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Research Article

Safety Dose Effect of Combining Some Artificial Sweeteners on Health by Rats

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Abstract

Artificial sweeteners (ASs), offer the sweetness of sugar without the calories. This research was to investigate the effect of mixing some the most widely used Ass on health using rat's models. 48 adult male albino rats fed on standard diet (one week) for adaptation then, they were divided into six groups (n=8). First group fed on standard diet only as a control group. Second group fed standard diet plus Aspartame (ASP), Acesulfame-k (ACS-k), and Sucralose (SUC). Third group fed standard diet +ASP, ACS-k and Saccharin (SAC). Fourth group fed standard diet + ACS-k, SUC and SAC. Fifth group fed standard diet + ASP, SUC and SAC. Sixth group fed standard diet + ASP, ACS-k, SUC and SAC for 4 weeks. Glutathione (GSH) and Malondialdehyde (MDA) were evaluated in liver, kidney and brain tissue of rats. Histopathology was examinated in same organs and others for all groups. GSH and MDA values of liver and kidney tissues had significant differences while brain tissue has no significant change between all groups. Histopathologic changes were mostly encountered in brain, liver, kidneys and esophagus. This study showed that organs tissue has changed it structure with safety dose from multi types of ASs.

INTRODUCTION

Artificial sweeteners (ASs) are substances used to replace sugar in foods and beverages. They can be divided into two large groups: nutritive sweeteners, which add some energy value (calories) to food; and non-nutritive sweeteners, which are also called high-intensity sweeteners because they are used in very small quantities, adding no energy value to food [1]. (ASs) are synthetic sugar substitutes but may be derived from naturally occurring substances, including herbs or sugar itself. (ASs) are also known as intense sweeteners because they are many times sweeter than regular sugar [2].

(ASs) were introduced over a century ago as means for providing sweet taste to foods without the associated high energy content of caloric sugars. ASs consumption gained much popularity owing to their reduced costs, low caloric intake and perceived health benefits for weight reduction and normalization of blood sugar levels. For these reasons, ASs are increasingly introduced into commonly consumed foods such as diet sodas, cereals and sugar-free desserts, and are being recommended for weight loss and for individuals suffering from glucose intolerance and type 2 diabetes mellitus [3] .

There are five (ASs) that have been tested and approved by the U.S. Food and Drug Administration (FDA): ACS-K, ASP, SAC, SUC, neotame. The FDA (2014) established Acceptable Daily Intake (ADI) levels for the approved high-intensity sweeteners. ADI is the amount of the high-intensity sweetener that is considered safe to consume each day over the course of a person's lifetime. Acceptable Daily Intake (ADI) is in milligrams per kilogram body weight per day (mg/kg BW/d) [4].

Sucralose (SUC) is approved for use in food as a non-nutritive sweetener. SUC is approximately 600 times as sweet as sucrose. SUC is a general purpose sweetener that can be found in a variety of foods including baked goods, beverages, chewing gum, gelatins, and frozen dairy desserts. It is heat stable, meaning that it stays sweet even when used at high temperatures during baking. ADI: is 5 (mg/kg BW/d) [4].

Saccharin (SAC) is an artificial sweetener. The basic substance, benzoic sulfilimine, has effectively no food energy and it is 200 to 700 times sweeter than table sugar (sucrose), but has a bitter or metallic aftertaste, especially at high concentrations. ADI: is 15 (mg/kg BW/d) [4].

Acesulfame-K (ACS-K) and aspartame (ASP) are characterized as artificial high-intensity sweeteners. They are also called nonnutritive sweeteners. It is about 200 times sweeter than

sugar and is often combined with other sweeteners.ASP (NL-alpha-Aspartyl-L-phenylalanine 1- methyl ester) is a synthetic sweetener of low caloric value formed from the union of two amino acids, aspartic acid and phenylalanine, with sweetening power 180 to 200 times greater than that of sucrose [5]. ADI of ACS-K: is 15 (mg/kg bw/d) As ADI of ASP: is 50 (mg/kg bw/d) respectively [4].

