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BIOLOGICAL AND BIOCHEMICAL EVALUATION OF CHLORINATED SUCROSE DERIVATIVE ADMINISTERED TO ALBION RATS.

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ABSTRACT: Chlorinated sucrose products (tri- and tetrachlorosucrose) were administered to Albino rats at different levels (0.05, 0.1, 0.2 and 0.15, 0.3, 0.6 % respectively) , for 2 months. Serum samples were withdrawn at different intervals during feeding to follow the changes in the biochemical indicators. At the end of the experiment, the rats were killed, and body weight gain and organs weights were recorded. The results indicated that body weight was reduced in all rats receiving the chlorinated sugars, while the organ weights remained significantly unchanged by the different treatments. Also serum total protein and urea concentrations were reduced, while creatinine did not show any significant changes. Significant decreases in the activities of AST (GOT) and ALT (GPT) were recorded in all rats receiving chlorinated sugars. Liver glycogen content tended to increase in the rats administered trichlorosucrose and tetrachlorosucrose at all used levels for 2 months while liver protein level did not show significant changes from the controls . Feeding on either trichlorosucrose or tetrachlorosucrose has generally reduced serum glucose level. This reduction in serum glucose level was not sweetener concentration dependent but rather it was an indirect effect of eliminating sucrose in the meal. Serum total lipids, triglyceride and cholesterol were diminished in all experimental rat groups compared to control.

Key words: Sucrose, Trichlorosucrose, Tetrachlorosucore, Sweeteners, Serum glucose, Glycogen, Total protein.

INTRODUCTION

Sugar chlorination was proved to the effective in enhancing the sweetness of chlorodeoxy – derivatives of sucrose ((Grice and Goldsmith 2000). have concluded that chlorine substitution is merely enhancing the intrinsic sensory quality of sucrose. The hydrophobic of the stimulus affects its accession and its distribution onto the receptor . Hence, the increase in hydrophobic after chlorination proved to be one of the clues to enhanced sweetenes (Ford and Waites 1978). It has been established that the selective chlorination of the sucrose molecule produced remarkable changes to the sweetness intensity and stability of sucrose, without compromising taste quality. The resulting sweetener has a pleasant sweet taste similar to sucrose and has no unpleasant after taste.

Sucralose (1,6-dichloro-1,6-dideoxy β-D-fructofuranosyl 4-chloro 4-deoxy α-D-galacto-pyranoside) is a unique non caloric sweetener derived from sucrose. It is a novel intense sweetener with a potency about 600 times that of sucrose (Hough 1989).

Tetrachlorosucrose (1,6-di chloro 1,6-dideoxy β-D-fructofuranosyl 4,6- dichloro 4,6-dideoxy α- D- galactopyranoside) is a novel intense sweetener with a potency about 100 times that of sucrose (Wladyslaw 2002).

Sucralose and tetrachlorosucrose are not hydrolysed in the intestinal humen, poorly absorbed by experimental animals and excreted largely unchanged in the faeces (Grice and Goldsmith 2000). The small amount of absorbed sucralose or tetrachlorosucrose is distributed essentially to all tissues.

Studies show that there is no active transport of sucralose and tetrachlorosucrose across the blood brain barrier. In addition, the high water solubility and physico-chemical stability of sucralose allow it to be used in acidic beverages and backed goods without loss its sweetness during processing and storage.

The safety of sucralose has been established and reported in a comprehensive series of toxicological studies over a 13 year period. But, there are still some discrepancies in the results and hence the worries about its consuming are still persisting .

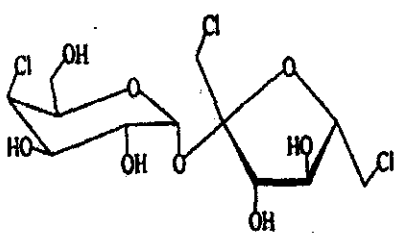
So, the present investigation is conducted to study the effects of consuming trichlorosucrose (sucralose) and tetrachlorosucrose

sweeteners on the general hygienic status of Albino rats measured through the levels of organs weight and clinical biochemical parameters in serum associated with liver and kidney function such as ALT, AST, urea, uric acid, creatinine, total protein, and albumin.

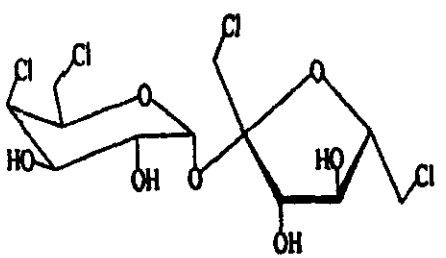
MATERIALS AND METHODS

Materials

Sucrose was obtained from the Sigma Chemical Co. The chlorinated sugars; 1',4,6' Trichlorogalactosucrose and 1',4,6,6' tetrachlorogalactosucarose were prepared according to Christopher *et al.* (1992) and Hough (1989) respectively.



