which evaluated the biological effects of aspartame on human subjects at different concentrations[20-22]. Studies suggest a possible association between intake of artificially sweetened drinks (containing aspartame as one of the ingredients) and an increased risk of preterm delivery in normal-weight and overweight women, indicating a possible negative impact of aspartame consumption and its use especially in pregnancy[23].

The importance of incretin axes in case of progressive beta abnormalities, i.e., type 2 diabetes mellitus is significant due to the fact that the incretin effect is severely reduced or absent in patients with type 2 diabetes mellitus (T2DM). Enteroendocrine cells in the small intestine possess type 1 receptor 2 and 3 (T1R2 and T1R3) taste receptors. Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are significantly affected by non-nutritive sweeteners and glucose. Research studies showed that release of GLP-1 and GIP from taste receptors in L cells is triggered in presence of glucose. A cross-over study evaluated the metabolic effects of dissolved aspartame (36 mg) taken before meals in 54 prediabetic patients divided randomly into two groups. The two groups were evaluated for some biochemical (fasting and postprandial blood glucose, fructosamine, alanine aminotransferase (ALT), creatinine, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides and insulin) and physical (weight, height, waist circumference etc.) parameters at 0, 3 and 6 months. Diet given to group first was found effective in weight loss at the end of first three months. The weight loss continued after the addition of aspartame for the

next 3 months. The second group, fed with aspartame and diet, showed weight loss for the first three months. However, during the next three months, weight gain occurred after aspartame was discontinued. Comparing the weight loss in the two groups, it was found greater ($p = 0.027$). In the first group compared to second group wherein aspartame was discontinued for the next three months. No significant changes were found in other parameters. Thus, the effect of aspartame on weight loss as described by the study needs further long-term experimental studies for investigating the mechanism of the relationship between change in weight and incretins[24,25].

Also, normal people and phenylketonurics demonstrated a sharp increment in plasma phenylalanine levels after ingestion of aspartame[26,27]. Despite the fact that aspartame-containing items have an alert for phenylketonurics however there is no data about the genuine sweetener content. Hence there is a worry that aspartame ingestion by the general population who don't have PKU could deliver particularly expanded phenylalanine levels which are sufficient to hurt the cerebrum. This worry is more if there should be an occurrence of the evaluated 2% of the overall public who conveys the quality for phenylketonuria (PKU) and consequently have a lessened limit with respect to phenylalanine digestion. Concentrates in rats demonstrate that heights of plasma phenylalanine levels, as happened in people after aspartame ingestion, create an expansion in mind phenylalanine fixation[28,29]. Then again, the pinnacle plasma phenylalanine levels subsequent to stacking measurements of aspartame in ordinary people and in PKU heterozygotes are still impressively lower than those in rationally impeded patients with PKU. The greater part of the examinations in people on the impacts of aspartame on plasma amino acids have utilized high dosages of the (Phe PKU LNAA Phenylalanine Phenylketonuria Large unbiased amino acids) sweetener[30] and have announced increments in plasma phenylalanine levels up to fourfold in typical and ten times in PKU heterozygotes. Additionally, no examinations in people have tended to the impacts of aspartame on the plasma proportion of phenylalanine to the next extensive impartial amino acids (valine, leucine, isoleucine, tyrosine and tryptophan), which is the determinant of phenylalanine accessibility to the brain[31]. These observations demonstrate that an expected normal measurement of aspartame does not complement hyperphenylalaninemia in patients with untreated PKU or non-PKU hyperphenylalaninemia, yet produces measurably huge heights in the plasma phenylalanine level in typical subjects and in PKU bearers. The measurements of aspartame utilized, 10 mg kg^{-1}, is one fifth the present ADI (acceptable daily intake) level set by the Food and Drug Administration, it was chosen to approximate the normal maximal measure of aspartame that an adult would devour in 10-15 min. For a 60 kg adult, 10 mg kg^{-1} aspartame is available in somewhat more than one quart of Kool-Aid (34.5 oz) or in three cans of an aspartame-sweetened soft drink[32]. Research into the mechanisms of potential NNS effects with continued efforts would ease the research in the area of energy compensation. This would incorporate, however not be restricted to, expanding the assortment of learning of oral, gut and neural receptors and hormonal reactions that add to modifications in sustenance inclinations, craving direction and satiety that are evoked by NNS. Research toward this path ought to advise the determination of sustenance, refreshments, particular NNS (or blends) and the concentration must be, particular populace subsets for investigations of pay impacts[10].