Some studies showed benefits for ASs consumption [6] and little induction of a glycemic response [7], whereas others demonstrated associations between ASs consumption and weight gain [8] and increased type 2 diabetes risk [9].

However, interpretation is complicated by the fact that (ASs) are typically consumed by individuals already suffering from metabolic syndrome manifestations. Most ASs pass through the human gastrointestinal tract without being digested by the host [10,11] and thus directly encounter the intestinal microbiota, which plays central roles in regulating multiple physiological processes [12]. Microbiota composition [13] and function [14] are modulated by diet in the healthy/lean state as well as in obesity [15,16] and diabetes mellitus [17], and in turn microbiota alterations have been associated with propensity to metabolic syndrome [18].

Consuming artificial sweeteners is associated with negative long-term effects on weight, heart disease, and diabetic [19].

AIM OF SEARCH

The sweeteners may be better for some people than others, and their use may depend on a person's individual health profile and prospects. For these reasons this study was carried out to investigate the effects of the most widely used Ass with ADI as a safety dose intake by rats for a reassessment of massive ASs usage, also the effects of combined ASs.

MATERIALS AND METHODS

Materials

- Sweeteners: ASP, ACS-K, SUC and SAC; Chemicals and other materials: DL-methionine, choline chloride, vitamins, and minerals were obtained from Morgan and El-Gomhorya Company for Chemicals, Cairo, Egypt.
- Skimmed milk and corn starch purchased from local market- Cairo. Egypt.
- Animals: forty eight healthy adult male albino rats "Sprague Dawley strain" weighing 150 ± 10g obtained from the animal colony, Helwan Farm, Vaccine and Immunity Organization, Helwan Governorate, Egypt.

The standard diet prepared as described by Reeves et al. [20].

Methods

Biological experiment: Forty eight healthy adult male albino rats "Sprague Dawley strain" kept in single wire cages with wire bottoms under hygienic conditions and controlled laboratory conditions of temperature (25°C), lighting and ventilation. Food and water were provided ad-libitum and checked daily and was approved by the experiments animal unit- National Nutrition Institute, Cairo Governorate, Egypt.

Experimental design: Adult male albino rats fed on standard diet for one week for adaptation then, they were divided into six groups (n=8). The first group fed on standard diet only and served as control group. The second group fed standard diet with added combined safety dose from ASP, ACS- k, and SUC. The third group fed standard diet with added combined safety dose from ASP, ACS- k and SAC. The forth group fed standard diet with added combined safety dose from ACS- k, SUC and SAC. The fifth group fed standard diet with added combined safety dose from ASP, SUC and SAC. The sixth group fed standard diet with added combined safety dose from ASP (50mg/kg BW/d), ACS- k (15mg/kg BW/d), SUC (5mg/kg BW/d) and SAC (15mg/kg BW/d) according to **FDA UAS** [4] each time segment of the experiment.

At the end of the experiment period (four weeks), the animals were sacrificed after being fasted (overnight). The organs (liver, kidney, testes, spleen, brain, stomach and esophagus) of each animal was quickly removed by careful dissection, washed in saline solution (0.9%), dried using filter paper then rapidly weighed and the portion from which put in 10% formaldehyde to examine histopathology and other portion from liver, kidney, and brain frozen at -20°C until biochemical analyzed.

Biochemical analysis: The assay of glutathione reduced levels was performed using the spectrophotometric method [21]. It depends on the reduction of 5, 5'-dithiobis 2-nitrobenzoic acid with glutathione to produce a yellow color whose absorbance is measured at 405nm. Lipid peroxidation as Malondialdehyde (MDA) reacts with thiobarbituric acid (TBA) in acidic medium giving a pink colored TBA- complex that could be measured calorimetrically according to the method of Uchiyama and Mihara [22].

Histopathological examination: Tissues were dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. Paraffin blocks were prepared and serially sectioned into 4 microns thick sections, then stained with hematoxylin and eosin for routine histopathologic study [23].

Statistical analysis: The data were expressed as means ± standard error (mean ± SE). All variables were tested for normal distribution using one way analysis of variance (ANOVA) ($P<0.05$). If the groups showed significant differences, Turkey's multiple comparison test was performed [24]. Statistical analysis was carried out using the program of Statistical Package for the Social Sciences (SPSS), PC statistical software (Version 16; Untitled–SPSS Data Editor).