1',4,6' Trichlorogalactosucrose



1',4,6,6' Tetrachlorogalactosucrose

Animals

Forty adult male albino rats, Sprague-Dawley strain, of mean weight 100 gm were obtained from the animal test farm, Faculty of Veterinary Medicine, Zagazig University. The animals were divided into eight groups and housed individually in stainless steel wire cages. With mesh bottoms and kept in a room maintained at 25-30°C. The room was lightened on a daily photoperiod of 12h. All animals were kept under normal healthy conditions and fed on control diet for two weeks. After a feeding period on control diet. The eight groups received the experimental diets. Scheme (1) shows the composition of the basal and experimental diets scheme. Food and water were provided ad libitum.

Body gain weight and food consumption were recorded weekly for 2 months.

Scheme 1 :Composition of the experimental diets (g/100g)

Ingredients dry base	Basic diet (G_1)	Sucrose 10% (G_2)	1',4,6 trichloro-galactosucrose 0.05% (G_3)	0.1% (G_4)	0.2% (G_5)	1',4,6,6'tertachlorogalactosucrose 0.15% (G_6)	0.3% (G_7)	0.6% (G_8)
Starch	65.0	55.0	64.95	64.90	64.8	64.85	64.7	64.4
Corn oil	10	10	10	10	10	10	10	10
Sucrose	-	10	-	-	-	-	-	-
Trichloro-galacosucrose	-	-	0.05	0.1	0.2	-	-	-
Tetrachloro-galactosucrose	-	-	-	-	-	0.15	0.3	0.6
Cellulose	5	5	5	5	5	5	5	5
Salt Mix.	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Vit. Mix.	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Casein	15	15	15	15	15	15	15	15

Tissue Preparation

After 60 days of feeding, animals were fasted for 16 hour and anesthetized with ether. Incisions were made into the abdomen, blood samples were obtained from the portal vein and left to clot then centrifuged at 4000 rpm for 15 min. at 4°C to obtain serum. Liver, kidney, heart, spleen, brain, testes and lung were excised, rinsed in chilled saline solution, then blotted on filter paper and weighed separately to calculate the absolute and relative organ weight. Two- thirds of liver were stored in saline solution at -20°C until analysis.

Analytical Procedures

Serum glucose, proteins, albumin, urea , uric acid, creatinine and total lipids were estimated by using kits prepared by Diamond Diagnostic for Laboratory Services, Cairo, Egypt, according to Trinder (1969), Sunderman *et al.* (1958), Webster, (1974), Fawcett and Scott, (1960) Henry (1974), Henry (1974), Thannhouser, (1958) respectively.

Serum total cholesterol, triglyceride, activities of glutamate oxaloacetate transminase (AST) and glutamate pyruvate transminase (ALT) were estimated by using enzymatic colorimetric method kits developed by Biocon, D-57299 Burbach / Germany, according to Richmond (1973), Fossati and Prencipe (1982), Reitman and Frankel (1957), respectively.

Total protein and glycogen content in liver were determined by the method of Lowry *et al.* (1951), Nicholas *et al.* (1956) respectively.

Statistical Analysis

Statistical analysis was done by completely randomized design in factorial arrangement (ANOVA, F-test) showed evidence of overall differences between diets, according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Serum Glucose

The data in Figure 1 represent serum glucose levels in Albino rats fed on diets containing different levels of trichlorosucrose or tetrachlorosucrose as compared to controls during different intervals of ; 14, 28, 42 and 60 days. It is clear that serum glucose level increased gradually in the two controls groups with no differences between the two controls, i.e the rats fed on diets containing 65 % starch and those

fed 55 % starch plus 10 % sucrose. Feeding on either trichlorosucrose or tetrachlorosucrose has generally reduced the serum glucose level and the decrease was more pronounced in case of trichlorosucrose. This was not sweetener concentration dependent.

It can be concluded that, the serum glucose reduction was not a direct effect of sweetener administration but rather to the elimination of sucrose from the meal, independently from the treatment diets. As all treatments diets contain about 65 % starch but no sucrose, glucose level was reduced to different levels within the same range and without distinction between the different diets. So it can be concluded that consuming these sweeteners as replacement of sucrose can reduce serum glucose level as an indirect effect of eliminating sucrose from the meal. Three concentrations of each sweetener were tested to make sure that increasing sweetener concentration does not have any adverse effects in any of the individuals used to different levels of sweetener powers. The present result opposes that of Goldsmith (2000) who observed statistically significantly increased glucose levels relative to control as a result of administrating 1 and 3 % sucralose to male Albino rats for 6 months. But these recorded high levels were less than those recorded pretest. The longer period and higher dose in the latter study may account for this increased glucose levels., and might have originated from a specific effect of the sweetener.

