BIOCHEMICAL STUDY ON SUGAR REPLACER (SACCHARIN®) IN FEMALE RATS
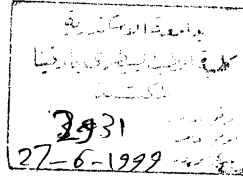
Thesis
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For
The M.V.Sc. Degree
Biochemistry and Clinical
Biochemistry

To
Faculty of Veterinary Medicine
Alexandria University

1995

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قرار لجنة الحكم والمناقشة
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قررت لجنة الحكم والمناقشة ترشيح السيد ط.ب/ محمد مصطفى يوسف الكـــــــدن
للحصــول على درجــة الماجستير فى العلـوم الطبية البيطرية تخصص (الكيمياء الحيويــــة
والاكلينيكيــــة)

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To My Father's
Spirit

To My Great
Mother

To My Sister

To My Sister's
Daughter Asmaa

To My sister's
Sons Ahmed
and Ashraf

Acknowledgement

At first, the prayerful thanks to our Merciful God who gives me everything I have.

I am very grateful to **Prof. Dr. Ibrahim Fatouh**, Professor of Biochemistry, Faculty of Veterinary Medicine, Alexandria University, for his extreme valuable advises, instructions, guidance, suggestions and encouragement throughout my investigation; under his supervision this work has been completed.

I would like to express my gratitude for the kindness and encouragement of **Prof. Dr. Nabil Taha**, Prof. of Biochemistry, Faculty of Veterinary Medicine, Alexandria University for his valuable help and continuous interest during the preparation of this work.

I am very grateful to **Dr. Mahdy Korshom**, Assistant Professor of Biochemistry, Faculty of Veterinary Medicine, Alexandria University for his interest, encouragement and facilities he offered in this work.

Great thanks for **Dr. Abdel-Wahab Mandour**, Assistant Professor of Biochemistry, Faculty of Veterinary Medicine, Alexandria University, for his help and valuable advises.

Special thanks and gratitudes to A. Lecturer **Samir El-Shazly**, Assistant Lecturer of Biochemistry, Faculty of Veterinary Medicine, Tanta University.

Finally, many thanks and appreciations are due **all staff members** of Biochem. Dept. in Faculty of Veterinary Medicine, Alexandria University for their constant help.

CONTENTS

INTRODUCTION

Saccharin is considered as one of the most important sugar replacers which used in several medical purposes such as a non caloric sweetener for diabetics and obsess persons (Finar, 1973).

The appetite of rats increases when kept on diet incorporated with saccharin solution (Tordoff and Friedman, 1989a,b,c). Berthoud *et al.* (1981); Woods and Bernstein (1980) stated that, saccharin stimulate cephalic phase insulin release. Cephalic phase response initiated by the sweet taste of saccharin shifts metabolic fuels away from oxidative pathways towards storage (Newsholme and Start, 1973).

Under some conditions, drinking non-nutritive sweeteners increases short-term food intake and the motivation to eat (Blundell and Hill, 1986 and Blundell *et al.*, 1988), and blocking the sweet taste of a drink decreases subsequent food intake (Brala and Hagen, 1983).

The literature seemed to be deficient in the effect of saccharin on liver and kidney function tests, therefore, the present study was conducted to throw light on the effect of sugar replacer (saccharin) on some liver and kidney function tests in female rats.

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REVIEW OF LITERATURE

I. Saccharin

Saccharin is used instead of sugar for many purposes, e.g., sweetening preserves, drinks, elicit is also used by diabetics and obese persons (Finar, 1973).

Studies with rats indicate that the preferred concentration of saccharin, judged by 24-hours consumed tests, is approximately a 0.25% solution (Stellar, 1962).

Female rats prefer significantly higher concentrations of saccharin than males do (Valenstein et al., 1967).

The sweet taste of saccharin also appears to stimulate the release of insulin (Deutsch, 1971).

It is hypothesized that sweet taste increases food intake by producing biochemical changes in the liver that increase fuel storage and consequently decrease fuel oxidation (Tordoff, 1988).

Saccharin increases the catabolism of dietary tryptophane to metabolites with known carcinogenic activity toward the rat bladder. (Sims and Renwick, 1983).

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Saccharin can stimulate cephalic-phase insulin release. (Berthoud *et al.*, 1981 and Woods and Bernstein, 1980).

Cephalic-phase response initiated by the sweet taste of saccharin shifts metabolic fuels away from oxidative pathways towards storage (Michael *et al.*, 1989).

The cephalic phase produced by the sweet tasting of saccharin leads to a series of interrelated mechanisms are brought into play, including increasing gastrointestinal secretion (Pavlov, 1902 and Farrel, 1928), enhanced gastrointestinal absorption (Threatte *et al.*, 1986), pancreatic enzyme and hormone release (Pavlov, 1902; Preshow *et al.*, 1966; Powley, 1977; Louis-Sylvestre and LeMagnen, 1980; Sjostrom *et al.*, 1980; Woods and Bernstein, 1980) and probably a change in hepatic metabolism to favor storage of fuels (Shimazu, 1983).

The motion of cephalic-phase hyperinsulinemia as a control of feeding may require reinterpretation within a wider metabolic context (Berridge *et al.*, 1981 and Berthoud *et al.*, 1984).

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Insulin facilitates amino acids uptake consequently enhancing protein synthesis and this inhibits breakdown and degradation of protein, (Malchester, 1972 and Morgan *et al.*, 1972).

II. Liver functions:

The liver is considered to be the main organ responsible for the biosynthesis, uptake and degradation of a number of biological materials in blood including proteins and enzymes.

Liver functions may, therefore, be reflected to some extent on the levels or the activities of these circulating biochemical compounds in serum. The infection of the liver results in cirrhosis characterized by fibrosis and absence of parenchymal regeneration (Salah, 1962).

Liver supports the intermediary metabolism of all food stuffs, it's the major nucleus of synthetic, catabolic and detoxifying activities in the body, it is crucial in the excretion of heme pigments and through its Kupffer cells, it participates in the immune response i.e. the liver is a complex organ which performs many of metabolic functions.

Many tests have been based on the hundred of reactions occurring in the liver.

Going on through the literature, the most important parameters used for assessment of liver functions are:

1.   Serum amino transferases:

These enzymes are now an indispensable part of diagnosis, this is especially true in the case of hepatic disease.

Serum glutamic oxalacetic transaminase (SGOT) or aspartate amino transferase (AS-T) is an enzyme that catalyses the reversible transfer of amino group from glutamic to oxalacetic. It is present in large quantities in liver, skeletal muscle, kidney, cerebral tissues, pancreas, spleen and lungs. And the serum level of the enzyme increases whenever these tissues are acutely destroys and elevation occurs due to release of enzyme from damaged cells. Very high values are found with hepatocellular necrosis (Sherlock, 1975). Serum glutamic pyruvic transaminase (SGPT) or alanine amino transferase (AL-T); is a cytosol enzyme that catalyses the reversible transfer of an amino group from glutamic to pyruvic acid.

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This enzyme is also present in liver and although the absolute amount is less compared with (AS-T), a greater proportion is present in liver compared with heart and skeletal muscles.

Serum glutamic oxalacetic transaminase (SGO-T) and serum glutamic pyruvic transaminase (SGP-T) increased in liver and heart diseases as hepatocellular damage (e.g. hepatitis), liver cirrhosis, biliary obstruction, hepatic necrosis and myocardial infarction (Cantarow and Trumper, 1962; Wroblewski and La Due, 1956; Molander *et al.*, 1955; Forster, 1957 and Davidsohn, 1974).

Cantarow and Trumper (1962) stated that, these enzymes had a wide tissue distribution. He found that, transaminases catalyze the transfer of amino group from an amino acid (glutamic) to a keto acid (oxaloacetic or pyruvic).