RESULTS AND DISCUSSION

The present study showed the effect of oral administration of safety dose of mix some ASs on oxidative stress biomarkers (GSH and MDA) for liver, kidney and brain tissues of male albino rats at time periods.

Data in (Figure 1) represented those oxidative stress biomarkers like GSH value in the liver, kidney and brain tissues after four weeks of mix ASs administration. Groups fed standard diet with added different safety doses of mix ASs had lower value of tissues GSH than normal group. These differences among normal group and other groups were significant except GSH values in brain tissues. (Group 6) had the lowest level of GSH

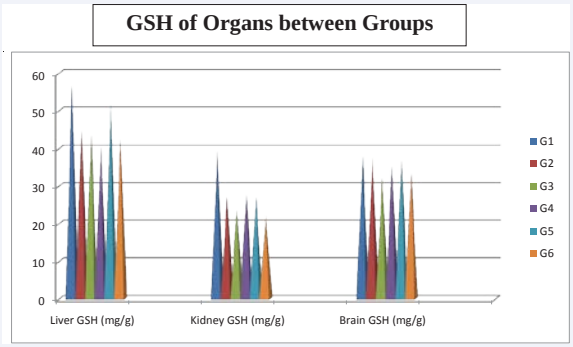

Figure 1 Mean of organs (liver, kidney and brain) tissues glutathione (GSH).

in kidney tissue and which was significantly different between other groups except (Group 3) fed standard diet with added mix from ASP, ACS- k and SAC. Brain tissue GSH value of groups orally with combined alternative sweeteners was lower than normal group and these differences were no-significant.ASP one of ASs was metabolized and broken down in the gastrointestinal tract to its constituents (i.e., aspartic acid, phenylalanine, and methanol) [25]. Given that ASP is completely metabolized in the gastrointestinal tract to phenylalanine, aspartic acid, and methanol, it may be concluded that the observed the oxidative stress markers were caused not by ASP itself but rather by its metabolites. In particular, it cannot be disregarded that the conversion of ASP methanol into formaldehyde in the liver may result in a generation of formaldehyde adducts [26]. Stegink and Filer [27], reported that the phenylalanine formed in the intestine following ingestion of ASP is excreted in the form of CO_2; most of it is incorporated into the pool of amino acids and contributes to protein synthesis like glutathione**.** Methanol is primarily metabolized by oxidation to formaldehyde and then to format, these processes are accompanied by the formation of superoxide anion and hydrogen peroxide [28]. Pankow and Jagielki [29] recorded the decrease in GSH activity have been caused by methanol, because methanol metabolism depends upon presence GSH**.** While Acesulfame K consists of a 1,2,3-oxathiazine ring, a six-heterocyclic system in which oxygen, sulfur, and nitrogen atoms are adjacent to each another [30]. The negative effects exerted upon hexitol metabolism by saccharin, and acesulfame k, may be due to their collective role in decreasing intracellular levels of phosphoenolpyruvate (PEP**)** [31]. These results were compatible with the study of Neacşu and Madar [31] who reported that the ASs are very unhealthy despite the fact that human consume them every day, and they are present almost in every sweet-flavored food.

Results in (Figure 2) were showed the differences among normal group and all groups remedy with mix non-nutritive sweeteners of tissue MDA level in liver, kidney and brain. MDA values showed that it's were the highest level in all organs examined of groups fed blend safety dose of ASs. There were significant differences between normal group and treated groups of tissue MDA in liver and kidney while brain tissue MDA wasn't significant. (Group 3) had the lowest value of kidney tissue MDA and had significant differences with compare treatment groups and normal group. The increase in MDA level observed in this study, which is an index of lipid oxidation, indicated organs cell membrane damage after ASP and other sweeteners within ASs administration. This is in harmony with Parthasarathy et al. [33], who showed that methanol administration significantly increased MDA level in the lymphoid organs; in addition, Zararsiz et al. [34], who observed a significant increase in MDA level in the kidney of rats after treatment with formaldehyde Abdel-Salam et al. [35], recorded that findings suggest impaired memory performance and increased brain oxidative stress by repeated aspartame administration. The impaired memory performance is likely to involve increased oxidative stress as well as decreased brain glucose availability. Saccharin has been found to cause bladder cancer in male rats and is considered a possible carcinogen by the American Environmental Protection Agency. In the US, food containing saccharin must be labeled with a warning that use of this product may be hazardous to health and has caused cancer in laboratory animals [36]. The toxicity of sweeteners has become increasing and has attracted the concern of many scientists to study their toxic effect. Several previous studies have revealed that the use of artificial sweeteners may entail some hazards to the users [37].

