

Comparative effect of Aspartame and Sodium Cyclamate on Lipid Profile, Histology and Biochemical Parameters of Kidney and Liver Function in Albino Rats

Jiddah Nafiu Usman^{1*}, Salisu Maiwada Abubakar² and Aisha Muhammad Gadanya²

¹Department of Biochemistry, Faculty of Science, Gombe State University,
P.M.B 127, Tudun wada, Gombe, Nigeria.

²Department of Biochemistry, Faculty of Basic Medical Science, Bayero University Kano,
P.M.B 3011, Kano state, Nigeria

*Corresponding author:
Jiddah Nafiu Usman,
Department of Biochemistry,
Faculty of Science,
Gombe State University,
P.M.B 127, Tudun wada,
Gombe,
Nigeria.
Email: jiddasnafiu@gsu.edu.ng

HISTORY

Received: 8th March 2022
Received in revised form: 4th April 2022
Accepted: 12th April 2022

KEYWORDS

Aspartame
Sodium cyclamate
Histological
lipid profile
kidney function

ABSTRACT

Aspartame and Sodium cyclamate are two synthetic sweeteners used extensively in developing world. The study aims to assess the comparative effect of these sweeteners on lipid profiles, biochemical parameters of liver and kidney function and histological changes. Twenty male albino rats were separated into 5 groups of 4 rats each. Group 1 were fed normal diet and water, whereas those in Groups 2, 3, 4, and 5 received 50 mg/kg sodium cyclamate, 500 mg/kg sodium cyclamate, 50 mg/kg aspartame, and 500 mg/kg aspartame, respectively. After 7 weeks, the animals were sacrificed; blood and tissue samples were taken to analyze lipid profiles, biochemical parameters, and histology. The results showed that rats administered with low and high doses of sweeteners had significant ($P < 0.05$) increase in serum triglyceride (TG) (0.92 ± 0.05 mg/dL to 1.95 ± 0.04 mg/dL), total cholesterol (TC) (4.88 ± 0.31 mg/dL to 8.18 ± 0.45 mg/dL), low density lipoprotein (LDL) (3.59 ± 0.26 mg/dL to 8.02 ± 0.25 mg/dL), high density lipoprotein (HDL) (0.76 ± 0.05 mg/dL to 1.11 ± 0.1 mg/dL), Creatinine (0.94 ± 0.08 mg/dL to 1.77 ± 0.04 mg/dL) and urea (42.35 ± 2.43 mg/dL to 57.3 ± 1.79 mg/dL) levels in the treated group compared to the control group. Significant increase ($P < 0.05$) in AST (47.33 ± 3.92 U/L to 158.01 ± 5.07 U/L), ALP (76.80 ± 8.58 U/L to 124.01 ± 1.51 U/L) and ALT (12.87 ± 2.64 U/L to 37.74 ± 5.76 U/L) levels was also observed in experimental group compared to the control group. Moreover, high doses of sweeteners caused more histological abnormalities in rats. Sodium cyclamate and aspartame maybe harmful to kidney and liver especially when consumed at high dose for a long period.

INTRODUCTION

Increased awareness concerning the dangers of excessive intake of refined sugars has driven people to substitute them with artificial sweeteners. Over the years, artificial sweeteners have received increased attention from consumers due to their low-calories, importance in body weight and diabetes management to avoid the effect of sucrose [1]. Artificial sweeteners are essentially sweeter than sucrose and therefore very small amounts are required for sweetening foods. They are extensively used in jams, carbonated drinks, baked products, powdered drink

mixtures, and dairy products in high amounts as low-calorie product. Although food and drug administration (FDA) has licensed many sweeteners, majority of the sweeteners used are synthetic and sweetener excessive consumption has been reported to cause adverse health effects in human and animal models [2].