Wroblewski and La Due (1956) gave the normal values of GoT and GpT in different tissues (in units per 10^{-4} grams of wet tissue homogenate) as shown in the following table:

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	Heart	Liver	Muscle	Kidney	Pancreas	Spleen
GoT	156	142	99	81	28	14
GpT	7.1	44	4.8	19	2	1.2

Doxey (1971) found that, chronic liver damage did not result in gross elevation of the serum enzyme levels. He concluded also that, liver disfunction could be confirmed by the activities of GoT, GpT and alkaline phosphatase. He recommended this to be sensitive and reliable especially in acute liver damage.

Cantarow and Trumper (1962) stated that, serum transaminases activities were increased in certain diseases involving tissue rich in these enzymes (specially the liver). This increase was due to the liberation of abnormal amounts from the damaged tissues.

Buk *et al*. (1961) referred the increase in SGOT activity to liver damage.

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Insulin facilitates amino acids uptake, consequently enhancing protein synthesis and this inhibits breakdown and degradation of protein (Malchester, 1972 and Morgan *et al.*, 1972).

No slight increase in S.AL-T activity after any administration of antidiabetic agents (Retiene *et al.*, 1969 and Muller *et al.*, 1969).

Rats which injected with insulin by an acute dose not showed marked elevation in S.AL-T during the starting period of treatment, then returns to its normal level (Beyer *et al.*, 1969).

Insulin causes a significant elevation in S.AL-T due to the fact that, insulin injection to rats or mice by an acute dose cause a damage of liver cells. This damage causing escape of serum transaminases into blood stream, then elevation occurred during the starting of experimental, but the normal level of transaminase occurred after that (Harper, 1975).

On contrast, Abdel-Khalek *et al.* (1978) stated that, rats which treated with insulin, produced no change in serum AL-T activity in normal and alloxan diabetic

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rats, but if these rats injected insulin for a long period or even by an acute dose, no elevation in serum transaminase.

2.   Serum total bilirubin:

Bilirubin is one of the end products of haemoglobin breakdown in the body and is excreted by the liver via the bile, and it's a bile pigment normally present in blood, it's formed in the cells of the reticuloendothelial system.

In qualitative and quantitative analysis, the term bilirubin is applied to the unconjugated bilirubin (Mallory and Evelyn, 1937).

It was shown that bilirubin present in serum in three forms as free bilirubin, the mono, and diglucuronide compounds.

All three are loosely bound to serum albumin.

Bilirubin is insoluble in water unless bound to albumin-react slowly the diazo reagent and requires the presence of alcohol.

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Serum bilirubin is also elevated in a number of diseases of bones and liver, haemolytic and obstructive jaundice or hepatotoxic drugs (William *et al.*, 1969 and Bauer *et al.*, 1974).

3.   Serum total proteins:

The plasma proteins represent a complete mixture containing a number of components which differ in properties and function.

The liver is usually considered to be the site of formation of the plasma proteins, although other parts of the body may also have a function in this connection. The plasma proteins aid in the maintenance of a normal acid-base balance. Plasma proteins serve as a vehicle for the transport of many required water insoluble nutrients and metabolites.

It's claimed that 90-95% of plasma proteins are synthesized by the liver.

Miller *et al.* (1951) reported that, the liver plays an important role in the production of plasma proteins, and observed that the liver synthesized practically all of the plasma fibrinogen, the albumin fraction and probably more than 80% of globulin fraction.

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Some gamma globulins, however, are produced by lymphoid tissues.

Serum protein abnormalities are of value clinically in indicating the presence of diseases.

Ismail *et al.* (1957); Ramirez *et al.* (1961); Mousa *et al.* (1976) and Saleh *et al.* (1976) recorded that, there is an alterations in serum proteins as a host response to infection have frequently been observed in experimental animals and patients.

4.   Serum albumin:

Albumin is a globular protein, the two main functions of albumin are to transport small molecules through the plasma and extracellular fluid and to provide osmotic pressure. Therefore, albumin serves as a carrier for metals, ions, fatty acids, amino acids and enzymes (Ganong, 1977).

An apparent reduction of serum albumin is found in several pathological conditions including various diseases of the liver, this may be due to increased capillary permeability of expansion of extra cellular space, resulting in the presence of an increased proportion of albumin in extravascular compartment (Martin and

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Neubergar, 1957). It may be also caused by increased albumin catabolism, the normal quantity being reduced.

The absolute fall in serum albumin concentration resulting from a disturbances of normal synthesis by the liver is not an early biochemical alteration, and is found more commonly in chronic liver diseases such as subacute hepatitis or diffuse fibrosis. In portal fibrosis there is a characteristic decrease in serum albumin and elevation in gamma globulin (Cantarow and Trumper , 1962).

The albumin level in serum is generally inversely related to the severity of the liver damage (Osserman and Takatsuki, 1963 and, Mantwyler, 1964).

5.   Serum globulins:

Globulins are protein molecules that are insoluble in plain water but soluble in salt water.

The serum globulins are heterogenous, complex mixture of protein molecules that are frequently designated as alpha-, beta- and gamma-globulins based on their electrophoretic mobility.

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Chronic inflammatory diseases of the liver cause hyper globulinemia (Talwar *et al.*, 1989).

Many diseases are characterized by alterations in plasma albumin and globulins with no or little variation in total plasma proteins.

The increase in albumin is associated with a decrease in globulin and vice versa (West and Todd, 1966).

6.   **Albumin/globulin ratio (A/g ratio):**

In mammals the albumin-globulin ratio is more than 1.

III. Kidney functions:

The kidneys perform a series of functions, they excrete the end products of protein metabolism (urea, uric acid and creatinine) and about half the water eliminated from the body.

They maintain and regulate the composition of essential blood constituents, maintain the internal composition of the body to be compatible with life and help to preserve normal acid-base balance of the body fluids.

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Renal function tests generally estimate an average performance of the total kidney.

The most important and useful parameters for evaluation of renal function are:

1.   Serum urea:

Virtually, all of the body tissues produce ammonia, which is present predominantly as ammonium ions, these ammonium ions arise primarily from the catabolism of amino acids. In mammals most ammonium ions are detoxified in the liver where they are converted to urea, a form of nitrogen much less toxic to the central nervous system. If the urea synthesizing system falls as a result of malfunctioning liver, ammonia intoxication results.

Urea represents the main end product of protein catabolism, the liver is the sole site of urea formation as it's the only organ that contains all the enzymes necessary for urea biosynthesis through successive stages of deamination of amino acids, the formation of ammonia, the incorporation of this ammonia into the Kreb's cycle with the resultant formation of urea.

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After urea is formed in the liver, it passes into the blood and is excreted in the urine.

Urea diffuses freely through capillary walls and cell membranes.

It is present in virtually identical concentration per unit of water in extracellular and intracellular fluids i.e. plasma, serum,cerebrospinal fluids, saliva and intestinal secretions.

Blood urea nitrogen (BUN)depends upon the relationship between urea production (protein ingestion and catabolism) and urea excretion, the minor amount of urea destroyed by microorganisms in the intestinal tract may be disregarded (Davidsohn and Henary, 1974).

Urea is excreted mainly by the kidneys. It's through to be cleared at the glomerulus, but partly diffuse back to the blood during the tubular reabsorption of water (White *et al.,* 1976).

The nitrogen content of urea varies from 9-15 mg/dl. The range of normal values may be greater depending on age, sex and diet.

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It has been also reported that, the concentration is higher during the day than during night (White *et al.,* 1976).

Blood urea nitrogen increases in nephritis, prostatic obstruction, renal insufficiency, and decreases in acute yellow atrophy of liver, liver cirrhosis and pregnancy.

Blood urea concentration does not began to increase until glomerular filtration has fallen below 50% (White *et al.,* 1976).

In contrast to serum creatinine levels which vary at a minimum with the protein intake (Zilva and Pannall, 1979) obtained a 400% variation in serum urea levels and this due to the differences in protein intake.

2.   **Serum creatinine:**

Creatinine is derived from the metabolism of tissue creatine and so it is not affected by diet. The synthesis of creatine and parts of two amino acids (arginine and methionine).