RESEARCH ON MICE MODEL

As a matter of fact, animal models are used to test and check the safety status of anything to be used for human consumption. Therefore, numerous studies conducted before and after the approval of aspartame to be used as a food ingredient found that aspartame is responsible for the induction of hepato-carcinoma and bronchial carcinoma in mice model especially male mice when it was introduced prenatally (12th day of gestation) through the life span (until death) via feed. Aspartame induces significantly increased and dose-dependent incidence of hepatocellular carcinomas ($p<0.01$). This increased incidence was found to be more significant at a dose level of 32,000 ppm ($p<0.01$) and 16,000 ppm ($p<0.05$) in male mice. Studies revealed the significant dose-dependent occurrence of alveolar/bronchiolar carcinomas in males ($p<0.05$). However, no carcinogenic effects were observed in any female mice. In conclusion, the study confirms that aspartame could be a carcinogenic agent in multiple sites in male and female rodents[33]. It has also been observed that aspartame (50 mg kg^{-1} b.wt./day) alters glucose homeostasis by causing an increment in fasting blood glucose of 1.6-fold, with a significant reduction in insulin sensitivity during an insulin tolerance test (ITT). On further analysis, a strong correlation was found between body weight at 6 weeks and body weight and fasting glucose levels at 17 weeks, suggesting that early body weight may be a predictor of glucose homeostasis in later life[34]. The metabolic breakdown products (phenylalanine, aspartic acid and methanol) of aspartame-induced chromosomal aberration (CA) at all

concentrations (3.5, 35 and 350 mg kg^{-1} b.wt.) without any sister chromatid exchange (SCE) in the bone marrow cells of Swiss albino male mice. However, aspartame neither affects mitotic index nor induction of genotoxicity low concentrations[35,36].

Experimental studies on neurobehavioral and biochemical disturbance revealed that aspartame administration caused many changes in fear and anxiety behavior. Non-social and social behavior was reported to be significantly affected in exposed mice showing an increase in the former and a decrease in the later stages, respectively. Social behavioral elements (attack, number of fights and bites, Naso-nasal and Naso-genital contacts) were found to be decreased significantly. Reduced locomotor activity and neuromuscular coordination (grip strength) were observed in treated male albino mice compared to the control group. Moreover, hematological parameters (red blood cell count, packed cell volume, hemoglobin concentration, white blood cell count and platelets count) and testosterone hormone were significantly decreased followed by a decrease in acetyl cholinesterase enzyme activity in the treated males. All the above alterations significantly contribute to drawing the conclusion that aspartame as a food additive was threatening to mice in case of behavior and biochemical analysis and thus suggest for further scientific investigation on other parameters[37,38].

Aspartame induces changes in blood glucose levels, spatial learning and memory along with weight gain in C57BL/6J mice when exposed to chronic aspartame treatment beginning in utero[39]. Biological effects of aspartame consumption were determined by investigating the changes in the expressions of key oncogenes (c-myc and Ha-ras) and a tumor suppressor gene (p53) in 5 weeks old female inbred CBA/CA mice. Not only *in vivo* experiments but even some *in vitro* studies also support the role of aspartame in angiogenesis[40].

RESEARCH ON RAT MODELS

Systemic effects of aspartame administration on rat models suggest that aspartame caused significant changes in some oxidative stress parameters in many organs of male albino rats. Male albino rats treated with aspartame showed significant changes in some hepatic and renal oxidative parameters. Increased lipid peroxidation (LPO) levels were observed in the liver tissue after 4 and 6 weeks of aspartame administration. A significant decrease in lipid peroxidation (LPO) level after 2 weeks was followed by a significant increase in the renal tissue after 6 weeks of aspartame administration. Significant decrease in hepatic tissue superoxide dismutase (SOD) activity and a significant superoxide dismutase SOD and catalase CAT activity in the renal tissue, following 2, 4 and 6 weeks of aspartame treatment was also reported. Significant decrease in glutathione (GSH) content alongside a significant increase in the glutathione-S-transferase (GST) activity in liver tissue after 2, 4 and 6 weeks was attributed to aspartame exposure[41]. Aspartame administration (54.8 ± 7.3 mg kg^{-1} b.wt., day) resulted into elevated blood pressure, increased body weight, short-term increase in the blood pressure and in plasma values of glucose and triglycerides accompanied by a transitory reduction in plasma urea, which may affect cardiovascular risk factors[42]. However, long-term consumption of aspartame could lead to an increment in glycemic and lipid profile, followed by erythrocytes oxidative stress. Increased value of reduced glutathione (GSH) and increased activity of catalase (CAT) were also proved to be associated aspartame administration. Aspartame administration (40 mg kg^{-1} b.wt.) results in increased values of serum glucose, cholesterol and triglycerides compared to control[43].