Some of the low-calorie artificial sweeteners frequently used by food industries include; aspartame, saccharin, acesulfame-K to serve as substitute to sucrose [3]. Aspartame is a synthetic methyl ester of the dipeptide consisting of L-aspartic acid and L-phenylalanine which is about 180-200 times sweeter

than sugar with low calorie value used in several countries. After ingestion and absorption, aspartame is metabolized into components like aspartic acid, phenylalanine, methanol. The products are further metabolized to release formaldehyde, formic acid and diketopiperazine [4,5]. Aspartame metabolite especially formaldehyde and format which is metabolite of methanol, are associated with most of its toxicity [6].

Sodium cyclamate is a synthetic non-nutritive sweetener that is 30 times sweeter than sugar. In combination with saccharin, cyclamate has a good sweetness. Even though cyclamate dissolves in water, the solubility can be enhanced by forming sodium or calcium salt [7]. Cyclamate has very low toxicity but produces cyclohexylamine when broken down by gut bacteria which exhibit higher toxicity [8]. Intestinal flora metabolism of cyclamate and rate of elimination from circulation are the major factors that determine the plasma concentrations of cyclohexylamine after ingestion of cyclamate [7].

Owing to their use as food additives, numerous studies have shown that artificial sweeteners interfere with many physiological processes. Some researchers have reported the effect of artificial sweeteners on biochemical parameters [9,10]. Others focuses on the effect on behavioral and histological changes in rat brain [11], hematological and brain catalase activity [12].

Even though research works have been done on the effects of artificial sweeteners, the data available is not sufficient and the outcomes of various research are not consistent, maybe because of dissimilarities in the chosen sweeteners, dose administration or duration of study. Furthermore, little or no information is available on the comparative study of sodium cyclamate and aspartame sweeteners at low and high doses. In this study, we aim to compare the effect of aspartame and sodium cyclamate on the biochemical parameters of liver, kidney and lipid profile in male albino rats. Also the histological study of liver and kidney was conducted. The result of the histological assessment showed infiltration in kidney and liver of treated groups at low and high dose of both sweeteners. The research will provide more insight into the toxicity of the sweeteners.

MATERIAL AND METHODS

Sweeteners

Sweeteners were selected based on the frequency of usage in the local food and drinks. It was purchased from solag Allied Chemicals Nigeria limited.

Experimental Animals

Twenty (20) male albino rats weighting 65 – 80 grams were purchased from the Biological Science Department of Bayero University Kano and used for the study. The albino rats were kept in cages and maintained under standard laboratory condition for temperature and controlled environment (12 hours light/dark cycle). Acclimatization was done for one week prior to experimentation with water and rat feeds provided *ad libitum*.

Ethical consideration

All the experimental procedures in the present study were done in accordance with “Institutional animal ethics committee guidelines for the use of animals for research”.

Experimental Design

The sweeteners were administered orally once daily to the rats for seven weeks based on their individual body weight using gavage technique. Two solutions of sodium cyclamate and aspartame (low and high dose) were prepared by dissolving the component in distilled water respectively. Group I (normal control) were fed normal diet and water *ad libitum*; Group II were fed sodium cyclamate (50 mg/kg bw); Group III were fed sodium cyclamate (500 mg/kg bw); Group IV were fed aspartame (50 mg/kg bw); Group V were fed aspartame (500 mg/kg bw). The lower dose was the recommended daily intake (RDI) of the sweeteners while the higher dose ($\times 10$ of the lower dose) was administered to the animals to ascertain the effect on the biochemical parameters of the animals.

Sample collection and preparation

Prior to dissection, both control and treated rats were fasted overnight. The animals were sacrificed after seven weeks under anesthesia (chloroform suffocation) and blood samples were collected through slaughter into tubes. Centrifugation at 4000 rpm for 15 minutes at room temperature was used to separate blood sera from cells. The serum was then used for the measurement of the biochemical parameters. For histological investigations, specimens were harvested from the liver and kidney, formalin fixed-paraffin embedded technique (FFPE) was used. Samples were immediately submerged in cold phosphate buffer and cut into small slices. Fixation was done in cold neutral buffered formalin (10%) for 24 hours and grossing carried out.