Histopathological results

Histopathologic changes were mostly encountered in liver, brain, kidneys and esophagus. N.B. Atypia (mild dysplasia) was noticed in brain, liver and esophagus.

Figure 3 Liver tissues, in all treated groups; with hyperchromatic nuclei, coarse clumped chromatin and increased nuclear: cytoplasmic ratio. Bi-nucleated liver cells were also noticed. Liver fibrosis was noticed in (Group 5,6) with fibrous tissue septae extending from portal tract fibrosis, entangling inflammatory cells.

A: (Group 1) Control rat liver showing preserved liver architecture. A central vein is seen surrounded by hepatocytes arranged in single cell plate pattern. Nuclei are bland, vesicular with ordinary nuclear: cytoplasmic ratio.

B: (Group 2) rat liver showing preserved liver architecture. Hepatocytes showed nuclei with prominent basophilia and clumped coarse chromatin. Scattered hepatocytes showed binucleation. Hypertrophied Kupffer cells are seen.

C: (Group 3) rat liver showing binucleated hepatocytes with increased nuclear: cytoplasmic ratio**.**

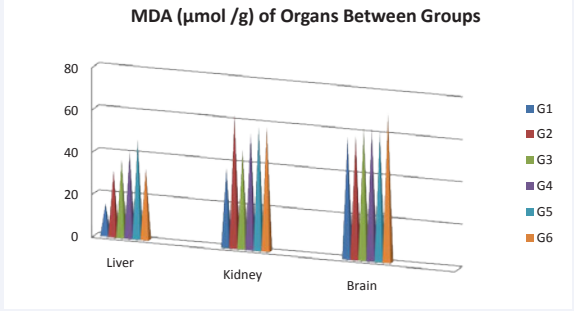

Figure 2 Mean of organs (liver, kidney and brain) tissue malondialdehyde (MDA).

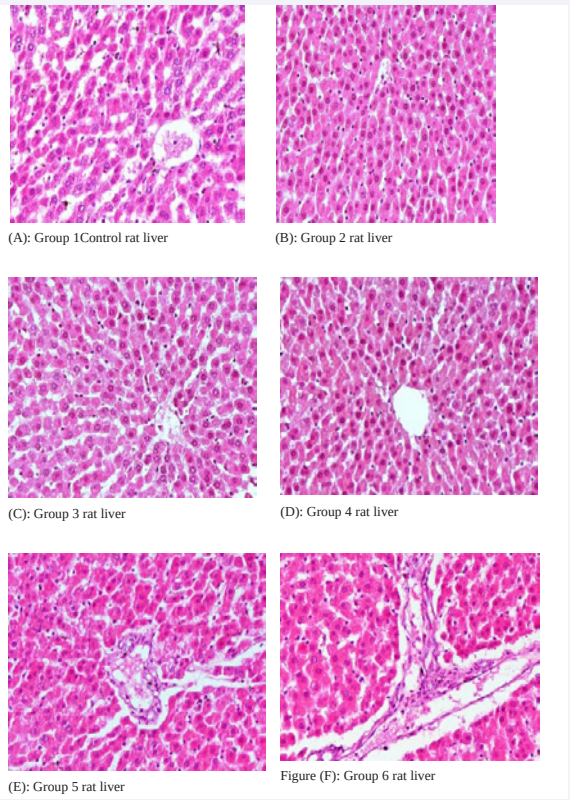

Figure 3 Liver histology.

