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A STUDY ON THE NATURAL; NUTRITIVE AND NON-NUTRITIVE SWEETENERS IN RATS

By

NAGWA I. HASSANIN

Biochemistry and Nutrition Department, Girl's College, Ain Shams University, Cairo , Egypt.

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SUMMARY

A challenge design was used in eight separate studies using young adult male albino rats (mean weight ± 69.5g) to investigate the effects of natural; non-nutritive sweeteners ingestion on weight gain, food intake and clinical biochemical parameters. The result revealed that, the patterns in feed consumption were less clear than the growth data. For the sucrose diet, the rats receiving added either saccharin, or aspartame had significantly smaller weight gains than did the corresponding sucorse group. Rats fed saccharin diet incorporated with aspartame; and with sucrose, consumed diet than did the corresponsing saccharin group, while gaining a smaller amount of weight. All organs weights were reduced in aspartame and saccharin groups (except liver) than did the corresponding rats served as controls. Serum total protein concentration was reduced with an increase in urea level, while creatinine did not show any significant changes. There was a significant relationship between dietary regimen and serum total lipids and triacylglycerols. Also there was significant decrease in the activities of ASAT (S.Got) in all experimental rats group, except in saccharin regimen group, on the other hand, there were significant elevations in ALAT (S.GPT) activities in all experimental rat groups than did the corresponding control group. Using the casein diet, there was significant differences in liver total protein ($P<0.05$) and glycogen ($P<0.01$) concentration between animals on aspartame: Saccharin diet (9.36g/100g tissue); (49.98 mg/100g tissue), and those on sucrose: aspartame (7.57g/100g tissue; 59.8 mg/100 g tissue) respectively. The examined brain sections in rats receiving aspartame:saccharin mixture in a 1:3 ratio, showed necrosis cellularity (stage of tumors).

Numerous questions remain unanswered, particularly with respect to the possible effects of

aspartame on human beings who may consume the compound over years of daily use.

INTRODUCTION

Aspartame is the methyl ester of the dipeptide L-aspartyl-L-phenylalanine with a sweetening power 180-200 times that of sucrose (Mazur et al., 1969; Cloninger and Baldwin, 1970). It is approximately 40% by weight aspartic acid (Lewis, 1984).

Aspartame may be absorbed and metabolized in one of two ways. It may be hydrolyzed in the intestinal lumen to aspartate, phenylalanine, and methanol by proteolytic and hydrolytic enzymes (Oppermann et al., 1973; Ranney et al., 1976; Ranney and Oppermann, 1979). These components are absorbed from the lumen in a manner similar to that of amino acids and methanol arising from dietary protein or polysaccharides. Alternatively, aspartame may be absorbed directly into intestinal mucosal cells by peptide transport mechanisms (Addison et al., 1975), with subsequent hydrolysis within the cell to asparate, phenylalanine, and methanol.

Refined sugar (especially sucrose) and aspartame have each been considered a possible cause of hyperactivity and other behavior problems in childern (Crook, 1975; Wurtman, 1983). The presumed reaction to sucrose has been attributed to several possible causes, including a rise in blood sugar shortly after ingestion, reactive hypoglycaemia several hours after ingestion, and an allergic response (Wender, 1991). The presumed reaction to aspartame has be attributed to the possibility that its metaboli results in elevation of plasma phenylalan concentrations, which in turn may alter transport of essential amino acids to the br (Wurtman, 1983; Pardridge, 1986; Elsas a Trotter, 1988).

So, the present investigation is conducte study the effect of nutritive and non-nutri sweeteners on growth rate; relative org weight; clinical biochemical parameters in se associated with liver and kidney function also on gross pathological changes in so organs.

MATERIAL AND METHODS

Materials:

1- Aspartame was obtained from the Nutras Company, 175 Lake Cook Road, P.O B 730, Deerfield, Illinosis 60015, U.S.A.
2. Saccharin was obtained from Kahira Ph and Chem. Ind. Co. Cairo-Egypt.

Animals:

Eight groups of ten young adult male albino Sprague-Dawley Strain, of mean weight 6 were used. They were obtained from the Hel breeding farm, Cairo, Egypt. The animals v divided into eight groups and housed individu in stainless steel cages with wire mesh bott in a room maintained at 25-30°C with about relative humidity. The room was lightened c daily photoperiod of 12h.

Experimental diets:

The composition of the basal diet is represented in table (1).Each rat received a leaf of lettuce 3 day intervals, for six weeks. Food and water were provided and libitum. Body weight gain and food consumption were recorded periodically.

Tissue preparation:

After 42 days, animals were fasted for 16 hour and anesthetized with ether. Incisions were made into the abdomen and blood samples were obtained from the portal vein and left to clot and centrifuged at 1300xg for 15 min. at 4°C to obtain serum. Liver, kidney, heart, spleen and brain were excised, rinsed in chilled saline solution, then blotted on filter paper, weighed separately to calculate the absolute and relative organ weight. Two-thirds of liver stored in saline solution at -20°C until analysis. Specimens of brain and one-third of liver were kept in 10% formalin until processed for histopathology. Histological sections from brain, and liver organs were stained with Haematoxylin (HX), according to Carlton, et al. (1967).

Analytical procedures:

(1) Serum glucose concentration was estimated by enzymatic colorimetric procedures kits prepared by E.Merck, 64271 Darmstadt, Germany, according to Trinder , (1969).

(2) Serum total protein was determined by using colorimetric method kits No.3327, E. Merck, Postfach 4119, D-6100 Darmstadt, Germany, according to Sunderman et al. (1958) whereas serum albumin content was determined by using colorimetric procedures kits developed by Biocon, D-57299 Burbach/Germany, according to Webster, (1974), Globulin concentration and albumin/globulin ratio (A/G) were calculated.

(3) Serum total lipids level was determined by colorimetric procedures kits developed by the Egyptian American Company EAC. for Laboatory Services, Cairo, Egypt, according to Thannhouser, (1958). Serum total cholesterol was determined by using enzymatic colorimetric method kits developed by Biocon, D-57299 Burbach/Germany, according to Richmond (1973). Serum triacylglycerol level was determined by using enzymatic colorimetric method kits developed by Biocon, D-57299 Burbach/Germany, according to Fossati and Prencipe (1982).

(4) Serum activities of glutamate oxaloacetate transminase (ASAT) and glutamate pyruvate transaminase (ALAT) were estimated by colorimetric procedures kits supplied by Biocon, D-57299 Burbach/Germany, according to Reitman and Frankel (1957), whilst serum level of uric acid was analyzed by enzymatic colorimetric procedures kits supplied by Diamond Diagnostics for Laboratory Services, Cairo, Egypt, mentioned by Henry (1974). Serum level of creatinine was analyzed by Kinetic method kits supplied by Diamond Diagnostic, described by Herny (1974). Whereas serum level of urea was analyzed by enzymatic colorimetric method kits supplied by E. Merck, Frankfurter Str. 250-D-6100 Darmstadt 1/Germany, according to Fawcett and Scott, (1960).

(5) The analysis also included determination of

total protein and glycogen contents in liver, total protein was extracted from liver by the method of Lowry et al. (1951), whilst glycogen extracted from liver by the method of Nicholas et al. (1956).