Serum biochemical analysis

The serum activities of ALT and AST were evaluated based on the method of Reitman and Frankel, [13]. The ALP level was estimated using colorimetric method. TC was estimated by enzymatic colorimetric method of Kenny, [14]. TG was estimated by enzymatic colorimetric method. HDL was estimated by enzymatic colorimetric method using Randox kit according to the method of MCGOWAN et al., [15]. LDL was calculated using the formular; $LDL = [Total\ Cholesterol - (HDL + triglycerides/5)]$. The creatinine and urea levels were evaluated following the methods of Husdan and Rapoport, [16] and Patton and Crouch, [17] respectively.

Histological analysis

The liver and kidney fixed specimens were implanted in paraffin blocks after drying with ascending alcohol grades. Staining with haematoxylin and eosin (H and E) was done after cutting to a thickness of around 4 μ m. The slides were examined under microscope to check for any histological abnormalities in each organ.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 20 was employed for the analysis. The differences between test and control groups were analyzed by one-way ANOVA. The values reported as mean \pm standard deviation (SD). The results were considered significant at *P*-values of less than 0.05 ($P < 0.05$).

RESULTS

Lipid profile

The result of the effect of sweeteners on serum levels of TG, TC, HDL, and LDL in rats is presented in **Table 1**. There was significant increase ($P < 0.05$) in the levels TG, TC, LDL levels and a significant decrease in HDL level of experimental groups compared to the control group.

Similarly, there was significant increase ($P < 0.05$) in the lipid profile between groups administered low dose (50 mg/kg) aspartame and (50 mg/kg) sodium cyclamate and high dose (500 mg/kg) aspartame and (500 mg/kg) sodium cyclamate respectively. However, there was no significant difference between low dose of aspartame (50 mg/kg) and low dose of sodium cyclamate (50 mg/kg). Also, no significant difference between high dose of aspartame (500 mg/kg) and high dose of sodium cyclamate (500 mg/kg).

Table 1. Serum levels of TG, TC, HDL and LDL in rats orally administered with sweeteners for seven weeks.

Parameters	TG (mg/dL)	TC (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
Group I control	0.92±0.05 ^a	4.88±0.31 ^a	1.11±0.10 ^a	3.59±0.26 ^a
Group II SC 50 mg/kg	1.43±0.11 ^b	7.29±0.32 ^b	0.96±0.07 ^b	6.04±0.34 ^b
Group III SC 500 mg/kg	1.92±0.04 ^c	9.21±0.24 ^c	0.80±0.02 ^c	8.02±0.25 ^c
Group VI Asp 50 mg/kg	1.49±0.02 ^b	7.48±0.51 ^b	1.03±0.03 ^{ab}	6.15±0.53 ^b
Group V Asp 500 mg/kg	1.95±0.04 ^c	9.33±0.12 ^c	0.88±0.03 ^c	8.07±0.15 ^c

Values are Mean±SD of four estimations. Means having different letter(s) within a column are significantly different ($P < 0.05$) (Tukey's HSD test). Sodium cyclamate (SC), Aspartame (ASP).

Effect of sweeteners on Kidney

The result of the effect of sweeteners on kidney function indices (creatinine and urea) is presented in (Table 2). There was significant increase ($P < 0.05$) in creatinine and urea levels of rats administered both sweeteners (aspartame and sodium cyclamate) at high (500 mg/kg) and low (50 mg/kg) dose of aspartame compared to the control. There was significant increase in creatinine and urea levels between groups administered low doses of aspartame (50 mg/kg) and sodium cyclamate (50 mg/kg) and high doses of aspartame (500 mg/kg) and sodium cyclamate (500 mg/kg) respectively. However, no significant difference in urea level between groups administered low dose of aspartame (50 mg/kg) and low dose of sodium cyclamate (50 mg/kg). Furthermore, no significant difference in urea level between groups administered high dose (500 mg/kg) of both sweeteners.