D: (Group 4) rat liver showing hepatocytes with nuclei showing deep basophilia, hyperchromasia and increased nuclear: cytoplasmic ratio.

E: (Group 5) rat liver showing mild fibrosis with scattered chronic inflammatory cells.

F: (Group 6) rat liver showing moderate fibrosis within portal tract with fibrous tissue septae extending into liver tissue. Scattered chronic inflammatory cells are seen. Binucleated liver cells are observed. Liver has a major role in aspartame metabolism. Degeneration is a disturbance in the metabolism of the cell resulting in morphologic abnormalities. Hydropic degeneration means that mitochondrion is affected with the result of lack of energy [38]. ASP must be considered a trans-species carcinogenic agent in multiple sites, inducing a significantly increased incidence of malignant tumors in: (a) multiple tissues in male and female rats; (b) multiple organs in male mice; (c) an earlier occurrence in treated animals and a higher incidence and an anticipated onset of cancers when the treatment starts from fetal life [39].

Figure 4 Brain histology: Brain changes consisted mainly of hyper cellularity, enlarged nuclei, hyperchromasia and increased nuclear: cytoplasmic ratio (atypical changes- mildly dysplastic changes); being most evident in group 6 rats. Neovascularization was noticed with several newly formed vascular spaces, some showing glomeruloid endothelial proliferation.

A: (Group 1) Control rat brain showing normocellular brain tissue with ordinary astrocytes amidst glial tissue.

B: (Group 2) rat brain showing brain tissue with mild increase in cellularity with astrocytes showing slightly enlarged nuclei. One capillary vessel is seen at left side of the photograph.

C: (Group 3) rat brain showing hypercellularity with astrocytes showing hyperchromatic nuclei with increased nuclear: cytoplasmic ratio. Several thin walled capillaries are seen.

D1: (Group 4) rat brain showing neovascularization and glomeruloid vascular proliferation with prominent endothelial cells.

D2: (Group 4) rat brain showing two vessels and gliosis.

E: (Group 5) rat brain showing prominently hyper cellular brain tissue with astrocytes showing deeply basophilic, hyperchromatic nuclei and increased nuclear: cytoplasmic ratio.

F1: (Group 6) rat brain showing cellular brain tissue with astrocytes showing deeply basophilic, hyperchromatic nuclei and increased nuclear: cytoplasmic ratio. One capillary vessel is seen at the center of the photograph.

F2: (Group 6) rat brain showing congested blood vessel.

Soffritti et al. [40], who was studied effect of ASP on malignant

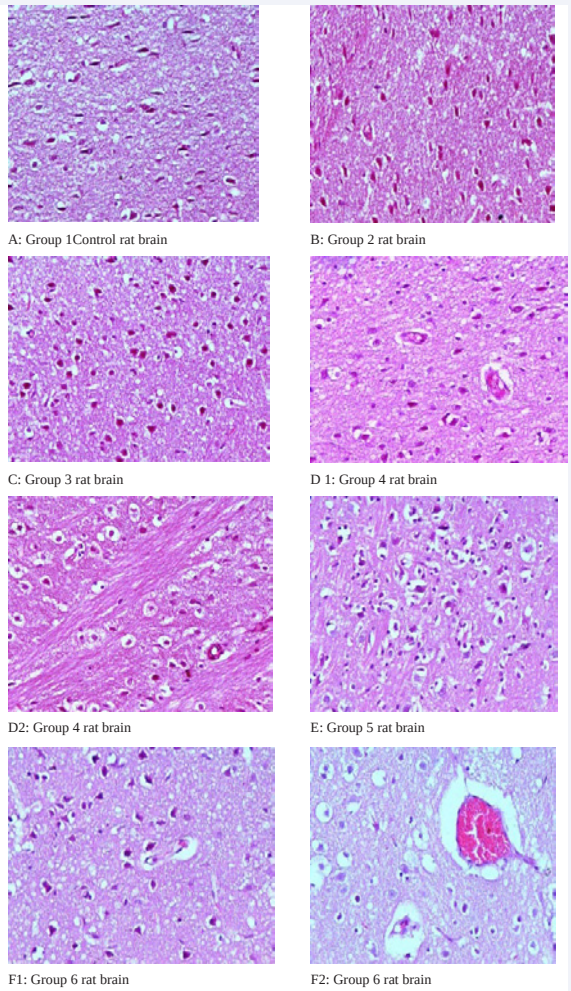

Figure 4 Brain histology.