Statistical analysis:

Statistical analysis was done by compl randomized design in factorial arrange (ANOVA,F-test) showed evidence of o differences between diets, according to Sne and Cochran (1980).

Table(1) *Composition of the experimental diets (g/100 g diet)*

Ingrediants dry base	Basic diet	Sucrose	Sacch-arine	Aspart-ame	Sucrose + Saccharine (1:3)	Sucrose + Aspartame (1:3)	Saccharine + Aspartame (1:3)	Aspartame + Saccharine (1:3)
	G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_8
Starch	60.2	20.2	60.111	59.989	50.133	50.042	60.02	60.08
Casein (96% protein)	20	20	20	20	20	20	20	20
Corn oil	10	10	10	10	10	10	10	10
Non-nutritive cellulose	5	5	5	5	5	5	5	5
Salt mix[7]	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Vit mix[8]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Dl-Methionine	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Sucrose	-	40	-	-	10	10	-	-
Saccharin	-	-	0.089[1]	-	0.067[3]	-	0.022[5]	0.067
Aspartame	-	-	-	0.211[2]	-	0.158[4]	0.158	0.053[6]

1- Saccharin weight equivalent "40g" sucrose.

2- Aspartame weight equivalent "40g" sucrose.

3- Saccharin weight equivalent "30g" sucrose.

4- Aspartame weight equivalent "30g" sucrose.

5-Saccharin weight equivalent "10g" sucrose.

6-Aspartame weight equivalent "10g" sucrose.

7- Mineral mixture g/kg: $CaHPO_4$, 500; NaCl, 74; $K_3C_6H_5O_7.H_2O$, 220; K_2SO_4, 52; Mago, 24; Manganous carbonate, 3.5; Ferric citrate, 6; Zinc carbonate, 1.6; Cupric carbonate, 0.3; KIO_3, 0.01, Na_2SeO_3. $5H_2O$, 0.01; [CrK $(SO_4)_2$. $12H_2O$], 0.55; Sucrose, f powdered to make 1,000g, (AIN, 1977).

8- Vitamin mixture mg/Kg (except as no Thiamin.HCl, 600; Riboflavin, 600; Ribofl 600; Pyridoxine. HCl, 700; Nicotinic acid D-calcium pantothenate, 1.6g; Folic acid, 2 D-Biotin, 20; Vit. B_{12}, 1.00; Retinol, 0.4 DL.α-Tocopheryl acetate, 5.000; Cholecalciferol, 2.5, Menaquinone 5.0; Su finely powdered to make 1.000 g (AIN, 1

RESULTS AND DISCUSSION

The effect different Sweetener diets on weight gain; food intake and tissue weight:

Table (2) summarizes the weight gain of the rats fed on the experimental diets for 6 weeks. The patterns in feed consumption were less clear than the growth data. For the sucrose diet, the rats receiving added either saccharin, G_5 or aspartame, G_6 had had significantly smaller weight gains ($P<0.05$) than did the corresponding sucrose group, G_2 (G_5, 129.5g, 183.4%); (G_6, 131.6g, 188%) and (G_2, 138.1g, 195.1%) respectively. In spite of food intake tended to be higher (11.44g/day) in rats consuming sucrose-saccha in mixture diet, G_5 in 1 1:3 ratio, rats gained less weight than those fed the diets containing sucrose-aspartame mixture diet in a 1:3 ratio, G_6 ($P<0.05$). Table (2) also shows the weight gains of the rats fed the experimental saccharin diets for 6 weeks. Unexpectedly, rats fed saccharin diet incorporated with aspartame, G_7; G_8 and G_5 consumed more diet than did the corresponding saccharin group, G_3, while gained a smaller amount of weight , (G_7 11.64g/day; 104.4g±31.4); (G_8, 12.17g/day; 115g±16.3); (G_5 11.44g/day; 129.5g±27.9), table (2). For the aspartame diet, the groups receiving added sucrose, G_6; saccharin G_7 and G_8 (all of the mixture equivalent 40g sucrose) had higher food intake and lower weight gain ($P<0.05$), when compared with aspartame feeding group, G_4. (G_6, 11.3g/ day; 131.6g±24.88); G_7, 11.3g/ day; 131.6g±24.88); G_7, 11.64/day; 104.4g±31.4) and (G_8, 12.17g/day; 115g±16.3) respectively, (table 2). There was a significant difference in feed efficiency among groups, $P<0.01$, (table 2). Wherein, all the experimental deits had almost feed consumption and feed efficiency except G_7 and G_8 , the feed consumption was increased and feed efficiency was decreased.

Relative organs weight:

Table (2) represents the effect of diets on liver, brain, kidney, heart and spleen relative weights. All organs weights, expressed as a percentage of body weight, were reduced in aspartame and saccharin groups (except liver) than did the corresponding rats served as control (G_1) (table 2, $P<0.01$). Addition of sucrose to the diet (G2) clearly elevated relative all organs weight except kidney and brain relative weight, ($P<0.01$). Final relative organs weights were significantly higher ($P<0.01$) for rats fed sucrose-saccharin 1:3 (G_5) except relative liver weight had slihgt increase. Whereas, sucrose-aspartame 1:3 (G_6) and saccharin aspartame 1:3 diets (G_7), had the same effect on relative organs weight, there was a significant increase in relative liver; brain ($P<0.01$) and spleen ($P<0.05$) weight, whilst there was less weight in relative kidney and heart ($P<0.01$).

Table (2) also shows the relative organs weight of rats fed the experimental aspartame-saccharin diet 1:3 (G_8) for 6 weeks. The diet clearly elevated relative brain and heart weights ($P<0.01$), wherein there was a slight increase in relative liver and kidney weight ($P<0.01$) and reduced relative spleen weight ($P<0.05$).

A challenge design was used in eight separate studies to investigate the effects of natural

Table (2) *Weight gain; % change in weight; food intake; feed efficiency and tissue weights of rats maintained on either sucrose, saccharin or aspartame treatments.*

	Groups [1]							
	G_1*	G_2*	G_3*	G_4*	G_5*	G_6*	G_7*	G_8*
Initial body weight, g	71.2	70.8	70.5	70.2	70.6	70.0	70.7	72.1
Final body weight, g	186.7	208.9	205	214.1	200.1	201.6	175.1	187.1
Body weight gain***, g	115.5±20.21	138.1±33.46	134.5±17.21	143.9±28.1	129.5±27.9	131.6±24.88	104.4±31.4	115±16.3
% chang in weight from initial weight	162.2	195.1	190.8	205	183.4	188	147.7	159.5
Food intake/day N.S, g	10.84±1.16	10.99±1.65	10.95±1.26	11.19±1.53	11.44±1.01	11.3±1.37	11.64±1.62	12.17±1.27
Feed eficiency**, g	0.255±0.04	0.299±0.063	0.291±0.03	0.307±0.043	0.271±0.06	0.273±0.048	0.216±0.06	0.225±0.02
Liver wt**, g	6.12±0.47	8.77±0.62	7.2±1.17	6.58±0.79	6.80±1.8	8.3±1.1	6.6±0.87	6.4±1.5
Liver wt/100 g body weight**	3.29	4.13	3.57	3	3.41	4.11	3.79	3.39
Brain wt**, g	1.19±0.19	1.3±0.28	1.04±0.23	0.8±0.19	1.5±0.26	1.6±0.39	1.4±0.19	1.6±0.15
Brain wt/100 g body weight**	0.642	0.604	0.504	0.345	0.745	0.702	0.810	0.830
Kidney wt**, g	1.13±0.11	1.2±0.12	0.8±0.18	0.8±0.09	1.3±0.16	1.2±0.12	1.01±0.10	1.2±0.15
Kidney wt/100 g body weight**	0.609	0.6	0.435	0.349	0.641	0.6	0.581	0.623
Heart wt**, g	0.5±0.07	0.7±0.11	0.5±0.14	0.4±0.11	0.6±0.08	0.6±0.15	0.4±0.11	0.6±0.14
Heart wt/100 g body weight**	0.282	0.34	0.245	0.174	0.314	0.269	0.257	0.333
Spleen wt**, g	0.5±0.09	0.6±0.15	0.4±0.13	0.5±0.12	0.6±0.20	0.6±0.12	0.5±0.18	0.5±0.16
Spleen wt/100 g body weight**	0.268	0.297	0.190	0.210	0.298	0.289	0.277	0.251