Table 2. Serum levels of creatinine and urea in rats orally administered sweeteners for seven weeks.

Parameters	Creatinine (mg/dL)	Urea (mg/dL)
Group I (Control)	0.97±0.05 ^a	42.35±2.43 ^a
Group II (SC 50 mg/kg)	1.19±0.05 ^b	47.69±1.84 ^b
Group III (SC 500 mg/kg)	1.47±0.02 ^c	55.16±3.16 ^c
Group VI (Asp 50 mg/kg)	1.56±0.05 ^c	45.20±1.79 ^{ab}
Group V (Asp 500 mg/kg)	1.77±0.04 ^d	57.30±1.79 ^c

Note: Values are Mean±SD of four estimations. Means having different letter(s) within a column are significantly different ($P < 0.05$) (Tukey's HSD test). Sodium cyclamate (SC), aspartame (ASP).

Effect of sweeteners on Liver Biochemical Indices

The result of the effect of sweeteners on liver biochemical indices; AST, ALT and ALP is presented in (Table 3). There was significant increase ($P < 0.05$) in levels of AST, ALT and ALP of rats administered both sweeteners at low (50 mg/kg) and high (500 mg/kg) doses compared to the control group. There was also significant increase between low doses of aspartame (50 mg/kg) and sodium cyclamate (50 mg/kg) and high doses of aspartame (500 mg/kg) and sodium cyclamate (500 mg/kg) respectively. However, no significant increase ($P > 0.05$) between aspartame and sodium cyclamate at low dose (50 mg/kg).

Table 3. Serum levels of AST, ALT and ALP in rats orally administered sodium cyclamate and aspartame for seven weeks.

Parameters	AST (U/L)	ALT (U/L)	ALP (U/L)
Group I (Control)	47.33±3.92 ^a	12.87±2.64 ^a	76.80±8.58 ^a
Group II (SC 50 mg/kg)	76.51±5.74 ^b	23.03±1.32 ^b	95.64±7.17 ^b
Group III (SC 500 mg/kg)	144.13±4.56 ^c	39.92±1.13 ^c	127.12±2.71 ^c
Group VI (Asp 50 mg/kg)	83.28±3.77 ^b	25.37±1.43 ^b	98.83±6.93 ^b
Group V (Asp 500 mg/kg)	158.01±5.07 ^d	45.75±4.44 ^c	131.41±3.17 ^c

Note: Values are Mean±SD of four estimations. Means having different letter(s) within a column are significantly different ($P < 0.05$) (Tukey's HSD test). Sodium cyclamate (SC), aspartame (ASP).

Histology of kidney

The present findings revealed moderate mesangial cell proliferation and narrowing of bowman's space in kidney of rats administered with low dose of sodium cyclamate (50 mg/kg) (Fig. 2A) compared to control (Fig. 1) while high dose of sodium cyclamate (500 mg/kg) showed focal mesangial cell proliferation and narrowing of bowman's space in the kidney of the rats (Fig. 2B). Findings of the present study revealed mesangial cell proliferation in the kidney of rats administered aspartame (50 mg/kg) (Fig. 3A) while high dose of aspartame (500 mg/kg) showed interstitial lymphocytic infiltrates in kidney of rats administered higher aspartame dose (Fig. 3B).

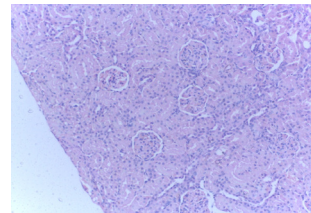


Fig. 1. Photomicrograph of kidney H & E x400 of normal control group.