A significant increase in free radical production due to oral administration of aspartame (40 mg kg^{-1} b.wt.) to folate-deficient rats for 15, 30 and 90 days consecutive days to mimic human methanol metabolism was observed. This increase was found to be responsible for affecting both the enzymatic (superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic (reduced glutathione and vitamin C) anti-oxidant status in various immune organs such as the lymph nodes, bone marrow, spleen, thymus of rats. A marked increase in lipid peroxidation and nitric oxide level was also reported. A marked decrease (especially in 30 and 90 days group) in the body weight of aspartame treated groups was observed compared to control and folate-deficient rats. Histological degeneration (cellular disruption and degeneration of the white pulp) of spleen and lymph nodes in aspartame-treated group (30 and 90 days) was also reported in this study[44].

Aspartame induces morphometric alterations in the rat fetal kidney during organogenesis. Significant nuclear variations in the cells of the glomerulus, proximal and distal convoluted tubules and collecting ducts were observed in the kidneys of aspartame-treated rat fetuses. Aspartame treatment resulted to significantly increased cell volume and decreased numerical cell density in fetal kidneys of rats. Serological parameters showed statistically significantly increased cell volume and decreased numerical cell density in fetal kidneys of rats treated with aspartame heated to 40° compared to controls. These findings revealed that the use of aspartame leads to nephrotoxicity[45]. Furthermore, evaluation

of the effects of aspartame on the fetal exocrine pancreas, through a morphometric study, at the end of gestational development demonstrated an alteration in minor diameter, mean diameter, ratio D/d, nuclear volume, perimeter, volume-area ratio, eccentricity, nuclear area[46].

Consecutive oral administration of aspartame 40 mg kg^{-1} b.wt., for 2, 4 and 6 weeks induced neurotoxicity, oxidative stress and inflammation in rat brain tissue, which was indicated by the significant increment in protein carbonyl content in association with a significant decrease in reduced glutathione concentration ($p<0.5$). Also, there was a significant increase in brain interleukin-1 IL-β and tumor necrosis factor-α (TNF-α) production accompanied with a significant decrease in brain-derived neurotropic factor (BDNF) and serotonin levels in aspartame treated rats for 6 weeks compared to 4 and 2 weeks intervals and their corresponding control rats ($p<0.05$). Furthermore, acetylcholine esterase (AchE) activity was significantly decreased accompanied by a significant increase in acetylcholine (Ach) concentration in brain homogenates in aspartame-administrated groups for 4 and 6 weeks[47].

Light and electron microscopic histological study revealed that oral administration of aspartame 250 mg kg^{-1}/day for 6 weeks could result in the marked affection of the frontal cortex. The pyramidal cells of the aspartame-treated animals showed significant morphological necrotic changes and appeared darkly stained or vacuolated, irregular in shape with pyknotic or faint nuclei. Neurons in the aspartame group were statistically significantly less stained by anti-neuron specific enolase (NSE) antibody than the control group. Glial fibrillary acidic protein (GFAP) immune-reactive astrocytes were also detected in a significant number. The authors conclude that the content of NSE of neurons and the number of glial fibrillary acidic protein (GFAP) (+) astrocytes could serve as molecular markers for neuronal injury, regeneration and astrocytes proliferation, respectively[48].

Aspartame administration at three different concentrations 15 mg, 35 mg and 70 mg kg^{-1} b.wt./day for 9 weeks was reported to enhance lipid imbalance in rats via the mechanism that involves oxidative stress and depletion of the glutathione-dependent system. Routine antioxidant parameters, viz., glutathione-s-transferase, glutathione peroxidase, superoxide dismutase, catalase and reduced glutathione of brain, kidney and liver were significantly affected. In addition, higher dose of aspartame significantly increased the levels of total cholesterol, triglycerides and low-density lipoprotein, alanine aminotransferase by 66 and 117%, aspartate aminotransferase by 21 and 48%, urea by 72 and 58%, gamma glutamyl-transferase 70 and 85% and conjugated bilirubin by 63 and 64%, respectively. Brain necrotic lesions, degeneration of hepato-architecture and nephro-architecture with monocytes infiltration were prominent in rats after aspartame treatment[49].