1 Values are mean. n = 10/group

* G_1: basic diet (control).
G_2: nutritive sweeteners (sucrose) diet.
G_3: non-nutritive sweeteners (saccharine) diet.
G_4: nutritive sweeteners (aspartame) diet
G_5: nutritive sweetener : nutritive sweetener (1:3) diet.
G_6: nutritive sweetener : nutritive sweetener (1:3) diet
G_7: non-nutritive sweeteners : nutritive sweetener (1:3) diet.
G_8: nutritive sweetener : non-nutritive sweetener (1:3) diet

** P < 0.01
*** P < 0.05
N.S. = Not significant

sweetener; non-nutritive sweetener and nutritive sweetener ingestion on weight gain, food intake and physiology parameter.

When aspartame diet (caloric sweetener) replaced sucrose diet, weight gain increased significantly ($P<0.05$), this result is diametrically opposed to the result obtained by the substitution of saccharin (non-caloric) for sugar. These findings are in accordance with those obtained by Blundell and Hill (1986), they have reported that the consumption of aspartame might lead to residual hunger and thus to a loss of control over appetite, i.e., a"paradoxixal effect". In addition, Stellman and Garfinkel (1986) have reported that persons who use artificial sweeteners are more likely to gain weight than nonusers. In a series of studies which evaluated the effect of aspartame on hunger, satiety, and body weight (e.g., the effect of covert caloric dilution with aspartame on energy intake and body weight), Porikos et al. (1977) and Katherine and Van Itallie (1984) have shown that when aspartame replaced sucrose, energy intake was 76 to 77% of baseline the first few days, 85% the next few days, and stable thereafter. Rolls et al. (1988) ; Rolls et al. (1989); Mattes, (1990) have shown that aspartame in some circumstances had appetite stimulating properties in comparison with ingestion of water. Moreover, the volunteers were left with a residual hunger with aspartame. The residual hunger is functional i.e., it leads to increased food consumption. On the other hand Rodin (1990) showed that aspartame sweetened drinks did not lead to significantly greater subsequent food intake than did a preload of plain water. In 1991, Rolls concluded that both short term and long term studies have shown that consumption of aspartame sweetened foods or drinks is associated with either no change or a reduction in food intake. For the aspartame diet, the rats received either sucrose: aspartame mixture in a 1:3 ratio (G_6); saccharin: aspartame mixture in a 1:3 ratio (G_7) or aspartame: or aspartame: saccharin mixture in a 1:3 ratio (G_8) (all of the mixture equivalent 40g sucrose/day) had higher food intake and lower weight gain ($P<0.05$) than did the corresponding experimental diets. These results are diametrically opposed to the results obtained by Saravis et al. (1990), who have demonstrated that aspartame combined with carbohydrate did not effect short-term energy or macronutrient intake as well as subjective feelings of hunger or mood, when compared to cyclamate with carbohydrate, in normal 9-10 year-old children. Canty and Chan (1991), also have shown that aspartame , compared to saccharin and sucrose, had no effect on subsequent food intake or subjective feelings of hunger, appetite, and satiety. In addition , searle conducted a two generation long-term toxicity study of aspartame (File E-70, (1974) and File E-87, 1975), groups of 40 male and 40 female rats received aspartame at 2 and 4g/Kg body weight/day as a diet admix for weeks. Decreased weight gain and food consumption were reported for the animals fed aspartame at 4g/Kg body weight/day, also this revealed that, heart-to-body weight ratios were significantly decreased in treated males and liver weight was increased in all treated females. These findings are in accordance with those obtained in this study for heart but not for liver, where heart relative weights were significantly decreased ($P<0.01$) in

all treated aspartame diets expect in (G_8), whilst liver relative weights were significantly decreased ($P<0.01$) in aspartame diet but significantly increased in aspartame mixture diets. The present study also revealed that, daily feed consumption was elevated and gain-to-feed ratios were significantly depressed by the Saccharine: aspartame (1:3); aspartame:Saccharine (1:3) diets only during the experimental period, indicating adaption to the Sweetener mixture diets with continued feeding.

The effect of different sweetener diets on S.glucose; S.total protein; albumin; globulin; total lipids; total cholesterol and triacylglycerols.

Table (3) gives the mean values and significance of S.glucose; S.total protein; albumin; globulin; (A/G) ratio; total lipids; total cholesterol and triacylglycerols. A significant increase ($P<0.05$) was observed in serum glucose concentration in all experimental sweetener rat groups (except sucrose: saccharin mixture diet in a 1:3 ratio) (G_5) as compared to control rat group, (table 3). In fact, the experimental aspartame diet significantly increase ($P<0.05$) S.glucose concentration, compared with the experimental saccharin and sucrose diets (G_4, 8.78 mmol/L; G_3, 8.36 mmol/L and G_2, 6.74 mmol/L) respectively, (tabel 3). Of the sucrose diet, a significant decrease (6.18 mmol/L,$P<0.05$) was observed in serum glucose when the rats fed sucrose: Saccharin mixture diet in a 1:3 ratio (G_5) (equivalent 40g sucrose/day). In contrast, there was a significant increase (8.61 mmol/L, $P<0.05$) in S.glucose in rats fed sucrose:aspartame mixture diet in a 1:3 ratio , (G_6) (table 3).

The differences in serum glucose concentra caused by the sweetener in the diet were rel principally to differences in the kind sweetener. For all six groups (without G_5) rate of increase in serum glucose significantly increased compared with co group ($P<0.05$), (table 3).

Table (3) depicts the changes in serum protein and its fractions that occurred i animals during each dietary treatment durin 42 days experimental period. After feeding all the experimental diets, serum total prote G3; G_4;G_6 and G_7 increased slightly (P< after 6 weeks, whereas there was a signifi increase observed in serum total protein w rats fed either sucrose (G_2) or sucrose: sacch (G_5) diets ($P<0.01$). Serum albumin did differ in sucrose: aspartame mixture (G_6) from control (3.62 g/dL and 3.36 respectively), whereas a significant increase observed in albumin in all other grou experimental sweetener diets. It was seen table (3) that, the results of globulin diametrically opposed to the results obtain serum total protein and serum albumin (G_2; where a significant increase was obser globulin; and G_6 serum globulin did not from control).