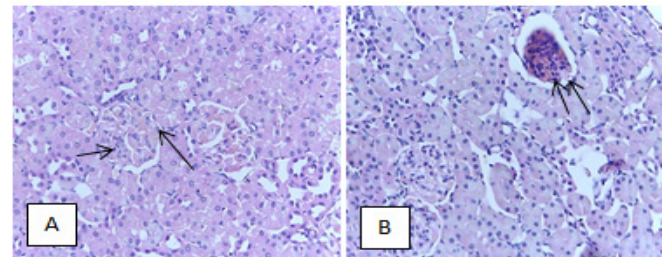


Fig. 2. A Photomicrographs of the kidney H&Ex400 of rats administered Sodium cyclamate at low and high dose.

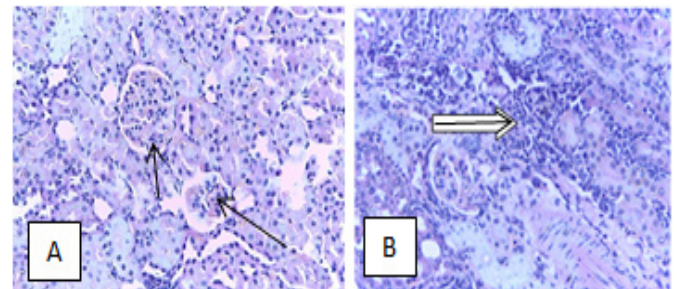


Fig. 3. A Photomicrographs of the kidney H&E X400 of rats administered aspartame at low and high dose

Histology Result of the Liver

The groups treated with low and high doses of sodium cyclamate and aspartame revealed severe histological changes in the liver. The present findings revealed periportal inflammatory infiltrates in rat's hepatocytes administered with low dose (50 mg/kg) of sodium cyclamate (**Fig. 5A**) compared to control (**Fig. 4**). High dose of sodium cyclamate (500 mg/kg) showed periportal/lobular inflammation with associated piecemeal necrosis (**Fig. 5B**). Furthermore, periportal & lobular inflammation in hepatocytes was observed in rats administered low (50 mg/kg) aspartame (**Fig. 6A**) and high dose (500 mg/kg) aspartame showed periportal lymphocytic infiltrates (**Fig. 6B**).

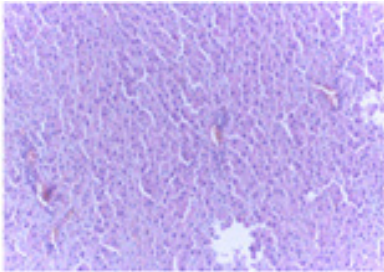


Fig. 4. Photomicrographs of the hepatocytes H&E (x400) in normal control group.

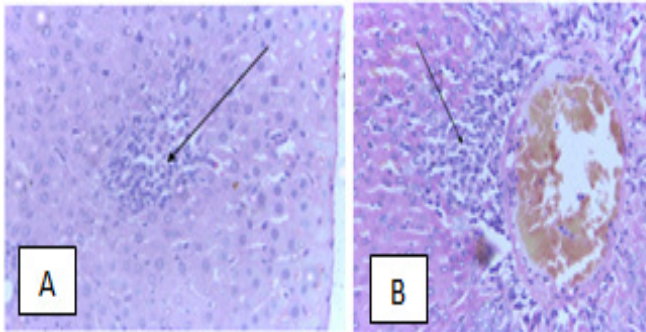


Fig. 5. Photomicrograph of Liver H&E X400 of rats administered sodium cyclamate at low and high dose.

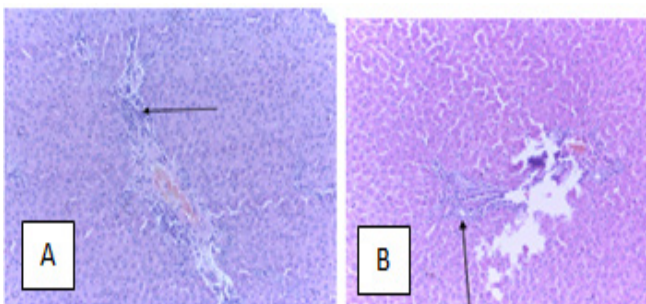


Fig. 6. Photomicrograph of Liver H&E X400 of rats administered aspartame at low and high dose