Guanidoacetate (glycocyamine) is formed by transamidination primarily in the kidneys; this is a reversible reaction mediated by transamidinase which is subject to feed back inhibition of dietary creatine.

Glycocyamine is then methylated with a second reaction requiring transmethylase and activated methionine in the liver to form creatine.

Creatine in the free state and as phosphocreatine is distributed from liver via blood to muscle and brain with trace amounts in the urine.

Phosphocreatine exists in high concentration, especially in muscle, where it is an important form of high-energy phosphate storage.

The dehydration of creatine results in a ringed compound creatinine which is readily excreted from the body by the kidneys. It is found in the plasma of adult animals in low quantities, in young growing animals, it is found in higher quantities.

Creatinine is highly diffusible substance and is evenly distributed in the body water.

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The measurement of creatinine level in serum yield the same diagnostic and prognostic information concerning renal function as that obtained by the measurement of urea nitrogen.

The determination of creatinine provides more accurate information. Creatine is cleared by the kidney at the glomerular filtration rate (Davidsohn and Henary, 1974).

The concentration of creatinine in the blood like that urea, will increase with decreased kidney function, and the main use of estimation of serum creatinine is in the assessment of kidney function (Taussky, 1954 and Hudson and Rappoport, 1968).

Plasma levels depend largely on glomerular function, while urinary concentration depend almost entirely on tubular function (Zilva and Pannall, 1979).

Creatinine is the least variable nitrogenous constituent of blood. Normal values for serum in man are in the range of 0.5 to 1.2 mg/dl in early nephritis, values of 2 to 4 mg/dl are noted, while in chronic hemorrhagic nephritis with uraemia values of 4 to 35 mg/dl may be obtained (White *et al.*, 1976).

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3.   Serum uric acid:

Mammals and most lower vertebrates are said to be prototrophic for purines and pyrimidines i.e. capable of synthesizing purines and pyrimidines nucleotides De NOVO and thus not dependent upon exogenous sources for these important compounds. In human the ultimate catabolite (end product) of purines is uric acid.

In lower primates and other mammals the enzyme uricase is responsible for the hydrolysis of uric acid to allantion, a highly water-soluble end product of purine catabolism in these animals.

MATERIALS AND METHODS

The present study was carried out at the Faculty of Veterinary Medicine, Alexandria University to clarify the effect of sugar replacer (sodium saccharin) on the liver and kidney functions in female albino rats.

Saccharin® (Imide of O-sulphobenzoic acid)

Upon oxidizing O-tolutene sulphonamide with potassium permanganate in alkaline solution, the sodium salt of O-sulphonamidobenzoic acid is formed, which upon acidifying with concentrated hydrochloric acid or warming passes spontaneously into the cyclic imide of O-sulphobenzoic acid or saccharin:

O-Toluene sulphonamide   Na O-sulphonamido-benzoate   Saccharin
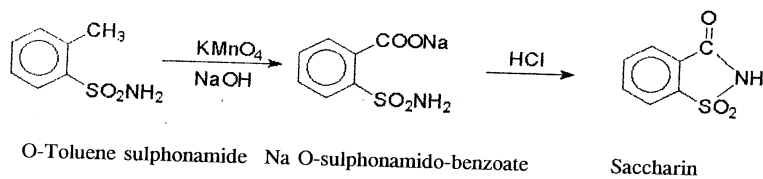
Saccharin itself is sparingly soluble in cold water, but the imino hydrogen is acidic and the compound as water-soluble sodium salt. The later is about 500 times as sweet as cane sugar (Vogel, 1956).

Saccharin, m.p. 224°C, is about 550 times sweeter than sugar. It is almost insoluble in water and hence is sold as its sodium salts, which is very soluble. Saccharin is very sweet in dilute solution but bitter in concentrated solution (Finar, 1973).

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Saccharin sodium tablets produced by Kahira pharm. & Chem. Ind. Co. Cairo-Egypt, as soluble tablets (each tablet contains: Saccharin Sod. 33 mg).

For performing the present study a total of sixty (60) healthy mature female albino rats obtained from Faculty of Veterinary Medicine, Zagazig University were used to avoid the possible sex related variations. Their weights ranged from 130-180 g body weight each.

The rats were kept on basal ration (Table I) formulated according to National Research Council (NRC) recommendation for two weeks prior to the experiment.

The rats were caged together under the same environmental conditions throughout the experimental period.

The rats were classified into three groups, each comprised of 20 rats.

The experimental rats were housed in separate cages throughout the experimental period.

Control group:	Kept on basal diet + water.
Group A:	Kept on basal diet + water containing 0.125% (W/V) sodium saccharin.
Group B:	Kept on basal diet + Water containing 0.25% (W/V) sodium saccharin.

Table I: Ingredient composition (%) of basal diet.

Ingredients	Basal diet (%)
- Yellow con	50
- Wheat bran	20
- Soybean oil meal	28
- Common salt	0.5
- Mineral mixture	1.10
- Vitamin mixture	0.40

Vitamin mixture:

According to NRC (1984) and these vitamins were manufactured by Alex. Pharm. & Chem. Ind. Company.

- Vitamin A   4000,000   Iu

- Vitamin D   1000,000   Iu

- Vitamin E   300,000   Iu

- Vitamin K . . . .   0.50   mg/kg

- Niacin . . . .   20.0   mg/kg

- Folic acid . . . .   1.0   mg/kg

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- Calcium pantothenate	20.0	mg/kg
- Riboflavine	3.0	mg/kg
- Thiamine	4.0	mg/kg
- Vitamin B_6	6.0	mg/kg
- Vitamin B_{12}	0.05	mg/kg
- Choline chloride	1000.00	mg/kg

Collection of blood samples:

Random samples of 5 rats each were chosen from control and treated group for collection of blood samples after 2, 4, 6 and 8 weeks from the beginning of the experiment.

After overnight fasting, the rats were anaesthetized by inhalation anaesthesia using diethyle ether (Stone, 1954).

Blood was drawn from the medial canthus of the eye by using heparinized hematocrit tube into a clean and dried centrifuge tube and left to clot at room temperature for one hour, then centrifuged at 3000 rpm for 15 minutes to separate out serum samples. After collection of blood samples, these rats were excluded from the experiment.

The clear non hemolyzed serum samples were separated and kept frozen at -20°C until analyzed.

The collected sera were subjected for determination of the following biochemical parameters:

A.   Liver functions:

1.   Serum Aspartate aminotransferase activity (S. As-T).

2.   Serum Alanine aminotransferase activity (S.Al-T).

3.   Serum total bilirubin.

4.   Serum direct bilirubin.

5.   Serum total proteins.

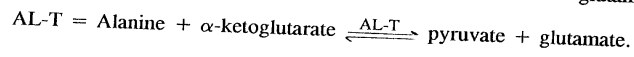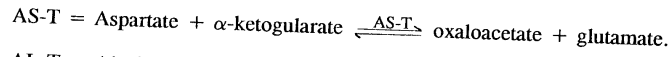6.   Serum albumin.

7.   Serum globulins.

8.   A/g ratio.

B.   Kidney functions:

9.   Serum urea.

10.   Serum creatinine.

11.   Serum uric acid.

1. Determination of serum Aspartate aminotransferase (As-T) and Alanine aminotransferase (Al-T) activities.

Serum Aspartate aminotransferase and Alanine aminotransferase activities were determined by using kits of Sclavo according to the method described by Reitman and Frankel (1957).

The determination of aminotransferase activity takes place according to the following formulate:

AS-T = Aspartate + α-ketogularate $\xrightarrow{\text{AS-T}}$ oxaloacetate + glutamate.

AL-T = Alanine + α-ketoglutarate $\xrightarrow{\text{AL-T}}$ pyruvate + glutamate.

The pyruvate produced by AL-T reacts with 2,4-dinitrophenyl hydrazine giving brown colored hydrazone.

The color was measured colorimeterically at 520-550 nm. The oxalacetate formed by AS-T was decarboxylated spontaneously to pyruvate.

The produced pyruvate was measured also by hydrazone formation as for AL-T.