Table (3) also gives the mean value significance of total serum lipids, cholesterol and triacyglycerols. There significant relationship between dietary and serum total lipids and triacyglyce significant increase ($P<0.01$) was obse

Table (3) *The effect of dietary regimen on serum glucose; total protein; albumin; globulin; total lipids; total cholesterol and triacylglycerols levels in albino rats.*

	Groups						
	G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_8
Serum glucose**, mmol/L	6.54±1.50	6.74±0.64	8.36±0.67	8.78±1.07	6.18±1.45	8.61±1.84	7.97±1.47	7.85±1.78
% changes from control	-	3.06	27.83	34.25	- 5.50	31.65	21.87	20.03
Serum total protein*, g/dL	6.40±0.66	7.01±0.36	6.58±0.42	6.55±0.26	7.42±0.46	6.50±0.37	6.52±0.37	6.72±0.34
% changes from control	-	9.53	2.81	2.34	15.94	1.56	1.88	5
Serum albumin*, g/dL	3.63±0.24	4.04±0.48	3.98±0.21	4.01±0.28	4.76±0.41	3.62±0.31	4.05±0.68	3.13±0.32
% changes from control	-	11.29	9.64	10.47	31.13	- 0.28	11.57	41.32
Serum globulins*, g/dL	2.77±0.48	2.97±0.47	2.60±0.33	2.54±0.48	2.59±0.63	2.72±0.16	2.35±0.72	1.38±0.40
% changes from control	-	7.22	- 6.14	- 8.30	- 6.50	- 1.81	- 15.16	- 50.18
Globulins ratio*	1.34±0.20	1.40±0.33	1.55±0.20	1.63±0.36	1.89±0.55	1.28±0.22	1.91±1.15	3.70±1.75
Serum total lipids*, mg/dL	184.8±19.92	206.2±27.73	429.6±64.46	294.4±28.26	269±88.42	213±19.87	267±89.66	250.4±63.83
% changes from control	-	11.58	132.47	59.31	45.56	15.26	44.48	35.50
Serum cholesterol*, mmol/L	1.89±0.19	2.75±0.55	2.24±0.44	2.39±0.41	1.94±0.08	2.39±0.27	2.03±0.42	1.73±0.24
% changes from control	-	45.50	18.52	26.46	2.65	26.46	7.41	- 8.47
Serum triacylglycerol*, mmol/L	0.81±0.11	1.57±0.28	1.56±0.24	1.18±0.10	1.17±0.25	1.64±0.16	1.06±0.38	0.92±0.34
% changes from control	-	93.83	92.59	45.68	44.44	102.47	30.86	13.58

* P < 0.01
** P < 0.05

serum total lipids and triacyglycerols concentration in all experimental sweetener rat groups (table 3), than in control group. On the other hand, mean total cholesterol was lower in aspartame: saccharin mixture diet (G_8), whereas, a slight increase ($P<0.01$) was observed in total cholesterol in either sucrose : saccharin diet (G_5) or saccharin :aspartame diet (G_7). Serum total cholesterol was almost similar for rats fed aspartame diet (G_4) and sucrose: aspartame diet (G_6), (table 3).

There was a significant change in S.glucose when aspartame and saccharin were substituted for sucrose. Studies involving healthy subjects have demonstrated that sucrose when consumed by itself (Jenkins et al., 1981) or as part of meal (Bantle et al., 1983) did not result in a greater rise in plasma glucose than isocaloric amounts of other common carbohydrates. The same has been demonstrated for diabetic subjects consuming sucrose as part of a meal (Slama et al., 1984). On the other hand, the present results are diametrically opposed to the results obtained by two studies done as following (Willard, 1984): a total of 43 of the diabetic patient who participated were under the care of the Department of Medicine, Tulane University School of Medicine, New Orleans, Louisiana while 26 were patients of the Jewish Hospital and Medical Center, Brooklyn, New York. Of the 43 patients studied at Tulane University, 18 showed fasting blood sugar concentrations which were below 100 mg/dl for the majority of their analysis. Of these 18 subjects, 7 had consumed aspartame, and 11 placebo (saccharin). But the present results are in agreement with another results which revealed by the same two studie whenin; there were 10 patients with a majority their blood sugar values above 110 and belo 130 mg/dL: 7 had consumed aspartame, and placebo. The remaining 15 patients showe blood sugar concentrations above 130 mg/dL f a majority of their analysis : 9 had consume aspartame, and 6 placebo. The remaining 2 patients, followed at the Jewish Hospital na Medical Center in Brooklyn, showed n statistically significant differences in blood sug concetrations between aspartame and placeb groups at weeks 1,5,9 and 14 of the study (th latter results are diametrically opposed to th results obtained by the present study).

On the other hand, there was a highly significan increase ($P<0.01$) in serum total lipids i saccharin regimen group (G_3) than did th corresponding sucrose or aspartame groups (G and G_4). The reversal of serum total lipid cholesterol, triacylglycerol levels in the a dietary groups after cross-over of the diets (tabl 3) is good evidence of a genuine effect of diet In this study, the difference in serum total lipid and its fractions levels can probably be attribute to the sweetener component of the diets. Thes results are diametrically opposed to the result obtained by (Porikos et al., 1982), they examined energy intake of volunteers (eight nonobese an five obese) eating foods sweetened wit sucrose or the low-caloric sweetener aspartam they found that, serum triacyglycerol level was at the upper limit of normal, (130 mg/dL), on th base-line diet. By the end of 12 days on th aspartame-swetened diet, triacyglycerol lev had fallen significantly to 87 mg/dL. On th

other, they also reported that, serum cholesterol and glucose levels were unchanged by the alteration in diet composition and the removal of sucrose, and there were no differences in the responses of obese and nonobese subjects.

The effect of different sweetener diets on ASAT, ALAT; Urea; Uric acid and Creatinine in Serum:

Fig. (1) and (2) depict the changes in ASAT (S.GOT); ALAT (S.GPT) activities; S. urea;' S. uric acid and S.creatinine that occurred in all animals during each dietary treatment during the 42 days experimental period. The study found significant decrease in the activity of ASAT in all experimental rat groups, except in saccharin regimen group (G_3) ($P<0.01$). On the other hand, there were significant elevations in ALAT activity in all experimental rat groups than did the corresponding control group Fig. (1). In a study on the efficacy of low -calorie sweeteners in reducing food intake, studies with aspartame, Katherine and Van Itallie (1984), they found that, during the base-line diet for obose and nonobese men, ALAT and ASAT activity rose significantly above admission levels with return to these initial levels during ingestion of aspartamne, that result is in agreement with this reported by present study in case of ASAT, but is not in accordance with ALAT. Fig. (2) shows that , 40% either sucrose (G_2); saccharin (G_3); aspartame (G_4) or sucrose: aspartame (1:3) (G_6) caused higher serum urea concentrations ($P<0.01$), wherein sucrose: saccharin (1:3) (G_5) and aspartame: saccharin (1:3) (G_8) diets were effective in lowering S.urea ($P<0.01$), whilst saccharin: aspartame (1:3) (G_7) diet slightly decreased S.urea than the correspodning rats served as contrlos (G1) $P<0.01$, Fig.(2). It was seen from table (3) that, S. creatininne did not show any significant changes Fig.(2). Overall analysis showed that serum uric acid and S.creatinine concentrations were significantly ($P<0.01$) lower in all experimental rat groups, except aspartame: saccharin (1:3) diet (G_8) which gave higher S.uric acid, wherein S. creatinine was increased in either aspartame (G_4) or sucrose: aspartame (G_6) diets, when compared with control feeding group but this increase was not significant Fig.(2). In a study on the effects of aspartame in young person during weight reduction, Willard, (1984) asministered sspartame and the placebo in 300 mg gelatin capsules. The substances were assigned and administered in a randomized double-blind fashion. Dieticans of the Thorondik Metabolic ward, Boston City Hospital, Boston, Massachusetts, obtained dietary histories on the initial visit and gave instruction for an individualized calorie restricted diet which was ingested for the 13 weeks that aspartame or the placebo were consumed. The daily intake of aspartame was 2.79g. Average plasma glucose measured at weeks 7 and 13 in both groups was significantly below initial concentrations, as would be excepted during weight loss, but there was no significant difference between the aspartame and placebo treatment (this result is not in accordance with present result). Measurements of blood urea nitrogen, creatinine, triacylglycerols, total cholesterol, cholesterol esters, direct, indirect and total bilirubin , total thyroxine,SGOT activity and utic acid gave no