Liver Glycogen and Proteins

The data in Figure 2 represent the levels of liver glycogen (A) and protein (B) in rats fed diets containing different levels of trichlorosucrose or tetrachloro-sucrose .It can be clearly noticed that glycogen was much higher in the rats fed 10 % sucrose & 55 % starch (control 2) than in rats fed 65 % starch (control 1). This may result from the quick absorption and assimilation of sucrose resulting in high serum glucose levels which may be consequently converted into glycogen in contrast to the slow release of glucose from assimilated starch.

Replacing sucrose by different levels of the two studied sweeteners has led to reduced glycogen levels as compared to the sucrose control (control 2). There was no significant differences between the different concentrations of each sweeteners,

i.e. the effect is not related to the sweetener itself but to the elimination of sucrose content in the diet from 65 to 55 % in the treatments than in sucrose .

However, glycogen in the rats fed different levels of the two sweeteners was still higher than the starch control (control 1).

In the same Figure 2 it can be observed that liver protein content was higher in sucrose control than starch control or treatments probably due to the surplus of absorbed and assimilated glucose than the body requirements. No distinction was observed between the different treatments and starch control probably due to the slow release of glucose from starch . These results agree with those of Hassanin (1998) who found that administration of both aspartame and saccharin to Albino rats led to significantly lower liver glycogen level and higher liver total protein level compared to control.

Body and Organ Weights

The data in Figure 3 show the body and organ weights in rats fed diets containing different levels of trichlorosucrose and tetrachloro-sucrose as compared to controls. It can be noticed that there was no difference in the final body gain between starch control (C_1) and sucrose control (C_2). However, the sweetener treatments showed significant reductions in body weight gain especially at the higher levels of sweeteners. This may suggest certain role of the sweeteners in reducing body weight, probably due to reduced appetite as a probable consequence of sweetener diets reduced platability. Corresponding reduced food intake were observed with sucrose chlorination in the range of 5 -6 %. This result agrees with that of Mann *et al.* (2000a) who stated that body weight gain and food intake were significantly decreased in a dose-dependent manner in sucralose-treated rats throughout a two-month treatment period (2-month) as compared to the controls. It was established that the reduction of body weight gain induced by using sucralose in the diet at concentrations of 1 % and below resulted from reduced food intake as a direct consequence of the unpalatable nature of sucralose.

There was no significant changes in rat organs final weights between the starch control rats, sucrose control rats and at all other treatments. This result may indicate that feeding on the studied sugars does not change the general

body physiology and hence the overall hygienic status. This result is in accordance with the finding of Mann *et al.* (2000b) who stated that organ weights were unaffected by administration of sucralose at a level of 0.3 – 3.0 % to mice during 104 weeks of feeding. Our results contradicts those of Goldsmith (2000) who recorded statistically significant decreases in the relative weight of rat and mice organs after administration of 5 % sucralose. This contradiction is apparently due to the relatively higher dose of sucralose used in the latter case.

Liver Functions

The data in Figure 4 represent serum contents of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), protein and albumin in rats fed on diets containing different levels of trichlorosucrose and tetrachloro-sucrose during different intervals of feeding as compared to controls. It is noticed that in both starch and sucrose control (C_1,C_2), ALT increased gradually with the duration of feeding. Feeding on both sweeteners has significantly increased the level of serum ALT at the early stage of feeding, i.e. after 14 days for all concentrations of the two sweeteners as compared to the two controls. The increasing effect diminished gradually with time and it was completely reversed by the end of the experimental period (2 months). It can be inferred that at early stages of feeding the increasing effect was due to the ingestion of unusual substance that required some biological mechanism to assimilate or counteract the new introduced substance. Once, this mechanism was established liver function as indicated by ALT level returned not only to normal level but to levels lower than the control. It can be also stated that after certain period of adaptation to the new sweetener, liver functions were greatly improved. In a similar study, Goldsmith (2000) found statistically significant decrease in ALT after 4 weeks of sucralose administration (5 %) and this level was returned to normal after 8 weeks. In the present study the ALT reduction was most evident after 6 weeks since the dose used is much inferior (0.05 – 0.2 %) to that used by Goldsmith (2000). Similar trend can be observed with the AST level in the rats fed trichlorosucrose. However rats fed tetrachlorosucrose showed relativity slight increases in AST at early stages but lower ones at late stages as compared to sucrose

control. There was no significant differences between rats fed trichlorosucrose or tetrachlorosucrose and control in either serum protein or serum albumin indicating the normality of liver functions.

In conclusion , it can be stated that liver function was maintained normal during feeding on diets containing different levels of either trichlorosucrose or tetrachlorosucralose especially after an adaptation period to the new substances.