Reagents:

Concentration of prepared reagents.

AS-T substrate:	DL-Aspartate	200 mM
	2-oxoglutarate	2 mM
	Phosphate buffer	(pH 7.45) 0.1 M
AL-T substrate:	DL-Alanine	200 mM
	2-oxoglutarate	2 mM
	Phosphate buffer	(pH 7.45) 0.1 M
Color reagent:	2,4 Dinitrophenyl hydrazine	1 mM
	Hydrochloric acid	1 N
Standard:	220 mg of Sod. pyruvate were dissolved in 100 ml	
	phosphate buffer	(pH 7.4).

Procedure for S.As-T

Test:

- 5 ml of AS-T substrate were incubated in water-bath at 37°C for 2-3 minutes.

- The tube was removed from the bath and immediately 0.1 ml serum was added, mixed and incubated in the water-bath at 37°C for 60 minutes.

- The tube was removed from the bath and immediately 0.5 ml of color reagent was added, mixed and kept at room temperature for 20 minutes.

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Blank:

5 ml of AS-T substrate were incubated in water-bath at 37°C for 2-3 minutes.

The tube was removed from the bath and immediately 0.1 ml of distilled water was added, mixed and incubated in the water-bath at 37°C for 60 minutes.

The tube was removed from the bath and immediately 0.5 ml of color reagent was added, mixed and kept at room temperature for further 20 minutes.

To test and blank, 5 ml of sodium hydroxide 0.4 N were added.

The tubes were allowed to stand at room temperature for 10 minutes.

The produced brown color was colorimetrically measured at 520-550 nm against the blank.

Procedure for AL-T:

Test:

- 0.5 ml of AL-T substrate was incubated in water-bath at 37°C for 2-3 minutes.

- The tube was removed from the bath and immediately 0.1 ml of serum was added, mixed and incubated in the water bath at 37°C for 30 minutes.

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- The tube was removed from the bath and immediately 0.5 ml of color reagent was added, mixed and kept at room temperature for 20 minutes.

Blank:

- 0.5 ml of AL-T substrate was incubated in water-bath at 37°C for 2-3 minutes.

- The tube was removed from the bath and immediately 0.1 ml of distilled water was added, mixed and incubated in the bath at 37°C for 30 minutes.

- The tube was removed from the bath and immediately 0.5 ml of color reagent was added, mixed and kept at room temperature for 20 minutes.

- To test and blank tubes, 5 ml of sodium hydroxide 0.4 N were added.

- The tubes were kept at room temperature for 10 minutes.

- The produced brown color was colorimetrically measured at 520-550 nm against the blank tube.

- The enzyme activity was determined by reference to the calibration curve.

Preparation of the calibration curve:

- The preparation of the calibration curve was done as follows:

Test tubes No.	1	2	3	4	5	6
Standard . . . . ml	0.05	0.10	0.15	0.20	0.25	-
Substrate (AS-T or AL-T) . . . . . . ml	0.45	0.40	0.35	0.30	0.25	0.50
Distilled water . . . . ml	0.10	0.10	0.10	0.10	0.10	0.10
Color reagent . . . . ml	0.50	0.50	0.50	0.50	0.50	0.50

- The tubes were allowed to stand at room temperature for 20 minutes.

- Then sodium hydroxide 0.4 N was added as follows:

Test tube No.	1	2	3	4	5	6
Sodium hydroxide 0.4 N ml	5	5	5	5	5	5

- The contents of the tubes were mixed and allowed to stand at room temperature for 10 minutes. The optical density of the produced brown color in the tubes from No. 1 to No. 5 were measured colorimetrically at 520-550 nm against the blank (Test tube No. 6).

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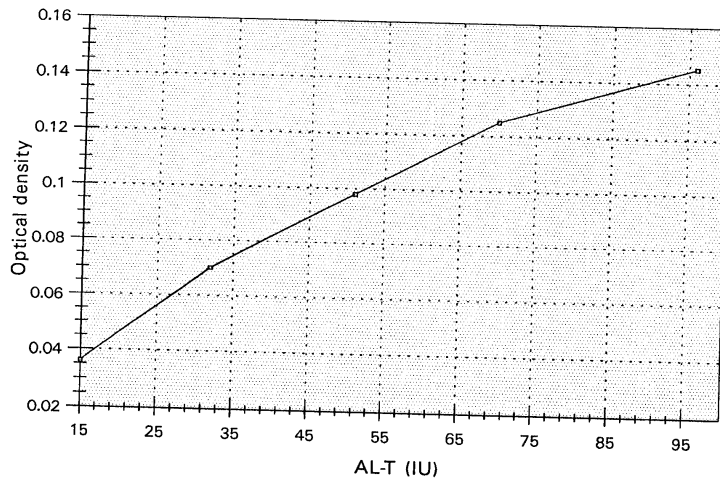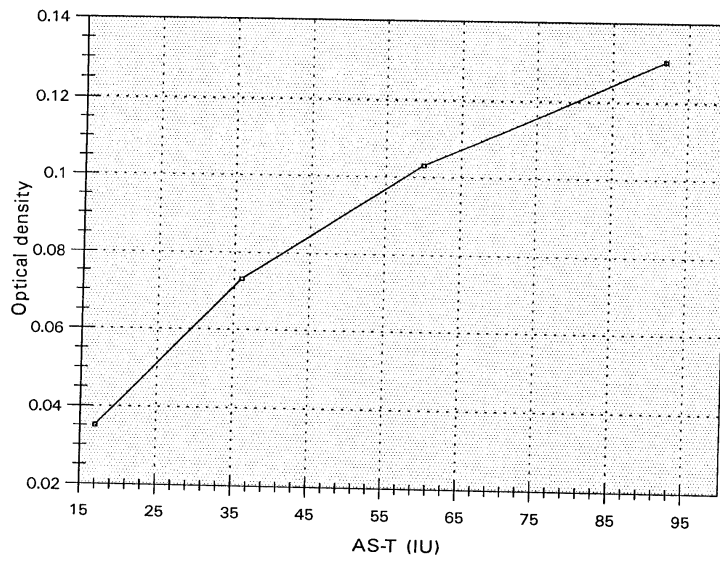
Calibration curve showing the relation between optical densities
and the corresponding values of AS-T and AL-T activities
in international units (IU)

- The corresponding values in international units for AS-T and AL-T were presented in the following table:

Test tubes No.	1	2	3	4	5
AS-T iu	17	36	60	92	-
Optical density	0.035	0.073	0.103	0.130	
AL-T iu	15	32	51	70	96
Optical density	0.036	0.07	0.098	0.125	0.145

2. Determination of serum total bilirubin:

Serum total bilirubin was estimated colorimetrically by using kits of Sclavo according method described by Wisten and Cehelik (1969).

Principle:

Sulfanilic acid reacts was sodium nitrite to give diazotized sulfanilic acid in the presence of detergent. Total bilirubin couples with diazotized sulfanilic acid to give azobilirubin. The formed color is directly proportional to the total bilirubin present in the sample.

Reagents:

Reagent A:	Sulfanilic acid	16.2 mM
	Hydrochloric acid	27 mM
	Detergent	25 mM
Reagents B:	Sodium nitrite	600 mM
Standard:		4.7 mg/dl

Procedure:

Two wave length technique:

The chromogenic reagent was prepared by mixing 1 volume of reagent B with 100 volumes of reagent A.

Standard tube:

1 ml of standard was added to 1.0 ml of chromogenic reagent.

Unknown tube:

0.1 ml of serum was added to 1.0 ml of chromogenic reagent.

Reagent blank tube:

0.1 ml of distilled water was added to 1 ml of chromogenic reagent.

The contents in the three tubes were mixed separately and let to stand for 10 minutes at 25°C, then the standard and unknown tubes were read colorimetrically against reagent blank at 540-560 nm (A_1) and 600-620 nm (A_2).