Chronic aspartame (50 mg and 1000 mg kg^{-1} b.wt., for 180 consecutive days) administration induced an alteration in ionic homeostasis, regional monoamine neurotransmitter concentrations, tyrosine hydroxylase activity and amino acids levels and monoamine synthesis, along with apoptosis in the brain. This dose-dependent alteration may affect electrolyte homeostasis and monoamine neurotransmitter synthesis which might affect cognitive functions[50].

OTHER INVESTIGATIONS

Very recent investigations indicate that even low intake of aspartame 6, 11 and 18 mg kg^{-1} b.wt./day for 42 days was able to increase appetite and weight gain and induced histopathological changes in brain and liver cells in Syrian weanling hamsters. A significant decrease was observed in total cholesterol, HDL levels treated groups together with a non-significant reduction in glucose concentration[51]. The metabolic products of aspartame, i.e., phenylalanine, aspartic acid and methanol are involved in the basic mechanism of aspartame-induced toxicity. Aspartame metabolites, aspartic acid, phenylalanine and diketopiperazine are responsible for the degeneration of neurons and astrocytes[52].

Studies report increased gene expressions in the genes investigated due to oral administration of different doses (40, 200 and 2500 mg kg^{-1} b.wt.) of aspartame. Organs such as lympho-reticular organs, bone marrow and kidney were found more sensitive due to their higher proliferation rate. The remarkable increase in gene expression was reported in liver, spleen and lungs only at a dose of 200 mg kg^{-1} b.wt. Inadequate absorption of aspartame 2500 mg kg^{-1} b.wt., was responsible to developed osmotic diarrhea, which according to the studies is responsible for lower gene expression at the high dose level. The authors conclude that aspartame is capable of inducing biological effects even at the admissible daily intake. Thus, any detectable biological effect due to the administration of aspartame would suggest the possibility of potential cancer-inducing effect[53].

CONCLUSION

Till date, the status of aspartame remained controversial due to the availability of research data in support and against its use. However, the adverse after-effects of aspartame consumption are clearly evident by the research done on the

human as well as animal models. Investigations are highly recommended in related novel directions to fulfill the existing research gaps to end the controversy related to aspartame use. The critical evaluation of the literature available in support of aspartame usage seems partially influenced by the producers or the funding agencies. Authors suggest bias-free comprehensive experiments for the safety assessment of aspartame on the different set of populations with different clinical conditions.

Therefore, authorities like the U.S. Food and Drug Administration (FDA), the European Food Safety Authority (EFSA) and the AgenceFrançaise de Sécurité Sanitaire des Aliments (French Food Safety Agency-AFSSA) the FSSAI (Food Safety and Standard Authority of India) Joint FAO/WHO Expert Committee on Food Additives (JECFA), should reconsider the acceptable daily intake (ADI) of aspartame among population.

SIGNIFICANCE STATEMENT

This study discovers the adverse effects of aspartame consumption on human as well as animal model in a single manuscript. No article is available till date to summarize the unbiased balanced status of aspartame consumption on various systems including human, rat, mice and others. Present study is not only beneficial for the researchers but it can be beneficial for the global mass to create awareness about possible health issues related to unbalanced, unrestricted aspartame consumption. Till date, the status of aspartame remained controversial due to the availability of research data in support and against its use. However, the adverse after effects of aspartame consumption are clearly evident by the research done on the human as well as animal models. Investigations are highly recommended in related novel directions to fulfill the existing research gaps to end the controversy related to aspartame use. Present review article is significant and novel as it incorporates various types of literatures available in an unbiased and comprehensive way which collectively represents various hypothesis and ideas along with research findings related to aspartame consumption. This study will help the researcher to uncover the critical areas of aspartame related harms at various doses as present article summarize various doses of aspartame on which various systems gets affected adversely. This review also beneficial for revealing serious cases of metabolism, glucose homeostasis, effects of aspartame metabolites individually, that many researchers were not able to explore. Thus a new theory on determining a safe or an alternative ADI of aspartame with negligible or no adverse effects may be arrived at.