Kidney Functions

Serum contents of urea, uric acid and creatinine in Albino rats fed different levels of trichlorosucrose and tetrachlorosucrose during different intervals are shown in Figure 5. It can be observed that serum urea was specifically reduced by trichlorosucrose especially at the higher concentrations of the sweetener (0.1 – 0.2 %). This effect was more pronounced at the late stage of feeding. With tetrachlorosucrose, no significant differences between the rat fed sweetener and controls in the serum urea level.

With trichlorosucrose, there was some reduction in serum uric acid with low concentrations (0.05 – 0.1 %) of sweetener at late stage of feeding. The values recorded for rats fed tetrachorosucrose were slightly higher than control but within the normal range.

Serum creatinine was insignificantly decreased in sweetener fed rats as compared to the control referring to the voidness of these sweetener of any side effect affecting kidney function. Similar effects on serum urea, uric acid and creatinine were reported (Hassanin, 1998) when some nutritive and non nutritive sweeteners (aspartame and saccharin) were fed to Albino rats.

Lipid Metabolism

The data shown in Figure 6 show the changes in serum total lipids, triglyceride and cholesterol in rats fed diets containing different levels of trichlorosucrose and tetrachlorosucrose during 60 days of feeding. It can be observed that total lipids increased in sucrose and starch controls with extending the duration of feeding. This trend was completely reversed in rats administered different levels of the two studied sweeteners . So, it can be concluded that eliminating sucrose from the diets supplemented with

the sweetener has led to reducing the serum total lipid with extending the time of feeding. This trend applies to the level of triglycerides which represent most of total lipid confirming the previous conclusion.

Likewise, rats fed different concentration of trichlorosucrose showed significantly reduced serum cholesterol levels especially when extending the time of feeding. It can be clearly seen, in both controls, serum cholesterol was increased gradually with time in contrast to the rat fed trichlorosucrose or tetrachlorosucrose which showed a reverse trend. However this cholesterol reducing effect was not influenced by the substance concentration. This result may be a direct effect of the used substance on cholesterol metabolism or to the relatively lower carbohydrate content in the treatment diets. A similar but less clearer trend can be observed with tetrachlorosucrose. The general results obtained here agree with those obtained by Hassanin (1998) who stated that sweeteners, such as aspartame and saccharin could reduce cholesterol level. However, they recorded high levels of total lipids and triglycerides in the rats fed sweeteners (aspartame and saccharin). This difference may be due to some specific effect of the artificial sweeteners used in that study. It might be more logic that the diets free of sucrose might not favour the accumulation of fatty substances in the serum. Hence, it is expected that rats fed diets containing sweeteners as replacers of sucrose component may favor lower serum total lipids and triglycerides level.

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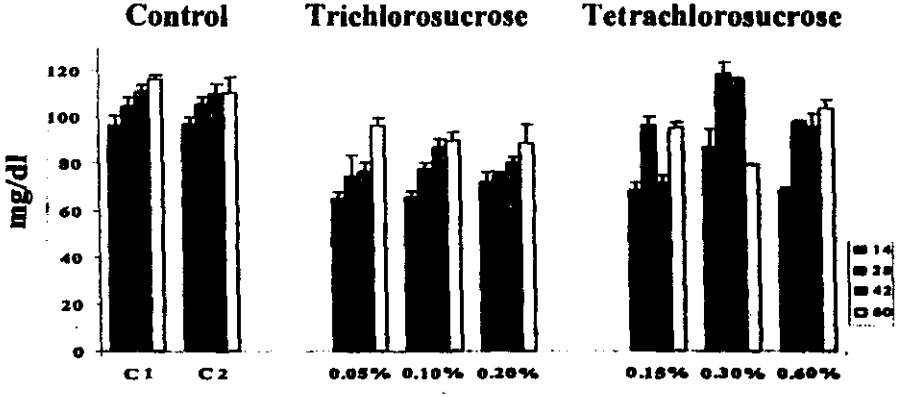

Figure 1 : Serum glucose levels in Albino rats fed diets containing different levels of trichlorosucrose and tetrachlorosucrose. Control 1: Normal basal diet (starch control). Control 2 : Normal basal diet contains 10 % sucrose at the expense of starch (sucrose control).