basis for suspecting toxicity of aspartame. Converntional urinalyses at weeks 7 and 13 likewise gave no evidence of important differences between treatment. These results are diametrically opposed to the results obtained by present study, wherein, there was a significant elevation in ALAT activity in all experimental rat groups than did the corresponding control group Fg.(1), these elevations are more specific indicator for acute liver damage and hepatic dysfunction.

The effect of dietary regimen on liver total protein and glycogen.

Data for the effect of different diets on liver total protein and glycogen are shown in table (4) . Using the casein diet, there was a significant difference in liver total protein ($P<0.05$) and glycogen ($P<0.01$) concentration between animals on aspartame: saccharin diet (G_8) (9.36 g/100 tissue; 49.98mg/100 tissue), and those on sucrose: aspartame (G_6) (7.57g/100g tissue; 59.8mg/100 tissue) respectively, (table 4). Sucrose diet (G_2) gave higher values for liver glycogen (73.84 mg/100g tissue) than the correspond experimental diets. On the other hand feed sucrose diet, in comparison with either feed saccharin or aspartame diets, led to progres increase in serum glucose, 6.74; 8.36 and mmol/L respectively, (table 3), while glycogens were 73.84, 17.73 and 39.68mg/ tissue respectively, (table 4). Overall anal showed that liver glycogen concetration significantly ($P<0.01$) lower in sucrose: saccl (G5); and saccharin diets (G3) (7.33 17.73mg/100 tissue respectively) than did corresponding experimental diets. The pre study revealed that, there was a slight increa liver total protein concentration in G_2;G_4 ; an in comparison with G_2 . On the other hand, total protein was elevated when Saccharin combined with aspartame in high doses (G_8) significantly depressed in low doses (G_7). T results may be due to, Saccharin (the oxida of O-tolenesulfonamide) combines well other sweeteners; synergistic effect w combined with aspartame (Adapted from multiple sweetener concept, June 1983. Ca Control Commentary. Calorie Control Cou 5775 Peachtree-Dunwoody Road, Suit 50

Table (4) *The effect of dietary regimen on liver total protein and glycogen*

	Groups							
	G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_8
liver total protein, g/100g tissue *	6.87±1.49	7.66±1.49	7.97±0.77	7.57±1.06	8.76±2.07	7.57±1.38	6.33±1.42	9.36±2.28
%change from control	-	11.50	16.01	10.19	27.51	10.19	-7.86	36.24
liver glycogen, mg/100g tissue **	3.48±1.39	73.84±33.16	17.73±9.1	39.68±9.2	7.33±7.87	59.8±23.32	36.68±30.85	49.98±63.71
%change from control	-	2021.84	409.48	1040.23	110.63	1618.39	954.02	1336.21

* $P < 0.05$
** $P < 0.01$

Atlanta, GA 30342).

Also the higher content of glycogen in liver of G_2 than G_3 and G_4 is attributed into change of sucrose into glycogen after its hydrolysis into glucose and fructose. On the other hand, liver glycogen in G_6 was elevated, in comparison with either feeding G_7 or G_8.

Histopathological Examinations

The brain sections in rats received control diet (G_1) showed, no significant microscopic lesions in brain and liver (Fig.3). Histopathlogical lesions were pronounced in the rats that received diet containing 40% sucrose/day, (G_2). The brain sections showed, aggregates of lymphocytes; inflammation in cells; reactive astrocytes and few congested blood vessels. While the portal tract in liver section showed, moderate infiltration by mononuclear inflammatory cells, mainly lymphocytes; and there was vascular congestion Fig.(4).

The examined brain sections in rats that received saccharin diet (G_3 equivalent 40g sucrose/day_ showed, inflammatory cells; and histocytes bizarre, whilst liver sections showed the same result mentioned before for 40% sucrose diet but mild Fig.(5). In rats receiving aspartame diet, (G_4 equivalent 40g sucrose/day), the examined brain section showed, focal areas of necrosis (infarction). The liver showed haemorrhage Fig. (6). Of the sucrose diet, the rats receiving added either saccharin, G_5 or aspartame, G_6 the examined brain sections, revealed inflammatory cells (lymphocytes; reactive astrocytes for G_5, while G_6 had inflammatory cells; histocytes bizarre and shaped astrocytes. Wherein liver sections showed, highly eosinophilic cells with pyknotic nuclei; and fatty change for G5 and G6 Fig. (7) and (8). Rats fed saccharin diet incorporated with aspartame, G_7, in a 1:3 ratio, equivalent 40g sucrose/day, the examined brain sections showed histiocytes bizarre; fatty cahnge and inflammatory cells. Whilist liver sections revealed very few inflammatory cells in portal tract and pyknotic nuclei Fig. (9).

The examined brain sections in rats receiving aspartame: saccharin mixture in a 1:3 ratio, equivalent 40g sucrose/day, showed necrosis cellularity; (Stage of tumors). The examined liver sections showed swollen cells with peripherally located; fatty change with congested dilated; blood sinusoids and central veins Fig. (10).