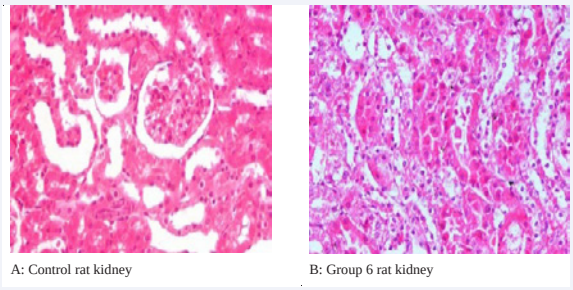

Figure 5 kidney histology.

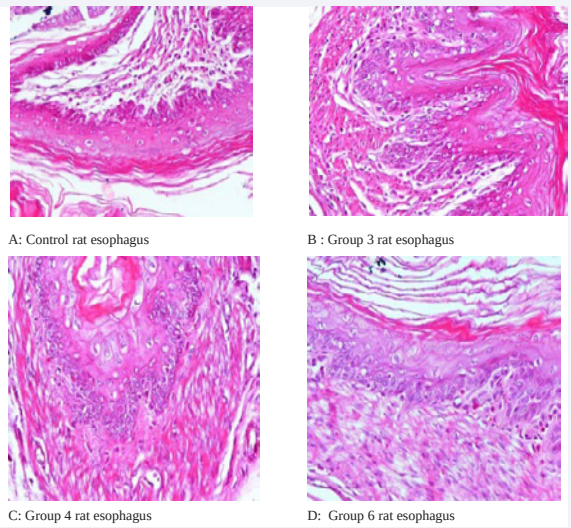

Figure 6 Esophagus histology

tumors in brain of male and female rats, observed tumors in both gender without dose relationship, in ASP-treated groups compare with control group.

(Figure 5) kidney histology: Kidneys showed marked cloudy swelling within renal tubules. Some tubules showed hydropic degeneration, others showed vacuolar degeneration within lining epithelium. Renal changes were most evident in group 6 rats. Other Groups (2-5) showed no histological change compared to control group.

A: Control rat kidney showing ordinary glomeruli and renal tubules with patent lumina.

B: Group 6 rat kidney showing prominent cloudy swelling within renal tubules. Other tubules showed hydropic and vacuolar degeneration within lining epithelium.

(Figure 6) Esophagus histology: Esophagus; Groups (3,4,6) showed stratified squamous epithelial lining cells with enlarged nuclei, coarse clumped chromatin and focal atypia.

A: Control rat esophagus showing benign stratified squamous epithelial lining.

B: (Group 3) rat esophagus showing acanthosis stratified squamous epithelial lining. Nuclei showed enlargement with clumped coarse chromatin.

C: (Group 4) rat esophagus showing stratified squamous epithelial lining with enlarged nuclei and coarse chromatin.

D: (Group 6) rat esophagus showing stratified squamous epithelial lining with enlarged nuclei and coarse clumped chromatin and focal atypia

Figure 7 Lung histology: Lung tissues showed thick walled blood vessels, inflammatory cell aggregates, averagely thick alveolar walls with pneumocytes shedded into alveolar Lumina and congestion within pulmonary interstitial tissues. Lung changes were seen in all treated groups.

A: Control rat lung showing ordinary alveolar spaces with averagely thick wall lined by regular pneumocytes.

B: (Group 2) rat lung showing a bronchus lined by benign regular columnar mucus secreting epithelium with adjacent averagely thick blood vessel.

C: (Group 3) rat lung showing alveolar spaces with averagely thick walls lined by benign pneumocytes. Shedding of the pneumocytes was seen surrounding a blood vessel.