Calculation:

mg/dl of total bilirubin =

$$\frac{A_1 \text{ speciment} - A_2 \text{ specimen}}{A_1 \text{ standard} - A_2 \text{ standard}} \times \text{standard conc.}$$

3. Determination of serum direct bilirubin:

Serum direct bilirubin was estimated colorimetrically by using kits of sclavo according to the method of Wisten and Cehelik (1969).

Principle:

Sulfanilic acid reacts with sodium nitrite to give diazotized sulfanilic acid, in the absence of detergents or accelerators. Direct bilirubin reacts with diazotized sulfanilic acid to give azobilirubin. The produced color was measured at 540-580nm.

The intensity of the color is directly proportional to the direct bilirubin content in the sample.

Reagents:

Reagent A: Sulfanilic acid   32 mM

                Hydrochloric acid   166 mM

Reagent B: Sodium nitrite   29 mM

Chromogenic reagent (A+B preparation):

    1 volume of reagent B was mixed with 100 volumes of reagent A.

Procedure:

Unknown tube:

    0.1 ml of serum was added to 1.5 ml of chromogenic reagent (A + B).

Serum blank tube:

    0.1 ml of serum was added to 1.5 ml of reagent A.

    The contents in the two tubes (unknown and serum blank) were mixed separately and allowed to stand for exactly 1 minutes at 25-37°C, then measured colorimetrically at 540-580 against distilled water.

Calculation:

    (unknown reading - serum bland reading) x calculation factor = mg/dl of direct bilirubin of the unknown.

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The calculation factors was read from the following table according the used wave length.

Wave length	Calculation factor
540	19.1
545	18.1
550	17.6
555	17.1
560	17.0
565	17.0
570	17.2
575	17.6
580	18.2

4. Determination of serum total proteins:

The serum total proteins were determined colorimetrically using biuret reagent according to the method of Weichselbaum (1946).

Principle:

In this method the protein content was determined by the biuret method in which the peptide bonds in the protein structure react with alkaline copper solution

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to give a violet coloration, which is proportionally directed to the concentration of protein present. The color was measured at 530 nm.

Reagents:

1.   Sodium chloride solution:

9 gm of sodium chloride (AR) were dissolved in 1000 ml of distilled water.

2.   Stock biuret reagent:

45 gm of sodium potassium tartarate were dissolved in 400 ml of 0.2 N sodium hydroxide, 15 gm of copper sulphate ($CuSO_4.5H_2O$) were added and dissolved by stirring, then 5 gm of potassium iodide were added and made up to volume of 1 liter with 0.2 N sodium hydroxide.

3.   Working biuret reagent:

50 ml of stock biuret reagent were diluted to 250 ml with 0.2 N NaOH solution containing 50 gm of potassium iodide and completed to one liter with distilled water.

4.   Standard protein solution:

1 gm of Armour crystalline bovine albumin was dissolved in water and made up to 100 ml for use in the method, 10 ml of this solution were diluted to 100 ml with distilled water. (1 ml = 0.001 gm of albumin).

Procedure:

Test:

0.1 ml serum was pipetted into a test tube, 4.9 ml saline and 5 ml of working biuret reagent were added.

Standard:

For standard, 0.1 ml of standard protein solution was placed in test tube followed by 4.9 ml saline and 5 ml of working biuret.

The content of each tube was mixed well and placed in water-bath (37°C) for 10 minutes, after cooling, the developing violet color was measured colorimetrically at 530 nm.

Calculation:

Total protein in g/dl =

[(Reading of test)/(Reading of Standard)] x 0.5

0.5 = concentration of standard.

5.   Determination of serum albumin:

Serum albumin was determined colorimetrically by dye-binding method as described by Bartholomev and Delancy (1966).

Reagents:

a.   N-sodium citrate: 234 g of sodium citrate were dissolved in distilled water and made up to 1 liter.

b.   M-citric acid: 210 gm of citric acid were dissolved in distilled water and made up to 1 liter.

c.   0.01 M-bromocresol green: 0.0698 g of bromocresol green was dissolved in 9.8 ml of 0.1 N NaOH and made up to 100 ml.

d.   Buffer indicator reagent: To about 800 ml of water, 17.3 ml of N sodium citrate, 32.7 ml of M. citric acid and 6 ml of 0.07 M. bromocresol green were added, mixed well and diluted to 1 liter.

-   The pH was adjusted to 3.8, if necessary with a drop of citrate or acid.
-   The reagent was stored at 4°C.

e.   Standard: Bovine albumin was used as standard in the following concentrations (6.4, 3.2 and 1 gm/dl).

Procedure:

4 ml of buffer indicator and 0.02 ml of serum were mixed and measured at 637 nm against blank of buffer indicator.

Calculation:

$$\frac{Reading\ of\ standard}{Reading\ of\ unknown} = \frac{Concentration\ of\ standard}{Concentration\ of\ unknown}$$

6. Determination of serum globulins:

Globulins value was determined by subtracting the albumin value from the total proteins in the same sample according to Coles (1974).

7. Determination of A/g ratio:

A/g ratio was calculated using serum albumin and globulin values for each individual sample.

8. Determination of serum urea:

Serum urea was determined colorimetrically by Nessler method cited in Varley (1976).

Principle:

The serum was incubated with urease, which converted urea into ammonia.

After protein had been precipitated, the color was produced when the ammonia treated with Nessler's reagent. The obtained colour was matched with that produced under the same condition using standard urea solution.

Reagents:

1.   **Isotonic sodium sulphate:**

30 gm of sodium sulphate ($Na_2SO_4.10H_2O$) or 13.2 gm anhydrous sodium sulphate (Na_2SO_4) per liter in distilled water.

2.   **Urease solution:**

5 gm of jack been meal were shaken with 100 ml of 70% glycerol and kept at room temperature over night and then centrifuged and kept in the refrigerator. The solution will keep its activity for 2-3 months.

3.   **Standard urea solution:**

100 mg of vacuum dried urea were dissolved in 100 ml of distilled water. The solution was preserved with a drop of chloroform and kept in the cold until used.

4.   **Zinc sulphate solution:**

10 gm of crystalline zinc sulphate ($ZnSO_4.7H_2O$) were dissolved in 100 ml distilled water.

5.   **Sodium hydroxide 0.5 N:**

This solution must be accurately prepared and checked against the zinc sulphate. 10 ml of zinc sulphate were taken and diluted to about 50 ml with distilled water and a few drops of phenolphthalein indicator were added and run in sodium

hydroxide from a burette, 10.8-11.2 ml which should be required to produce a permanent pink color.

6. **Iodide solution:**

2 g of iodine crystals were dissolved in a solution containing 3 g potassium iodide in 15 ml distilled water. The volume was completed to 100 ml.

7. **Nessler reagent:**

11.3 g of iodine crystals were weighed on a rough balance and added to a solution containing 15 g of potassium iodide in 10 ml of distilled water.

Most of this solution was added to 15 g of mercury in a glass stoppered reagent bottle. The mixture was cooled under tape water and shacked until the supernatant liquid had lost nearly all its color, then filtered into a-100 ml volumetric flask and tested by a drop of 1% starch. If no color obtained, more of the iodine solution was added till a faint reaction obtained with starch. The total solution was diluted to 100 ml and poured into 485 ml of 10% sodium hydroxide, if the solution was turbid it should be filtered or allowed to settle before used and should be kept in a bottle with rubber stopper. The solution gives best results when aged.

Method:

1.   Test:

Into a centrifuge tube, 4.4 ml of isotonic sodium sulphate, 0.1 ml of serum and 0.1 ml of urease solution were mixed.

2.   Standard:

Into a centrifuge tube, 4.4 ml of isotonic sodium sulphate, 0.1 ml of standard urea solution (100 mg per 100 ml) and 0.1 ml of urease solution were mixed.

Into a centrifuge tube, 4.5 ml of isotonic sodium sulphate and 0.1 ml of urease solution were mixed.

The tubes were stoppered with rubber plugs, and the contents were mixed and incubated at 37°C for 20 minutes.

After incubation 0.2 ml of zinc sulphate and 0.2 ml of 0.5 N-sodium hydroxide were added, mixed and very through mixed after each addition. The tubes were then centrifuged.