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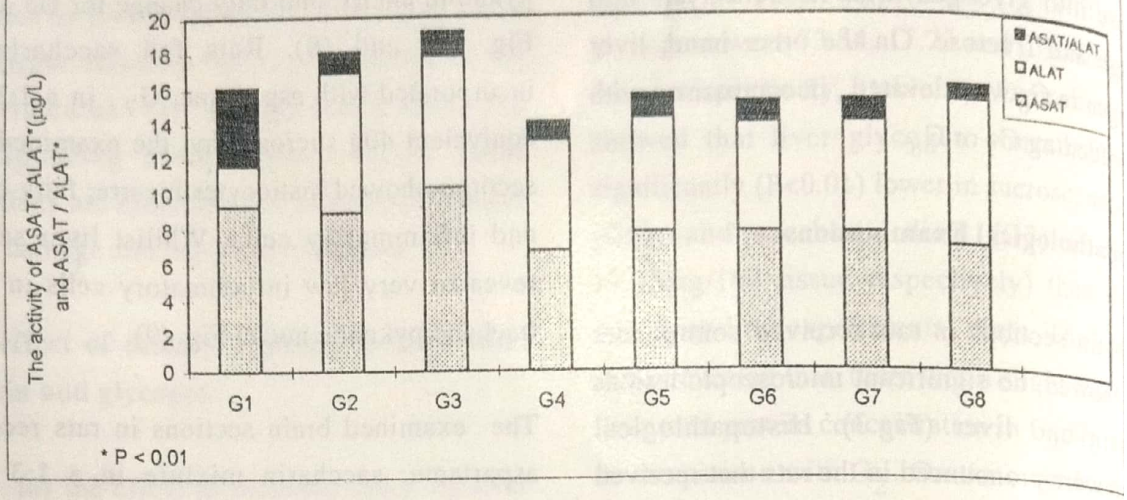
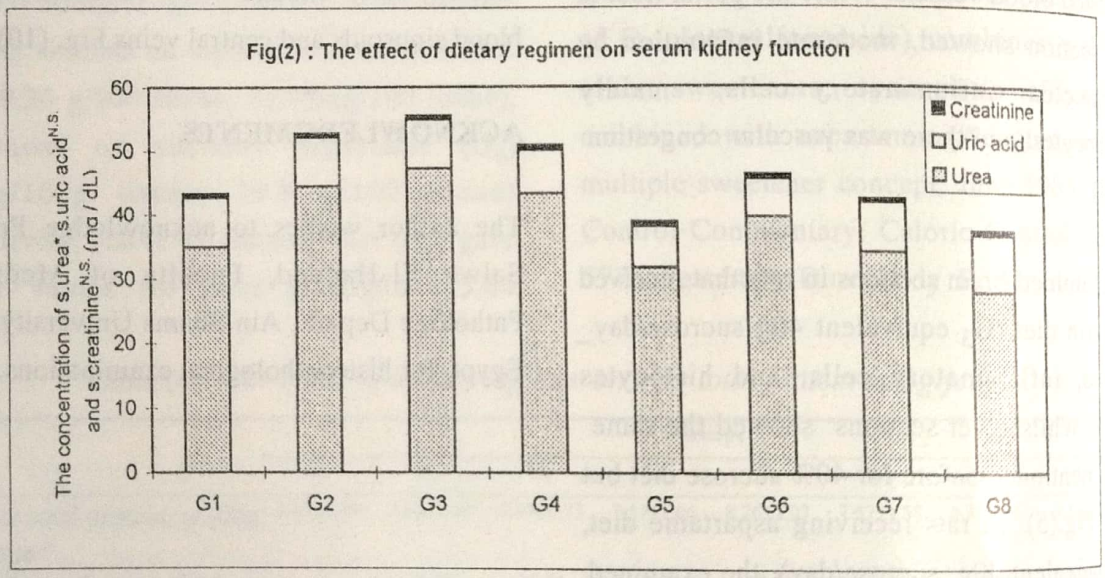
Fig(1) : The effect of dietary regimen on serum liver function



Fig(2) : The effect of dietary regimen on serum kidney function



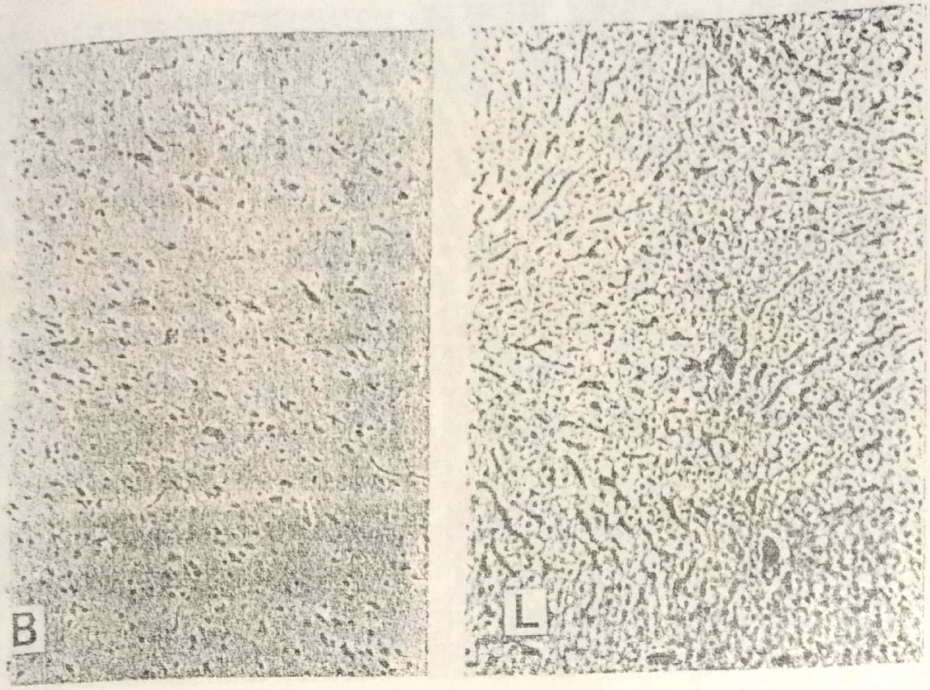


Fig.(3) *The brain and liver sections in rats received control diet showed, no significant lesions (x 100)*

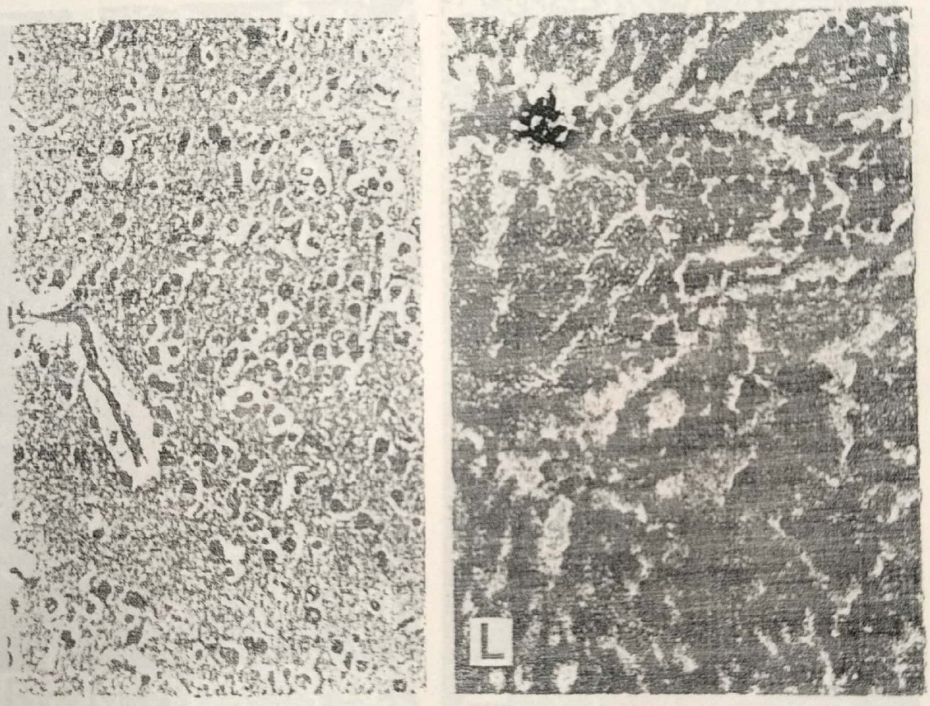


Fig.(4) *The brain sections showed, aggregates of lymphocytes; inflammation in cells; reactive astrocytes and few congested blood vessels (x 250). The liver sections showed, moderate infiltration by mononuclear inflammatory cells, mainly lymphocytes and vascular congestion (x 500).*