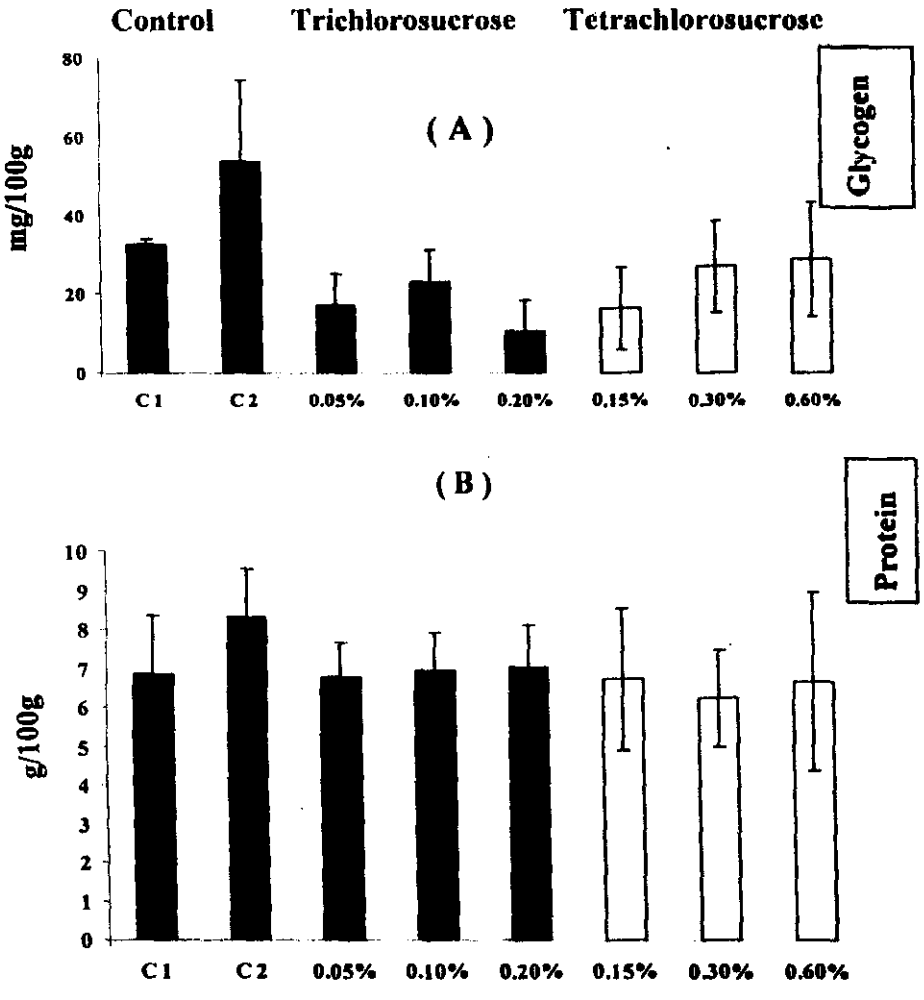

Figure 2: Liver glycogen (A) and protein (B) contents of Albino rats fed diets containing different levels of trichlorosucrose and tetrachlorosucrose. Control 1: Normal basal diet (starch control).Control 2 : Normal basal diet contains 10 % sucrose at the expense of starch (sucrose control).