DISCUSSION

The persistent increase in sweeteners consumption is expected to increase in the nearest future and cuts across all ages and genders [18]. The present study was conducted to determine toxicological effect of sodium cyclamate and aspartame commonly used worldwide, both at a low and high dose. The low dose is the RDI while the higher dose is x10 the RDI. The present study showed significant increase in TC, TG and LDL levels whereas HDL levels were significantly decreased in all groups administered

low and high dose of sodium cyclamate and aspartame compared with the control. Levels of lipid profile components in groups administered high dose of sodium cyclamate and aspartame is more significant than the groups administered low dose when compared with control. The results are in accordance with the findings of Helal et al., [9] for acesulfame-k and aspartame treated rats. The effect of sweetener on lipid metabolism can be seen in increased in lipid profile. It may be due to biotransformation of the artificial sweeteners since different sweeteners have different properties and are broken down differently [18]. Damage in the liver affects lipid metabolism due to the disruption of cell membrane integrity causing some membrane lipids to be released into circulation and leading to compromise of tissue effectiveness in regulating lipid metabolism. Significant increase in the levels of ALT, AST and ALP were observed in groups administered both low and high dose of sodium cyclamate and aspartame compared with the control.

The levels of liver enzymes were more significant in the groups administered high dose of both sodium cyclamate and aspartame. The findings is in line with the result obtained by Helal et al., [9] who reported increased in ALT and AST levels in rats administered acesulfame-k and aspartame. Similarly, Abdallah, [19] reported increase in serum ALT, AST and ALP activities in rats administered low and high dose of saccharin. AST and ALT are important determinant for liver dysfunction and damage since damaged cells release cytosolic enzymes into the blood [20]. According to Kim et al., [21], the increase in the activity of aminotransferase indicates an early diagnosis of hepatotoxicity and considered a biomarker of tissue damage. The more significant increase in groups administered high dose of sodium cyclamate and aspartame agreed with Prokic et al., [22], who stated alterations in levels of liver enzymes may depend on exposure time and dose. The increase in serum aminotransferase activity could be due to breakdown of liver parenchyma or related to cellular membranes interaction with free radical [23]. The hepatocellular impairment could be the cause of changes in liver function [24] and subsequently, causes the release into blood of intracellular enzymes in high levels.

Furthermore, increase in both levels of creatinine and urea in rats administered high and low dose of sodium cyclamate and aspartame are in accordance with the result from Azeez et al., [25] who reported increased in serum creatinine levels in rats administered 2.5mg/kg, 5mg/kg and 10 mg/kg of saccharin for 6 and 120 days. Similarly, Aboshanady et al., [26] also reported increase in urea and creatinine levels in rats treated with aspartame (250 mg/kg bw) for 6weeks. This could be because of disruption in kidney functions leading to decreased glomerular filtration rate and subsequently, withholding creatinine and urea in the blood [27].

The biochemical changes observed in the liver and kidney were supported by the histological findings. Periportal inflammatory aggregates, mainly lymphocytes infiltration, lobular inflammatory infiltrates, piecemeal necrosis in the rat's liver meanwhile, narrowing of the bowman's space, interstitial lymphocytic infiltrates, and periportal inflammation were observed in the rats' kidney in all treated groups. The histological changes were more pronounced in rats treated with high dose of sodium cyclamate and aspartame. The findings are in agreement with the work of Othman and Jumah, [28] who reported infiltration of inflammatory cells and congestion in liver and kidney respectively for mice administered 500 mg/kg aspartame for a month.

CONCLUSIONS

In view of the results obtained for biochemical parameters and histological changes for both low and high doses of aspartame and sodium cyclamate, it may be obvious that the sweeteners consumption especially at a higher dose for a long time may lead to hepatic and renal damage. Therefore, consumption should be minimized or be replaced with natural form of sweeteners.