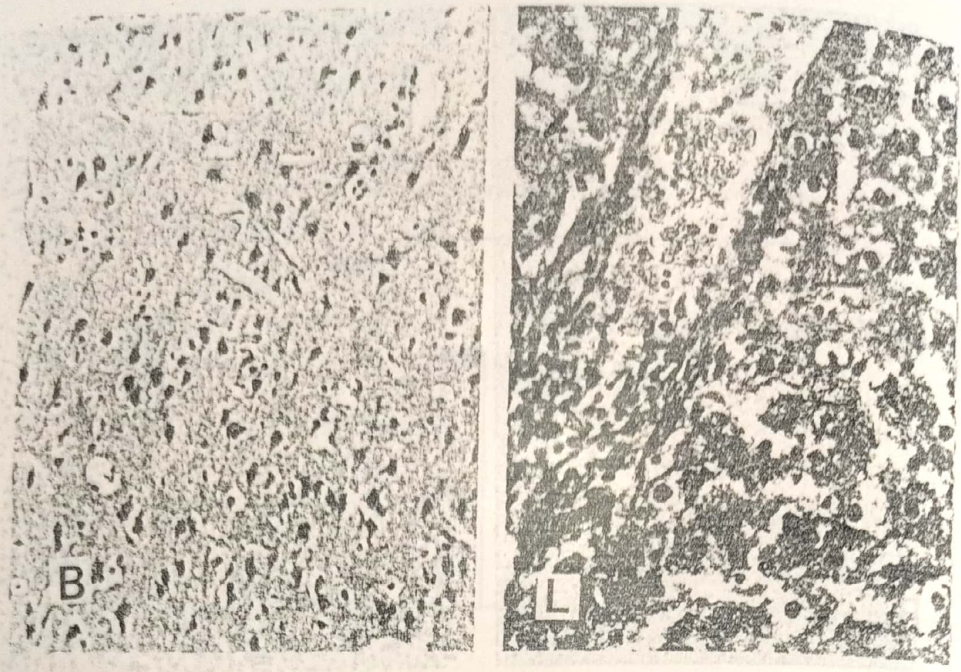


Fig.(5) *The brain sections showed, inflammatory cells; and histiocytes bizarre (x 250). The liver sections showed, the same result mention for Fig.(3) but mild (x 500).*

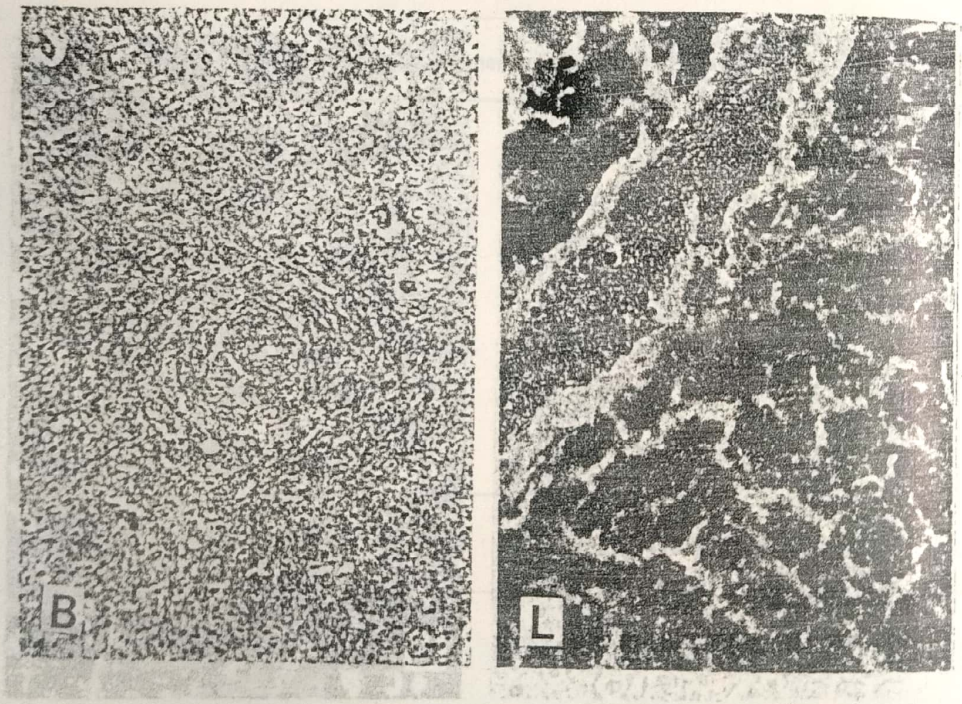
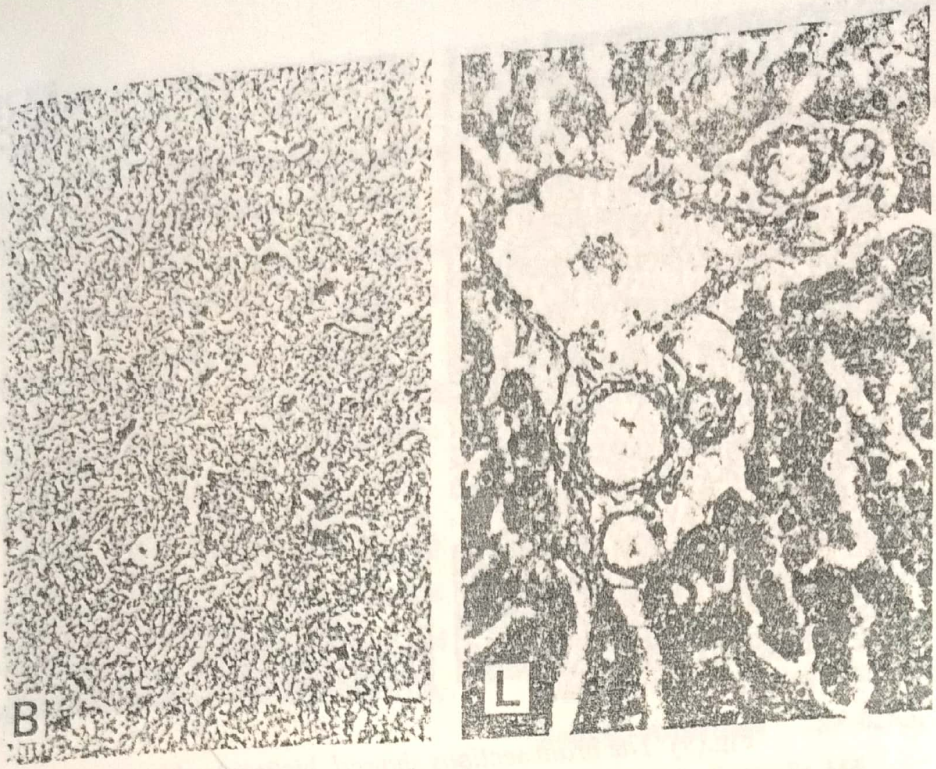