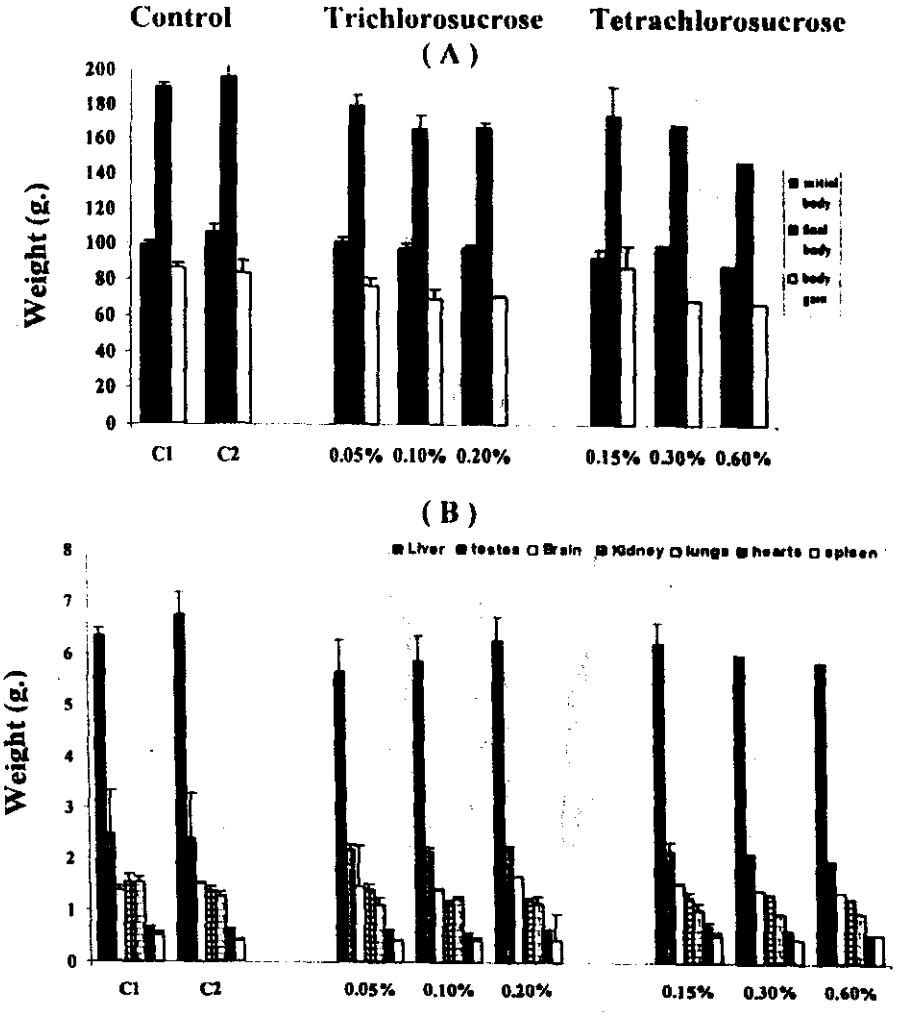

Figure 3: Weight of body (A) and organs (B)of Albino rats fed diets containing different levels of trichlorosucrose and tetrachlorosucrose. Control 1: Normal basal diet (starch control). Control 2 : Normal basal diet contains 10 % sucrose at the expense of starch (sucrose control).

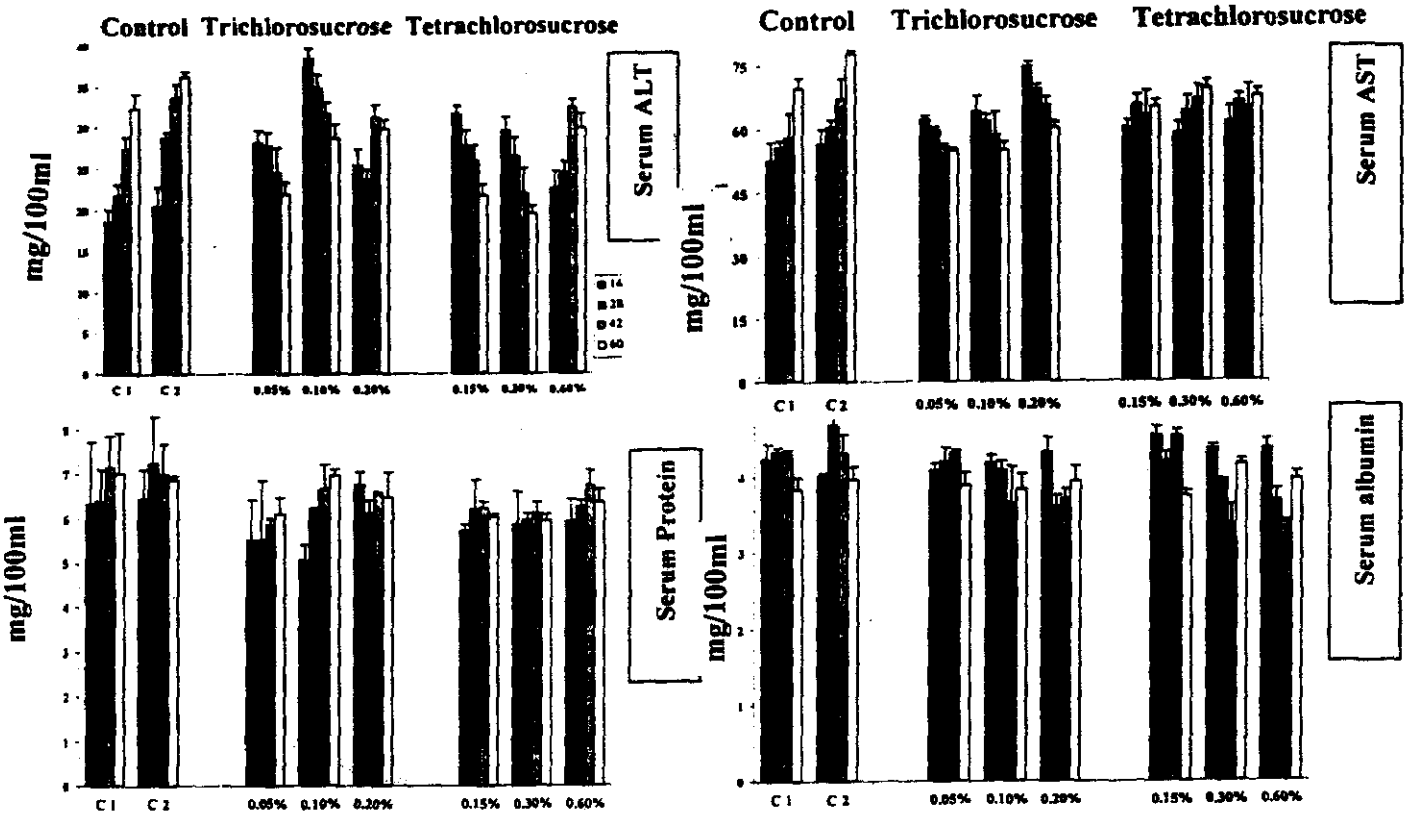

Figure 4: Serum ALT, AST, protein and albumin levels in Albino rats fed diets containing different levels of trichlorosucrose and tetrachlorosucrose. Control 1: Normal basal diet (starch control). Control 2 : Normal basal diet contains 10 % sucrose at the expense of starch.