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REFERENCES

1. Magnuson, B.A., G.A. Burdock, J. Doull, R.M. Kroes and G.M. Marsh *et al*., 2007. Aspartame: A safety evaluation based on current use levels, regulations and toxicological and epidemiological studies. Crit. Rev. Toxicol., 37: 629-727.
2. Mattes, R.D. and B.M. Popkin, 2008. Nonnutritive sweetener consumption in humans: Effects on appetite and food intake and their putative mechanisms. Am. J. Clin. Nutr., 89: 1-14.
3. El Haliem, N.G. and D.S. Mohamed, 2011. The effect of aspartame on the histological structure of the liver and renal cortex of adult male albino rat and the possible protective effect of *Pimpinella anisum* oil. Egypt. J. Histol., 34: 715-726.
4. Abd Elfatah, A.A.M., I.S. Ghaly and S.M. Hanafy, 2012. Cytotoxic effect of aspartame (diet sweet) on the histological and genetic structures of female albino rats and their offspring. Pak. J. Biol. Sci., 15: 904-918.
5. Whitehouse, C.R., J. Boullata and L.A. McCauley, 2008. The potential toxicity of artificial sweeteners. Aaohn J., 56: 251-261.
6. Tandel, K.R., 2011. Sugar substitutes: Health controversy over perceived benefits. J. Pharmacol. Pharmacother., 2: 236-243.
7. Hunty, A.D.L., S. Gibson and M. Ashwell, 2006. A review of the effectiveness of aspartame in helping with weight control. Nutr. Bull., 3: 115-128.
8. Duffey, K.J., L.M. Steffen, L. Van Horn, D.R. Jacobs, Jr. and B.M. Popkin, 2012. Dietary patterns matter: Diet beverages and cardiometabolic risks in the longitudinal Coronary Artery Risk Development in Young Adults (CARDIA) study. Am. J. Clin. Nutr., 95: 909-915.
9. Fitch, C. and K.S. Keim, 2012. Position of the academy of nutrition and dietetics: Use of nutritive and nonnutritive sweeteners. J. Acad. Nutr. Dietet., 112: 739-758.
10. Gardner, C., J. Wylie-Rosett, S.S. Gidding, L.M. Steffen, R.K. Johnson, D. Reader and A.H. Lichtenstein, 2012. Nonnutritive sweeteners: Current use and health perspectives: A scientific statement from the American Heart Association and the American Diabetes Association. Circulation, 126: 509-519.
11. Zafar, T., Q.A.B. Naik and V.K. Shrivastava, 2017. Aspartame: Effects and awareness. MOJ. Toxicol., Vol. 3. 10.15406/mojt.2017.03.00046.
12. Burke, M.V. and D.M. Small, 2015. Physiological mechanisms by which non-nutritive sweeteners may impact body weight and metabolism. Physiol. Behav., 152: 381-388.