Fig.(6) *The brain sections showed, focal areas of necrosis (infarction) (x 100). The liver sections showed, haemorrhage (x 500).*



(7) *The brain sections showed, inflammatory cells; reactive astrocytes (x 100). The liver sections showed highly eosinophilic cells with pyknotic nuclei, and fatty change (x 500).*

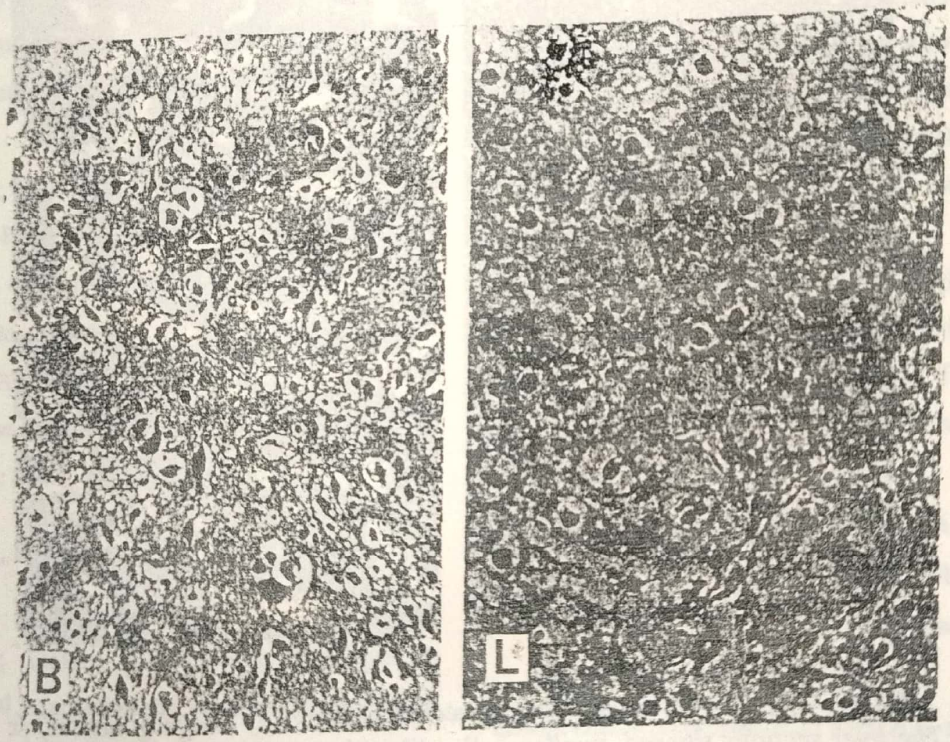


Fig.(8) *The brain sections showed, inflammatory cells; histiocytes bizarre and shaped astrocytes (x 250). The liver sections showed, the same result mention for Fig.(6) (x 500).*

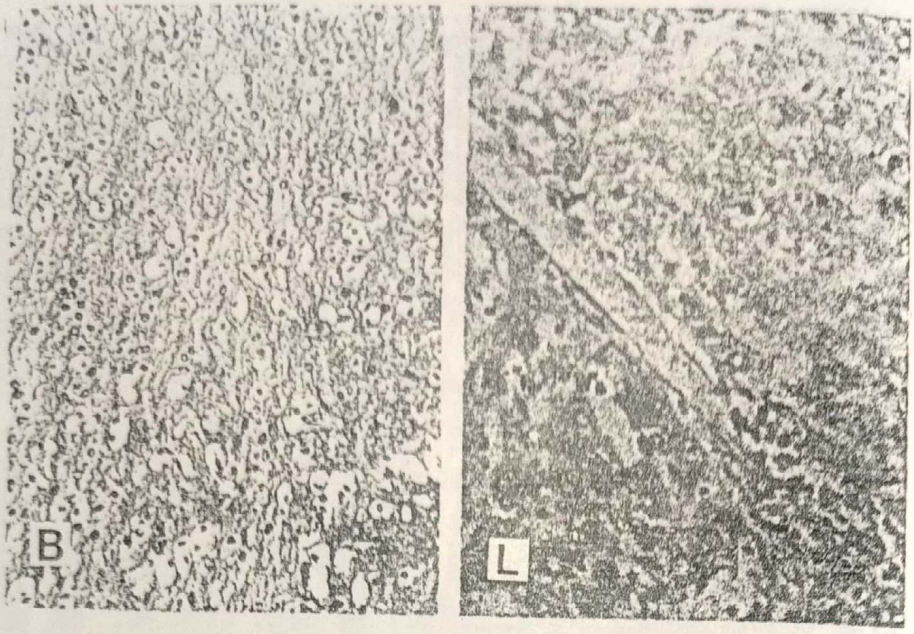


Fig.(9) *The brain sections showed, histiocytes bizarre; fatty change and inflammatory cells (x 250). The liver sections showed, very few inflammatory cells in portal tract and pyknotic nuclei (x 500).*

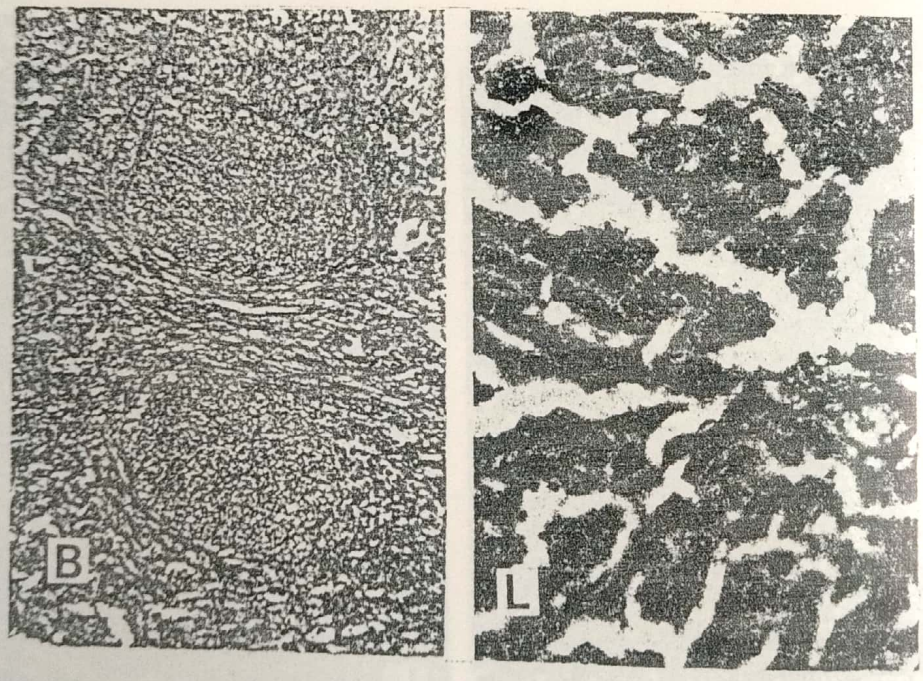


Fig.(10) *The brain sections showed, necrosis cellularity (stage of tumors) (x 100). The liver sections showed, swallen cells with peripherally located; fatty change with congested dilated; blood sinusiods and central veins (x 500).*

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