D: (Group 4) rat lung showing aggregates of chronic

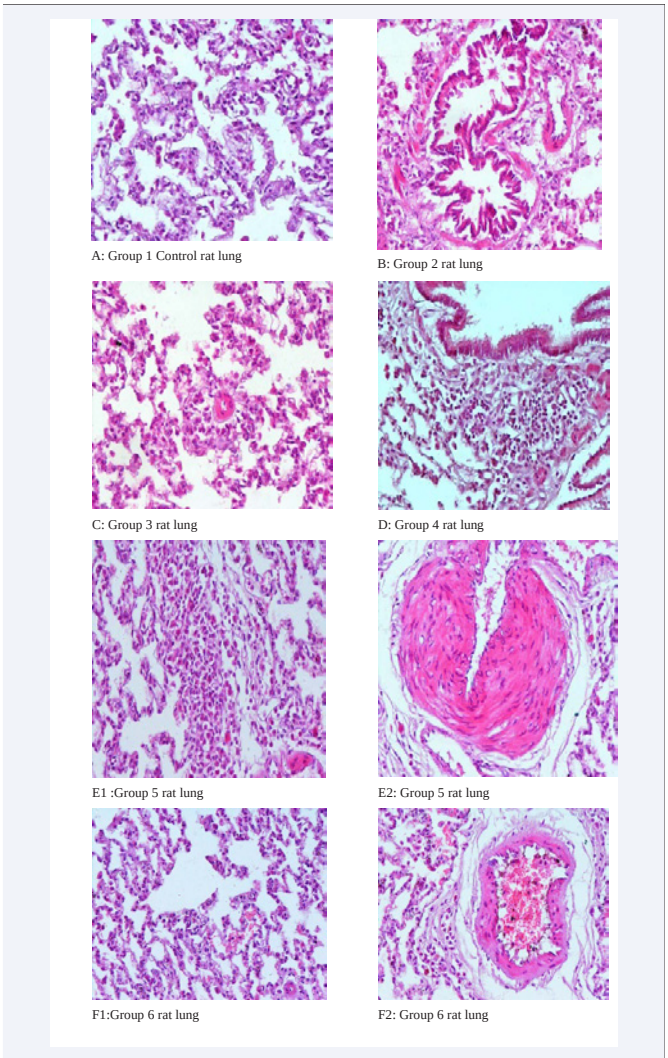

Figure 7 Lung histology.

inflammatory cells surrounding a bronchus lined by benign, regular, columnar mucus secreting epithelium.

E1: (Group 5) rat lung showing aggregates of chronic inflammatory cells and macrophages. Sloughed pneumocytes were seen shedded within alveolar lumina.

E2: (Group 5) rat lung showing a markedly thick walled blood vessel.

F1: (Group 6) rat lung showing congestion within interstitial tissues.

F2: (Group 6) rat lung showing congestion within a blood vessel and within pulmonary interstitial tissues.

(Figure 8) Spleen histology: Spleen showed only numerous foamy and hemosiderin laden macrophages within red pulp in group 6 rats.

A: Control rat spleen showing white pulp in form of lymphoid follicles with prominent germinal centers. Intervening red pulp is seen.

B: (Group 2) rat spleen showing white pulp in form of lymphoid follicle with prominent spiral arteriole. Surrounding red pulp is seen.

C: (Group 3) rat spleen showing preserved architecture. Lymphoid follicle of white pulp is seen surrounded by congested red pulp.