13. Zafar, T. and V.K. Shrivastava, 2017. Does monosodium glutamate induce obesity in female mice? Iran. J. Diabet. Obesity, 9: 112-116.
14. Zafar, T. and V.K. Shrivastava, 2017. Effect of monosodium glutamate consumption on health of female mice population. J. Macro Trends Applied Sci., 5: 11-16.
15. Fowler, S.P., K. Williams, R.G. Resendez, K.J. Hunt, H.P. Hazuda and M.P. Stern, 2008. Fueling the obesity epidemic? Artificially sweetened beverage use and long-term weight gain. Obesity, 16: 1894-1900.
16. Laska, M.N., D.M. Murray, L.A. Lytle and L.J. Harnack, 2012. Longitudinal associations between key dietary behaviors and weight gain over time: Transitions through the adolescent years. Obesity, 20: 118-125.
17. Cohen, L., G. Curhan and J. Forman, 2012. Association of sweetened beverage intake with incident hypertension. J. General Internal Med., 27: 1127-1134.
18. Miller, P.E. and V. Perez, 2014. Low-calorie sweeteners and body weight and composition: A meta-analysis of randomized controlled trials and prospective cohort studies. Am. J. Clin. Nutr., 100: 765-777.
19. Fagherazzi, G., A. Vilier, D. Saes Sartorelli, M. Lajous, B. Balkau and F. Clavel-Chapelon, 2013. Consumption of artificially and sugar-sweetened beverages and incident type 2 diabetes in the Etude Epidemiologique aupres des femmes de la Mutuelle Generale de l'Education Nationale-European Prospective Investigation into cancer and nutrition cohort. Am. J. Clin. Nutr., 97: 517-523.
20. Dhingra, R., L. Sullivan, P.F. Jacques, T.J. Wang and C.S. Fox *et al*., 2007. Soft drink consumption and risk of developing cardiometabolic risk factors and the metabolic syndrome in middle-aged adults in the community. Circulation, 116: 480-488.
21. Lutsey, P.L., L.M. Steffen and J. Stevens, 2008. Dietary intake and the development of the metabolic syndrome: The atherosclerosis risk in communities study. Circulation, 117: 754-761.
22. De Oliveira Otto, M.C., D. Mozaffarian, D. Kromhout, A.G. Bertoni, C.T. Sibley, D.R. Jacobs, Jr. and J.A. Nettleton, 2012. Dietary intake of saturated fat by food source and incident cardiovascular disease: The multi-ethnic study of atherosclerosis. Am. J. Clin. Nutr., 96: 397-404.
23. Halldorsson, T.I., M. Strom, S.B. Petersen and S.F. Olsen, 2010. Intake of artificially sweetened soft drinks and risk of preterm delivery: A prospective cohort study in 59,334 Danish pregnant women. Am. J. Clin. Nut., 92: 626-633.
24. Koyuncu, B.U. and M.K. Balci, 2014. Metabolic effects of dissolved aspartame in the mouth before meals in prediabetic patients; a randomized controlled cross-over study. J. Endocrinol. Diabet. Obes., Vol. 2.
25. Brown, R.J. and K.I. Rother, 2012. Non-nutritive sweeteners and their role in the gastrointestinal tract. J. Clin. Endocrinol. Metab., 97: 2597-2605.
26. Stegink, L.D., L.J. Filer, Jr. and G.L. Baker, 1977. Effect of aspartame and aspartate loading upon plasma and erythrocyte free amino acid levels in normal adult volunteers. J. Nutr., 107: 1837-1845.
27. Koch, R., G. Schaeffler and K.N.F. Shaw, 1976. Results of loading doses of aspartame by two phenylketonuric (PKU) children compared with two normal children. J. Toxicol. Environ. Health, 2: 459-469.
28. Glaeser, B.S., T.J. Maher and R.J. Wurtman, 1983. Changes in brain levels of acidic, basic and neutral amino acids after consumption of single meals containing various proportions of protein. J. Neurochem., 41: 1016-1021.
29. Yokogoshi, H., C.H. Roberts, B. Caballero and R.J. Wurtman, 1984. Effects of aspartame and glucose on plasma and brain large neutral amino acids and brain hydroxyl indoles. Am. J. Clin. Nutr., 40: 1-17.
30. Stegink, L.D., L.J. Filer, Jr., G.L. Baker and J.E. Mcdonnell, 1980. Effect of an abuse dose of aspartame upon plasma and erythrocyte levels of amino acids in phenylketonuric heterozygous and normal adults. J. Nutr., 110: 2216-2224.
31. Partridge, W.M., 1977. Regulation of Amino Acid Availability to the Brain. In: Nutrition and the Brain, Wurtman, R.J. and J.J. Wurtman (Eds.)., Vol. 1, Raven Press, New York., pp: 141-204.
32. Caballero, B., B.E. Mahon, A.B.F.J.R. Rohar, H.L. Levy and R.J. Wurtman, 1986. Plasma amino acid levels after single-dose aspartame consumption in phenylketonuria, mild hyperphenylalaninemia and heterozygous state for phenylketonuria. J. Pediatric., 109: 668-671.
33. Soffritti, M., F. Belpoggi, M. Manservigi, E. Tibaldi, M. Lauriola, L. Falcioni and L. Bua, 2010. Aspartame administered in feed, beginning prenatally through life span, induces cancers of the liver and lung in male Swiss mice. Am. J. Ind. Med., 53: 1197-1206.
34. Collison, K.S., N.J. Makhoul, M.Z. Zaidi, R. Al-Rabiah and A. Inglis *et al*., 2012. Interactive effects of neonatal exposure to monosodium glutamate and aspartame on glucose homeostasis. Nutr. Metab, Vol. 9. 10.1186/1743-7075-9-58.
35. AlSuhaibani, E.S., 2010. *In vivo* cytogenetic studies on aspartame. Comp. Funct. Genomics, 10.1155/2010/605921.
36. Tabassum, Z., K.S. Vinoy and G. Narayanan, 2016. Protective effect of *Persia Americana* Mill. Fruit extract against 9 Gy gamma radiation insult in swiss albino mice. Int. J. Adv. Res., 4: 615-623.
37. Abu-Taweel, G.M., 2016. Effect of monosodium glutamate and aspartame on behavioral and biochemical parameters of male albino mice. Afr. J. Biotechnol., 15: 601-612.