3 ml of each supernatant were drawn into a test tube followed by 2 ml of distilled water and one drop of iodine solution (to prevent clouding).

Just before reading the optical densities, one ml of Nessler's reagent was added and mixed. The optical density was read off at once before turbidity developed. The colors were compared at 489 nm.

$$Serum\ urea\ (mg/dl)\ =\ \frac{T\ -\ B}{S\ -\ B}\ x\ 100$$

Where:

T = Test

S = Standard

B = Blank

9. Determination of serum creatinine:

Serum creatinine was estimated colorimetrically using sclavo following the method of Jaffe (1886) and cited in Henry (1969).

Principle:

The rate at which creatinine forms a color complex with alkaline picrate is measured.

This method reduces the effect of interfering substances. Some of the interfering substances react with the picrate at slower rates than the creatinine.

Reagents:

Picric acid . . . . 20.5 mM

Sodium hydroxide 5% . . . . 1.25 M

Standard:

Creatinine in stabilizing solution . . . . 2 gm/dl

Preparation of alkaline picrate solution:

9 volumes of picric acid were mixed with 1 volume of sodium hydroxide 5%.

Procedure:

0.2 ml of serum or standard was pipetted into a test tube, 2.0 ml of alkaline picrate solution were added, then the two test tubes (sample and standard) were separately mixed and the first reading (A_1) was taken 10 seconds after addition of sample or standard.

Exactly 1 minute after the first reading, the second reading (A_2) was read at 510 nm.

Calculation:

$$\frac{\Delta A \ Unknown}{\Delta A \ Standard} \ x \ 2 \ = \ Creatinine \ (mg/dl \ of serum)$$

$$\Delta A \; Unknwon \; = \; A_2 \; unknown \; - \; A_1 \; unknown$$

$$\Delta A \; Standard \; = \; A_2 \; standard \; - \; A_1 \; standard$$

2 = concentration of standard (2 mg/dl).

Determination of serum uric acid:

Serum uric acid was determined colorimetrically following (Caraway, 1955).

Principle:

The serum uric acid was determined colorimetrically after deproteinization of serum proteins using phosphotungstic acid in an alkaline medium (sodium carbonate).

Reagents:

1 Reagent 1 (Deproteinization reagent)	Tetrasodium pyrophosphate	40	g/L
	Metaphosphoric acid	0.28	g/L
	Sodium tungstate	111	g/L
2 Reagent 2 (Standard)	Uric acid	1	g/L
	Lithium carbonate	0.6	g/L
3 Reagent 3 (Alkaline reagent)	Sodium carbonate	140	g/L

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4   Reagent 4 (Color reagent)   Sodium tungstate   40   g/L

                                      Phosphoric acid   27.2   G/L

Procedure:

1.   Preparation of the working standard solution:

The working standard solution was prepared by adding 99 volumes of distilled water to 1 volume of reagent 2.

2.   8 ml of distilled water and 1 ml of reagent 1 were added to 1 ml of sample in a centrifuge tube, mixed and centrifuged for 15 minutes at 3000 rpm.

3.   Three test tubes were prepared as follows

	Reagent blank	Standard	Sample
Supernatant of the centrifuge tube	-	-	5 ml
Distilled water	5 ml	-	-
Working standard solution	-	5 ml	-
Reagent 3	1 ml	1 ml	1 ml

The contents of the three tubes were mixed and left for 10 minutes 1 ml of reagent 4 was added to each tube, then shacked vigorously and left for 30 minutes and then measured colorimetrically against the reagent blank.

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4.   **Calculation:**

$$Uric\ acid\ mg/L = \frac{OD\ sample}{OD\ standard}\ x\ 100$$

Statistical analysis:

In the present investigation, statistical analysis was done using the package for the social science (SPSS) for IBM personal computers.

The normal probability frequency distribution was carried out according to Turner (1970) for arithmetic mean and standard error.

a.   Measure of statistical reliability (Validity) or arithmetic sample mean.

$$S.E. = \pm \frac{\sqrt{\sum d^2}}{n(n-1)^2} \qquad \overline{X} = \frac{\sum (x)}{n}$$

Where:

Z   =   Arithmetic mean

n   =   Number of samples

Σd^2 =   sum of $(x\text{-}x)^2$

S.E.   =   Standard error

b.   **Analysis of variance (ANOVA):**

F. value was calculated according to Snedecor and Cochran (1969) as follows:

-    Obtain the correction factor (CF) from square of sum of all observations divided by the number of samples (N):

$$F = \frac{(\sum x)^2}{N}$$

-    Total sum of squares (TSS) = sum of squares of all observation - C.F.
-    Treatment between squares = [(square of Σ of each treatment)n]-C.F.
-    Error sum squares = total sum squares - treatment sum squares.

The numerical results of an analysis of variance are presented in an analysis of variance table.

F = (between mean square)(Error mean square)

The calculated F values compared with the tabulated F value at the corresponding degree of freedom of treatment and that of error, and the significance of difference among the means of treatment were evaluated as:

1.   Non significant (NS).

2.   Significant at 5% level of probability (*).

3.   Significant at 1% level of probability (**).

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Duncan test:

Duncan test is used to compare each treatment with every other treatment mean. This test is used only if the F value is significant. The steps of this test are Determination of (SX) = $\sqrt{}$(error mean square)N.

RESULTS

Biochemical results have been statistically summarized in terms of mean ± S.E., analysis of variance (ANOVA) test and finally Duncan's test to compare each treatment with every other treatment mean (Snedecor and Cochran, 1969).

A.   Liver function tests:

1.   Serum aspartate aminotransferase activity (IU):

The estimates of this parameter has been statistically summarized in Tables (1-2) and illustrated in Fig. (1).

2.   Serum alanine aminotransferase activity (IU):

This parameter has been statistically summarized in Table 3-4) and illustrated in Fig. (2).

3.   Serum total bilirubin (mg/dl):

This parameter has been statistically summarized in Tables (5-6) and illustrated in Fig. (3).

4.   Serum direct bilirubin (mg/dl):

This parameter has been statistically summarized in Table (7-8) and illustrated in Fig. (4).

5.   **Serum total proteins (gm/dl):**

This parameter has been statistically summarized in Tables (9-10) and illustrated in Fig. (5).

6.   **Serum albumin (gm/dl):**

This parameter has been statistically summarized in Tables (11-12) and illustrated in Fig. (6).

7.   **Serum globulins (gm/dl):**

This parameter has been statistically summarized in tables (13-14) and illustrated in Fig. (7).

8.   **A/g ratio:**

This parameter has been statistically summarized in Tables (15-16) and illustrated in Fig. (8).

B.   Kidney function tests:

1.   **Serum urea (mg/dl):**

This parameter has been statistically summarized in Table (17-18) and illustrated in Fig. (9).

2.   **Serum creatinine (mg/dl):**

This parameter has been statistically summarized in Tables (19-20) and illustrated in Fig. (10).

3.   **Serum uric acid (mg/dl):**

This parameter has been statistically summarized in Tables (21-22) and illustrated in Fig. (11).