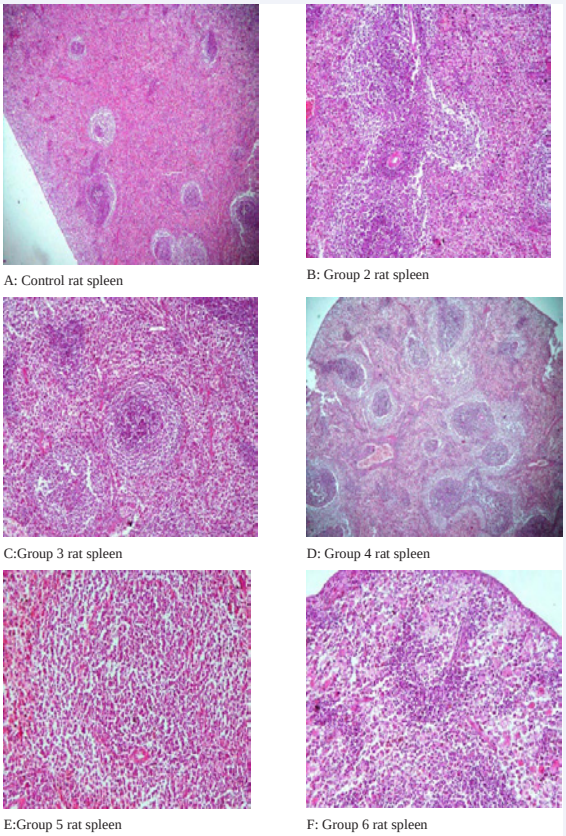

Figure 8 Spleen histology.

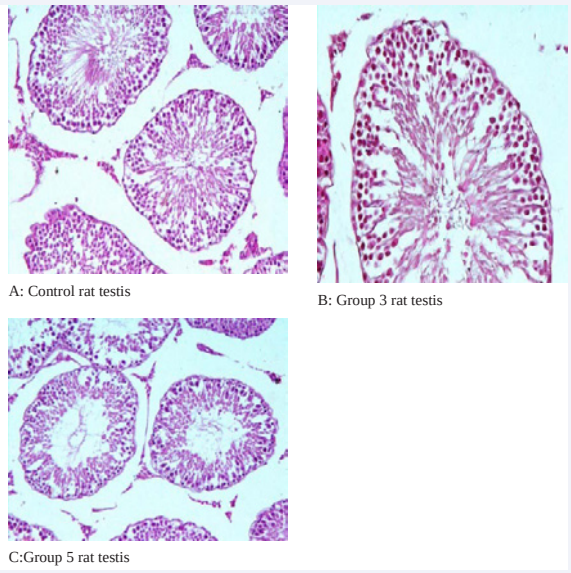

Figure 9 Testis histology.

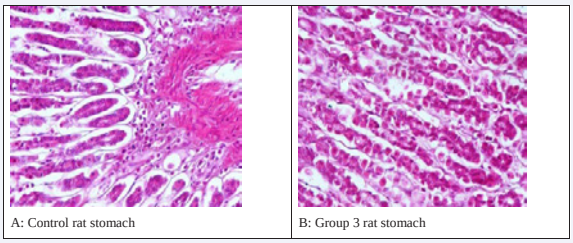

Figure 10 Stomach histology.

D: (Group 4) rat spleen showing preserved architecture, white pulp in form of lymphoid follicles with prominent germinal centers and intervening red pulp.

E: (Group 5) rat spleen showing preserved architecture, white pulp in form of lymphoid follicles with prominent spiral arteriole.

F: (Group 6) rat spleen showing red pulp rich in histiocytes; some foamy, others haemosiderin laden.

(Figure 9) Testis histology: Testis showed ordinary structure.

A: Control rat testis showing seminiferous tubules of average caliber. Basement membrane showed average thickness. Lining showed cells at different stages of spermatogenesis.

B: (Group 3) rat testis showing ordinary seminiferous tubules of average caliber. Basement membrane showed average thickness. Lining showed cells at different stages of spermatogenesis.

C: (Group 5) rat testis showing ordinary seminiferous tubules of average caliber. Basement membrane showed average thickness. Lining showed cells at different stages of spermatogenesis. (Figure 10) Stomach histology: Stomach showed ordinary structure. A: Control rat stomach showing ordinary mucosa formed of benign glands lined by regular mucus secreting epithelium. B: (Group 3) rat stomach showing ordinary

mucosa formed of benign glands lined by regular epithelium. C1: (Group 4) rat stomach showing mucosal glands lined by benign epithelium with focal areas of mucin secretion. C2: (Group 4) rat stomach showing mucosal glands lined by benign epithelium.

CONCLUSION

The oral administrations of safety dose of multi ASs affected on oxidative stress biomarkers (GSH and MDA) of liver and kidney. GSH and MDA brain tissue had no significant change between all groups although reduction in GSH value and increase in MDA value. Histopathologic changes were mostly encountered in brain, liver, kidneys, spleen and esophagus.

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