38. Zafar, T. and V.K. Shrivastava, 2017. Monosodium glutamate induced haematological alterations in female swiss albino mice *Mus musculus*. Ann. Res. Rev. Biol., 20: 1-9.
39. Collison, K.S., N.J. Makhoul, M.Z. Zaidi, A. Inglis and B.L. Andres *et al*., 2013. Prediabetic changes in gene expression induced by aspartame and monosodium glutamate in *Trans* fat-fed C57Bl/6 J mice. Nutr. Metab., Vol. 10. 10.1186/1743-7075-10-44.
40. Alleva, R., B. Borghi, L. Santarelli, E. Strafella, D. Carbonari, M. Bracci and M. Tomasetti, 2011. *In vitro* effect of aspartame in angiogenesis induction. Toxicol. *In vitro*, 25: 286-293.
41. Iman, M.M., 2011. Effect of aspartame on some oxidative stress parameters in liver and kidney of rats. Afr. J. Pharm. Pharmacol., 5: 678-682.
42. Martinez-Morales, F., E. Maldonado-Cervantes, M.A. Isiordia-Espinoza and O.H. Aragon-Martinez, 2015. Nutritional and biochemical effects of aspartame intake in rats under an experimental diet. J. Exp. Biol. Agric. Sci., 3: 298-306.
43. Prokic, M.D., M.G. Paunovic, M.M. Matic, N.Z. Djordjevic, B.I. Ognjanovic, A.S. Stajn and Z.S. Saicic, 2014. Prooxidative effects of aspartame on antioxidant defense status in erythrocytes of rats. J. Biosci., 39: 859-866.
44. Choudhary, A.K. and R.S. Devi, 2014. Imbalance of the oxidant-antioxidant status by aspartame in the organs of immune system of Wistar albino rats. Afr. J. Pharm. Pharmacol., 8: 220-230.
45. Martins, M.R.I., R. Azoubel, M. Martins and R. Azoubel, 2007. Effects of aspartame on fetal kidney: A morphometric and stereological study. Int. J. Morphol., 25: 689-694.
46. De Lima Leme, F.A.G. and R. Azoubel, 2006. Effects of aspartame on the exocrine pancreas of rat fetuses. Int. J. Morphol., 24: 679-684.
47. Kamel, E.A., 2015. Inflammation and neurochemical alterations induced by oral aspartame administration in experimental rats. Indian J. Applied Res., 5: 750-753.
48. Omar, S.M.M., 2009. Effect of aspartame on the frontal cortex of adult male albino rats. A light and electron microscopic study. Egypt. J. Histol., 32: 346-357.
49. Adaramoye, O.A. and O.O. Akanni, 2016. Effects of long-term administration of aspartame on biochemical indices, lipid profile and redox status of cellular system of male rats. J. Basic Clin. Physiol. Pharmacol., 27: 29-37.
50. Abhilash, M., M. Alex, W. Mathews and R.H. Nair, 2014. Chronic effect of aspartame on ionic homeostasis and monoamine neurotransmitters in the rat brain. Int. J. Toxicol., 33: 332-341.
51. Hassan, M.I., 2016. Low intake of aspartame induced weight gain and damage of brain and liver cells in weanling syrian hamsters. J. Food Nutr. Res., 4: 152-156.
52. Rycerz, K. and J.E. Jaworska-Adamu, 2013. Effects of aspartame metabolites on astrocytes and neurons. Folia Neuropathol., 51: 10-17.
53. Gombos, K., T. Varjas, Z. Orsos, E. Polyak and J. Peredi *et al*., 2007. The effect of aspartame administration on oncogene and suppressor gene expressions. In vivo, 21: 89-92.