Table 1: The effect of daily oral administration of different levels of saccharin on serum aspartate aminotransferase activity at the different periods in female rats (I.U.) (values are expressed as means ± S.E)

Item		No. of rats	X ± S.E
a. Effect of treatments:			
Control		20	64.90 ± 5.055a
Group A 0.125%		20	60.30 ± 5.760a
Group B 0.25%		20	66.30 ± 5.167a
b. Effect of treatments and periods			
Control	2 weeks	5	64.400 ± 6.836a
	4 weeks	5	56.400 ± 5.791a
	6 weeks	5	60.600 ± 4.429a
	8 weeks	5	58.650 ± 6.925a
Group A (0.125%)	2 weeks	5	66.200 ± 6.643a
	4 weeks	5	62.600 ± 5.334a
	6 weeks	5	57.600 ± 4.578a
	8 weeks	5	54.800 ± 2.165a
Group B (0.25%)	2 weeks	5	65.200 ± 8.644a
	4 weeks	5	70.600 ± 7.556a
	6 weeks	5	63.800 ± 6.630a
	8 weeks	5	66.200 ± 6.019a

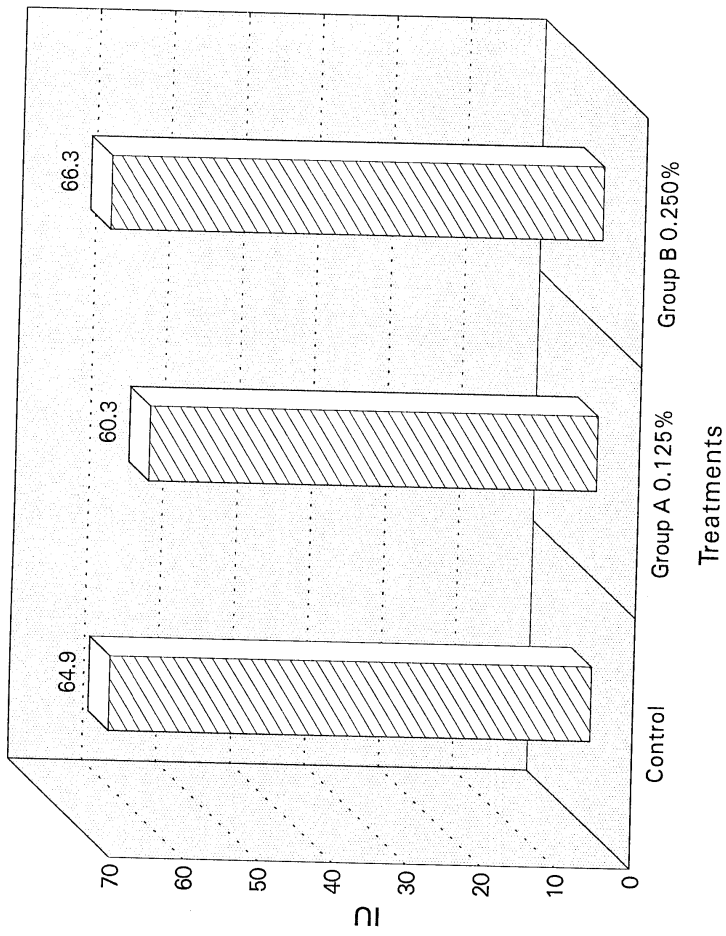Different letters mean significant while the same letters mean non significant

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Fig. 1: Effect of different levels of saccharin on serum aspartate aminotransferase activity AS-T (IU) in female rats

Table 2:   Analysis of variance of the effect of treatments and periods on the serum Aspartate aminotransferase activity (AS-T).

Source of variation	Degree of freedom	Mean square	F value	Pr > F
Treatments	2	445.007	1.42	0.2529
Treatments x periods	6	332.777	1.06	0.4005
Experiment error	48	314.458		

Table 3:   The effect of daily oral administration of different levels of saccharin
            on serum alanine aminotransferase activity at the different periods in
            female rats (I.U.) (values are expressed as means $\pm$ S.E)

Item		No. of rats	X $\pm$ S.E
a. Effect of treatments:			
Control		20	23.60 $\pm$ 2.055a
Group A 0.125%		20	26.50 $\pm$ 2.491a
Group B 0.25%		20	26.75 $\pm$ 2.658a
b. Effect of treatments and periods			
Control	2 weeks	5	24.000 $\pm$ 3.507a
	4 weeks	5	20.600 $\pm$ 3.529a
	6 weeks	5	26.400 $\pm$ 4.342a
	8 weeks	5	20.400 $\pm$ 1.363a
Group A	2 weeks	5	20.200 $\pm$ 2.009a
(0.125%)	4 weeks	5	28.600 $\pm$ 6.297a
	6 weeks	5	27.400 $\pm$ 5.801a
	8 weeks	5	23.800 $\pm$ 1.496a
Group B	2 weeks	5	22.000 $\pm$ 2.073a
(0.25%)	4 weeks	5	25.800 $\pm$ 7.895a
	6 weeks	5	28.200 $\pm$ 2.835a
	8 weeks	5	23.000 $\pm$ 1.974a

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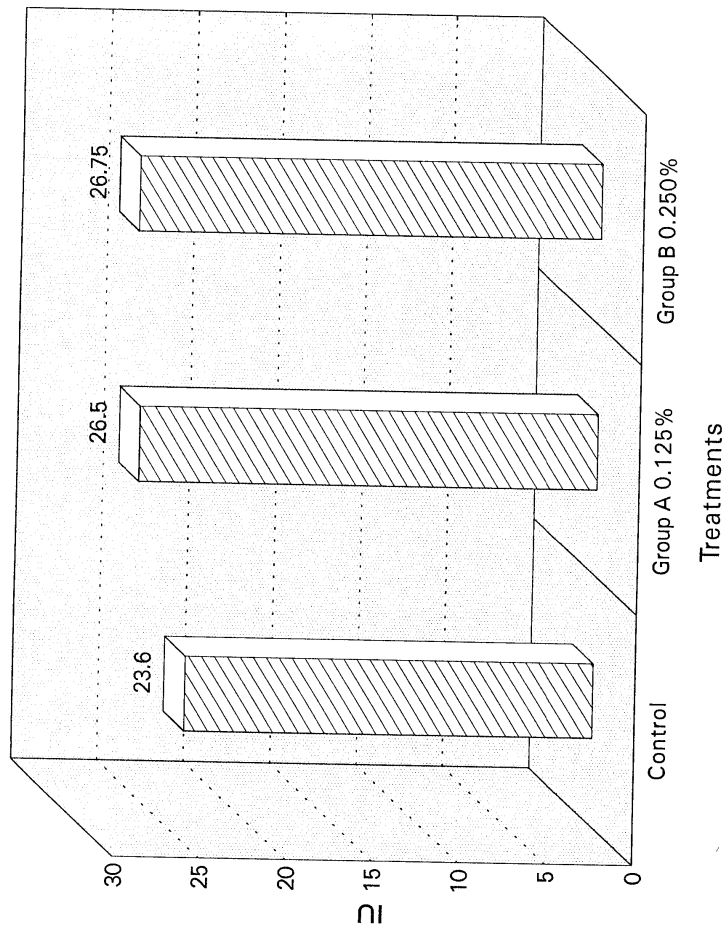
Fig. 2: Effect of different levels of saccharin on serum alanine aminotransferase activity AL-T (IU) in female rats

Table 4:   Analysis of variance of the effect of treatments and periods on the serum Alanine aminotransferase activity (AL-T).

Source of variation	Degree of freedom	Mean square	F value	Pr > F
Treatments	2	61.316	0.68	0.5128
Treatments x periods	6	62.227	0.69	0.6607
Experiment error	48	90.525		

Table 5: The effect of daily oral administration of different levels of saccharin on serum total bilirubin at the different periods in female rats (mg/dl) (values are expressed as means ± S.E)

Item		No. of rats	X ± S.E
a. Effect of treatments:			
Control		20	0.222 ± 0.0266a
Group A 0.125%		20	0.252 ± 0.0348a
Group B 0.25%		20	0.228 ± 0.0254a
b. Effect of treatments and periods			
Control	2 weeks	5	0.219 ± 0.0782a
	4 weeks	5	0.225 ± 0.0572a
	6 weeks	5	0.249 ± 0.0261a
	8 weeks	5	0.195 ± 0.0557a
Group A	2 weeks	5	0.249 ± 0.0772a
(0.125%)	4 weeks	5	0.193 ± 0.0456a
	6 weeks	5	0.256 ± 0.0737a
	8 weeks	5	0.211 ± 0.0657a
Group B	2 weeks	5	0.216 ± 0.0580a
(0.25%)	4 weeks	5	0.275 ± 0.1149a
	6 weeks	5	0.250 ± 0.0800a
	8 weeks	5	0.251 ± 0.0863a