REFERENCES

- Ni, Y., Xiao, W., & Kokot, S. A differential kinetic spectrophotometric method for determination of three sulphanilamide artificial sweeteners with the aid of chemometrics. *Food Chem.*, 2009 ;113(4); 1339-1345. <https://doi.org/10.1016/j.foodchem.2008.08.068>
- Lohner, S., Toews, I., & Meerpohl, J. J. Health outcomes of non-nutritive sweeteners: Analysis of the research landscape. *Nutr J.* 2017;16(1);1-21. <https://doi.org/10.1186/s12937-017-0278-x>
- Bandyopadhyay, A., Ghoshal, S., & Mukherjee, A. Genotoxicity testing of low-calorie sweeteners: Aspartame, Acesulfame-K, and saccharin. *Drug Chem Toxicol.* 2008;31(4):447-457. <https://doi.org/10.1080/01480540802390270>
- Trocho, C., Pardo, R., Rafecas, I., Virgili, J., Remesar, X., Fernández-López, J., & Alemany, M. Formaldehyde derived from dietary aspartame binds to tissue components in vivo. *Life Sci.* 1998;63(5):337-349. [https://doi.org/10.1016/s0024-3205\(98\)00282-3](https://doi.org/10.1016/s0024-3205(98)00282-3)
- George, V., Arora, S., Wadhwa, B. K., & Singh, A. K. Analysis of multiple sweeteners and their degradation products in lassi by HPLC and HPTLC plates. *J Food Sci Technol.* 2010;47(4):408-413. <https://doi.org/10.1007/s13197-010-0067-4>
- Finamor, I., Pérez, S., Bressan, C. A., Brenner, C. E., Rius-Pérez, S., Brittes, P. C., Cheiran, G., Rocha, M. I., Da Veiga, M., Sastre, J., & Pavanato, M. A. Chronic aspartame intake causes changes in the trans-sulphuration pathway, glutathione depletion and liver damage in mice. *Redox Biol.* 2017 ;11:701-707. <https://doi.org/10.1016/j.redox.2017.01.019>
- Chattopadhyay, S., Raychaudhuri, U., & Chakraborty, R. Artificial sweeteners – a review. *J Food Science and Technology*, 2011;51(4):611-621. <https://doi.org/10.1007/s13197-011-0571-1>
- Bopp, B. A., Sonders, R. C., Kesterson, J. W., & Renwick, A. G. Toxicological aspects of cyclamate and cyclohexylamine. *CRC Crit Rev Toxicol.* 1986;16(3):213-306. <https://doi.org/10.3109/10408448609037465>
- Helal, E. G., Abdelaziz, M. A., & El-Gamal, M. S. Effects of CANDEREL® and the ameliorative role of stem cell enhancer on some physiological parameters in male albino rats. *Egypt J Hosp Med.*2019;76(3):3702-3708. <https://doi.org/10.21608/ejhm.2019.39915>
- Khamise, N., Tayel, D., Helmy, M., & Aborhyem, S. Effect of aspartame and Sucralose artificial sweeteners on weight and lipid profile of male albino rats. *J High Inst Public Health.* 2020;0(0):87-100. <https://doi.org/10.21608/jhph.2020.108281>
- Erbaş, O., Erdoğan, M. A., Khalilnezhad, A., Solmaz, V., Gürkan, F. T., Yigittürk, G., Eroglu, H. A., & Taskiran, D. Evaluation of long-term effects of artificial sweeteners on rat brain: A biochemical, behavioral, and histological study. *J Biochem Mol Toxicol.* 2018;32(6):e22053. <https://doi.org/10.1002/jbt.22053>
- Ibragić-Emir Fehratović-Damir Suljević, S. Artificial sweeteners elicit oxidative stress in rat brain and development of microcytic anemia: Promising protective effects of vitamin C. *J Res Pharm.* 2021;25(2):117-123. <https://doi.org/10.29228/jrp.2>
- Reitman, S., & Frankel, S. A Colorimetric method for the determination of serum glutamic Oxalacetic and glutamic pyruvic transaminases. *Am J Clin Pathol.* 1957;28(1):56-63. <https://doi.org/10.1093/ajcp/28.1.56>