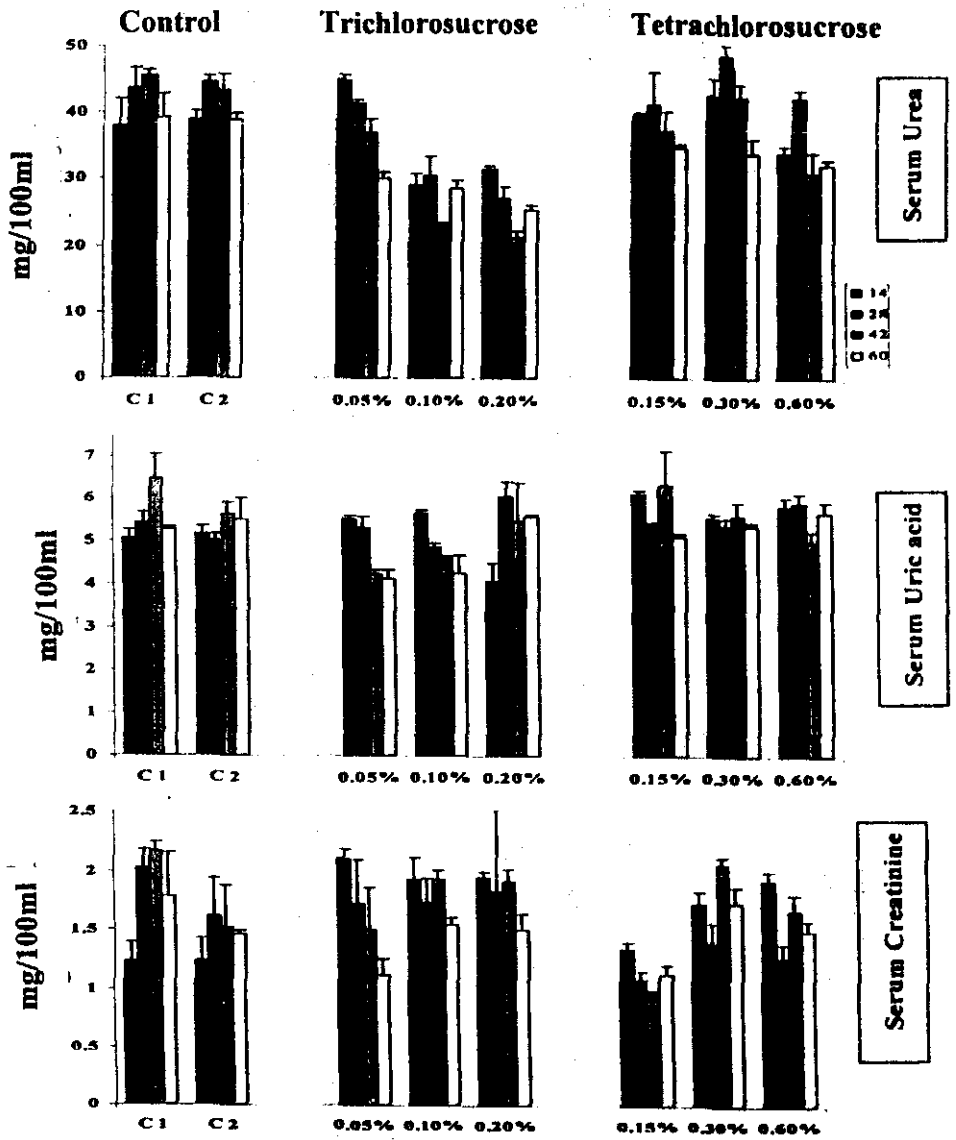

Figure 5: Serum urea, uric acid and creatinine levels in Albino rats fed diets containing different levels of trichlorosucrose and tetrachlorosucrose. Control 1: Normal basal diet (starch control). Control 2 : Normal basal diet contains 10 % sucrose at the expense of starch.

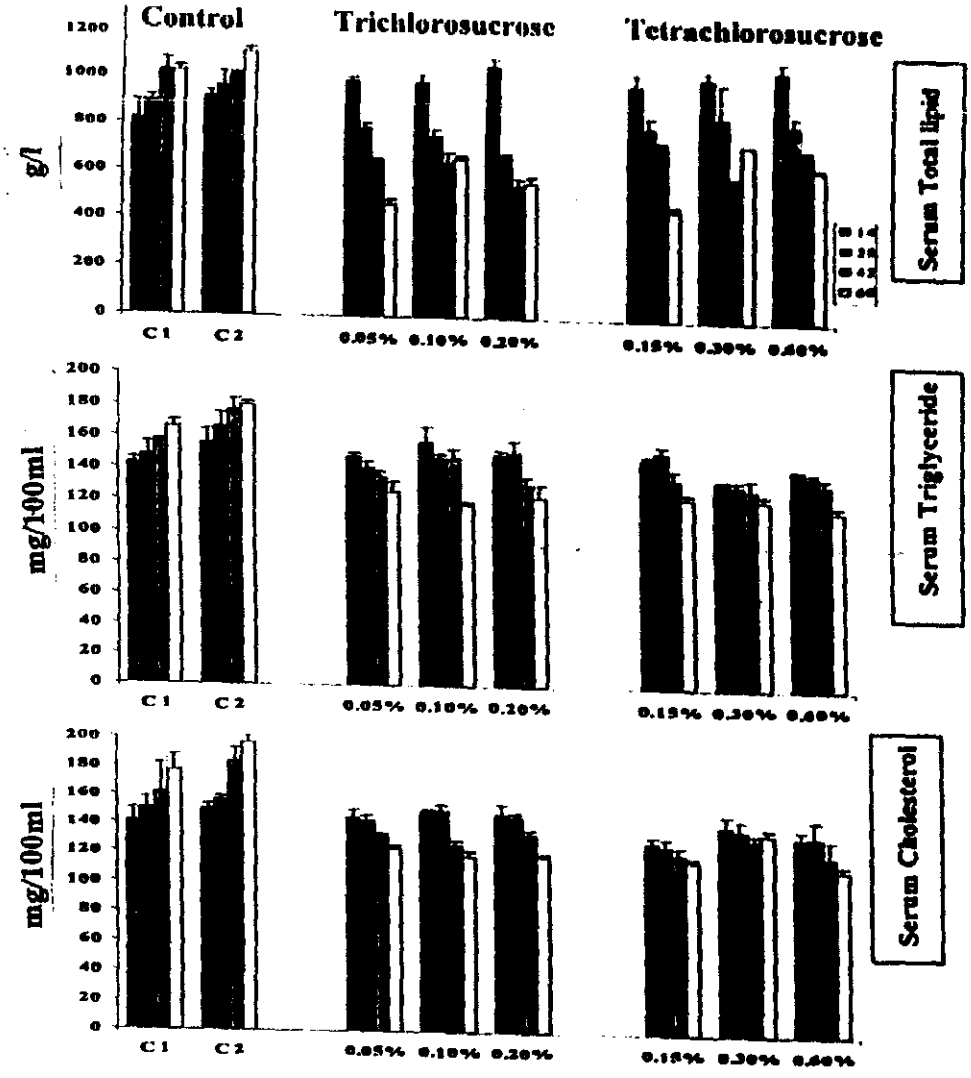

Figure 6: Serum total lipid , triglyceride and cholesterol levels in Albino rats fed diets containing different levels of trichlorosucrose and tetrachlorosucrose. Control 1: Normal basal diet (starch control). Control 2 : Normal basal diet contains 10 % sucrose at the expense of starch.

التقييم الكيميائى و البيولوجى لمشتقات السكروز المكلورة على الفئران البيضاء

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تم استخدم نوعين من مشتقات السكروز المكلورة وهما ثلاثى كلوريد السكروز ورباعى كلوريد السكروز كبدائل للسكروز فى الوجبات المقدمة للفئران البيضاء على ثلاث مستويات مختلفة (٠,٠٥و ٠,١ و٠,٢% للمشتق الاول و ٠,١٥ و ٠,٣ و ٠,٦ % للمشتق الثانى) واستمرت عملية التغذية لمدة شهرين . وقد أخذت عينات من سيرم الفئران على فترات أثناء فترة التغذية وفى نهاية التجربة تم وزن الفئران وذبحها ووزن الاعضاء. وقد أشارت النتائج الى إنخفاض الوزن الكلى لجسم الفئران نتيجة المعاملة بمشتقات السكروز بينما لم يتاثر وزن الاعضاء بهذه المعاملات. انخفض تركيز كل من البروتين الكلى واليوريا فى سيرم دم الفئران المغذاه على مشتقات السكروز المكلورة بينما لم يحدث اى تغيرات معنوية فى مستوى الكيرياتينين نتيجة هذه المعاملة. كما انخفض مستوى نشاط انزيمات ناقلات الامين Transaminases (ALT and AST) مقارنة بالكنترول. حدثت زيادة فى محتوى الجليكوجين بالكبد بينما لم يقابل ذلك اى تغير معنوى فى محتواه من البروتين مقارنة بالكنترول. وقد تزامن ذلك مع انخفاض مستوى الجلوكوز فى السيرم فى الفئران المعاملة. كما انخفض مستوى الدهون الكلية والجليسريدات الثلاثية والكوليستيرول الكلى فى سيرم دم الفئران المغذاه على مشتقات السكروز المكلورة مقارنة بالكنترول