- Kenny, A. P. The determination of cholesterol by the Liebermann-Burchard reaction. *Biochem J.* 1952;52(4):611-619. <https://doi.org/10.1042/bj0520611>
- McGowan, M. W., Artiss, J. D., & Zak, B. A procedure for the determination of high-density lipoprotein choline-containing phospholipids. *Clin Chem Lab Med.* 1982;20(11):807-812. <https://doi.org/10.1515/ccclm.1982.20.11.807>
- Husdan, H., & Rapoport, A. Estimation of creatinine by the Jaffe reaction. *Clin Chem.* 1968;14(3):222-238. <https://doi.org/10.1093/clinchem/14.3.222>
- Patton, C. J., & Crouch, S. R. Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. *Anal Chem.* 1977;49(3):464-469. <https://doi.org/10.1021/ac50011a034>
- Pang, M. D., Goossens, G. H., & Blaak, E. E. The impact of artificial sweeteners on body weight control and glucose homeostasis. *Frontier Nutr.* 2021;7:1-19. <https://doi.org/10.3389/fnut.2020.598340>
- Abdallah, I. Z. Physiological changes induced by long term administration of saccharin compared with aspartame to male albino rats. *The Egypt J Hosp Med.* 2002;8(1):70-81. <https://doi.org/10.21608/ejhm.2002.18747>
- Mitra, S., Venkataranganna, M., Sundaram, R., & Gopumadhavan, S. Protective effect of HD-03, a herbal formulation, against various hepatotoxic agents in rats. *J Ethnopharmacol.* 1998;63(3):181-186. [https://doi.org/10.1016/s0378-8741\(98\)00088-9](https://doi.org/10.1016/s0378-8741(98)00088-9)
- Kim, W. R., Flamm, S. L., Di Bisceglie, A. M., & Bodenheimer, H. C. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. *Hepatology*, 2008 ;47(4):1363-1370. <https://doi.org/10.1002/hep.22109>
- Prokic, M., Paunovic, M., Matic, M., Djordjevic, N., Ognjanovic, B., Stajin, A., & Saicic, Z. Effect of aspartame on biochemical and oxidative stress parameters in rat blood. *Arch Biol Sci.* 2015;67(2):535-545. <https://doi.org/10.2298/abs141009016p>
- Muriel, P. Role of free radicals in liver diseases. *Hepatol Int.* 2009;3(4):526-536. <https://doi.org/10.1007/s12072-009-9158-6>
- Abdelaziz, I., & Ashour, A. E. Effect of saccharin on albino rats' blood indices and the therapeutic action of vitamins C and E. *Human Exp Toxicol.* 2010;30(2):129-137. <https://doi.org/10.1177/0960327110368695>
- Azeez, O. H., Alkass, S. Y., & Persike, D. S. Long-term saccharin consumption and increased risk of obesity, diabetes, hepatic dysfunction, and renal impairment in rats. *Medicina*, 2019;55(10):681. <https://doi.org/10.3390/medicina55100681>
- Aboshanady, A., El-Sawaf, M., Abdel-Aziz, A., & Attia, M. The effect of aspartame ingestion in pregnant female albino rats on placental and fetal weights, umbilical cord length, and histology of fetal pancreas. *Tanta Med J.* 2018;46(2):114. https://doi.org/10.4103/tmj.tmj_5_18
- Turley, S. D., & Dietschy, J. M. Sterol absorption by the small intestine. *Curr Opin Lipid.* 2003;14(3):233-240. <https://doi.org/10.1097/00041433-200306000-00002>
- Othman, S. I., & Jumah, M. B. Histopathological effect of aspartame on liver and kidney of mice. *Int J Pharmacol.* 2019;15(3):336-342. <https://doi.org/10.3923/ijp.2019.336.342>