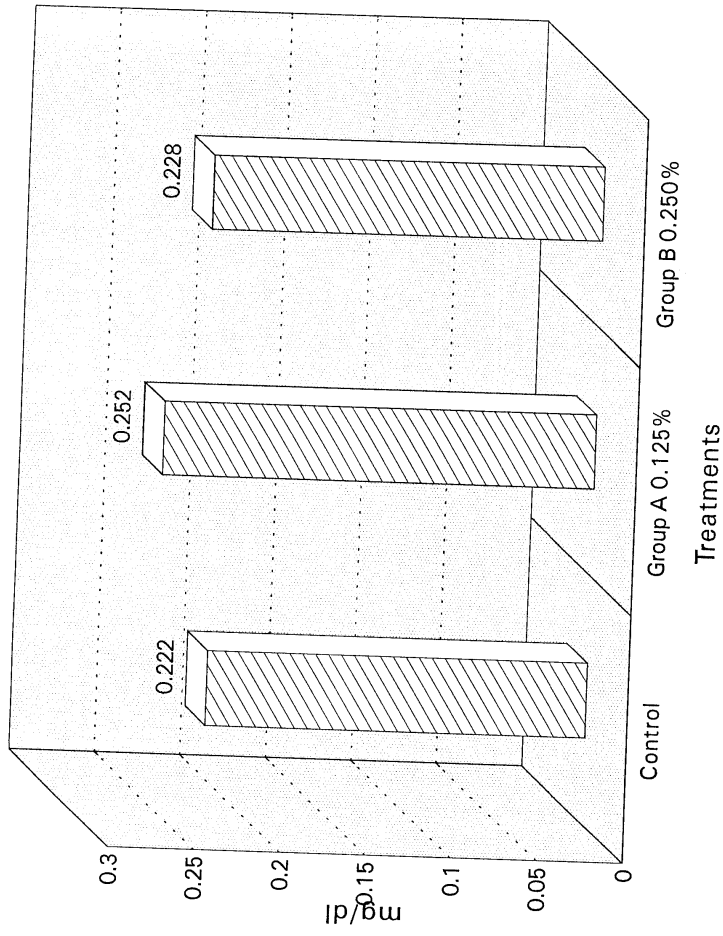Different letter mean significant while the same letters mean non significant

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Fig. 3: Effect of different levels of saccharin on serum total bilirubin (mg/dl) in female rats

Table 6: Analysis of variance of the effect of treatments and periods on the serum total bilirubin.

Source of variation	Degree of freedom	Mean square	F value	Pr > F
Treatments	2	0.00521	0.20	0.8217
Treatments x periods	6	0.0160	0.61	0.7247
Experiment error	48	0.0264		

Table 7: The effect of daily oral administration of different levels of saccharin on serum direct bilirubin at the different periods in female rats (mg/dl) (values are expressed as means $\pm$ S.E)

Item		No. of rats	X $\pm$ S.E
a. Effect of treatments:			
Control		20	0.0528 $\pm$ 0.0117a
Group A 0.125%		20	0.0651 $\pm$ 0.0211a
Group B 0.25%		20	0.0668 $\pm$ 0.0195a
b. Effect of treatments and periods			
Control	2 weeks	5	0.0424 $\pm$ 0.0178a
	4 weeks	5	0.0574 $\pm$ 0.0282a
	6 weeks	5	0.0574 $\pm$ 0.0201a
	8 weeks	5	0.0440 $\pm$ 0.0162a
Group A (0.125%)	2 weeks	5	0.0360 $\pm$ 0.0120a
	4 weeks	5	0.0304 $\pm$ 0.0174a
	6 weeks	5	0.0580 $\pm$ 0.0232a
	8 weeks	5	0.0302 $\pm$ 0.0192a
Group B (0.25%)	2 weeks	5	0.0402 $\pm$ 0.0202a
	4 weeks	5	0.0308 $\pm$ 0.0101a
	6 weeks	5	0.0452 $\pm$ 0.0089a
	8 weeks	5	0.0382 $\pm$ 0.0180a

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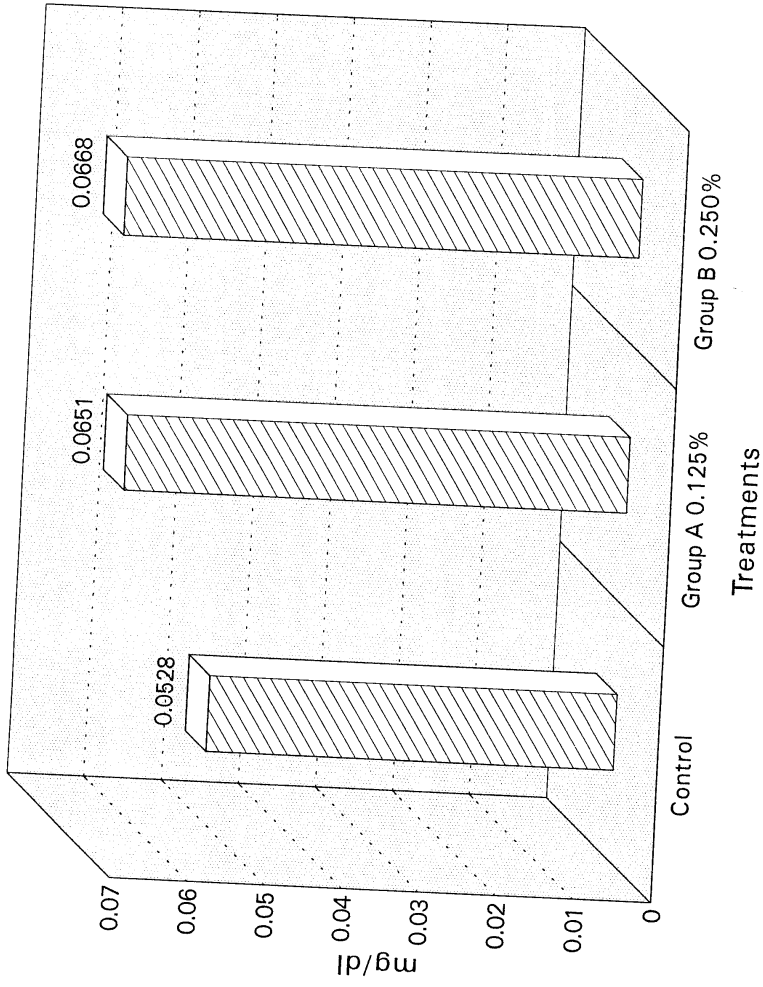
Fig. 4: Effect of different levels of saccharin on serum direct bilirubin (mg/dl) in female rats

Table 8: Analysis of variance of the effect of treatments and periods on the serum direct bilirubin.

Source of variation	Degree of freedom	Mean square	F value	Pr > F
Treatments	2	0.00358	0.59	0.5570
Treatments x periods	6	0.00692	1.15	0.3510
Experiment error	48	0.00604		

Table 9:   The effect of daily oral administration of different levels of saccharin
on serum total proteins at the different periods in female rats (gm/dl)
(values are expressed as means ± S.E)

Item		No. of rats	X ± S.E
a. Effect of treatments:			
Control		20	6.518 ± 0.0982a
Group A 0.125%		20	6.910 ± 0.0796a
Group B 0.25%		20	6.8147 ± 0.1020a
b. Effect of treatments and periods			
Control	2 weeks	5	6.354 ± 0.218a
	4 weeks	5	6.600 ± 0.244a
	6 weeks	5	6.350 ± 0.169a
	8 weeks	5	6.770 ± 0.124a
Group A (0.125%)	2 weeks	5	7.044 ± 0.129a
	4 weeks	5	6.870 ± 0.178a
	6 weeks	5	6.700 ± 0.165a
	8 weeks	5	7.028 ± 0.157a
Group B (0.25%)	2 weeks	5	6.778 ± 0.311a
	4 weeks	5	6.970 ± 0.130a
	6 weeks	5	6.740 ± 0.194a
	8 weeks	5	6.900 ± 0.251a

Different letter mean significant while the same letters mean non significant

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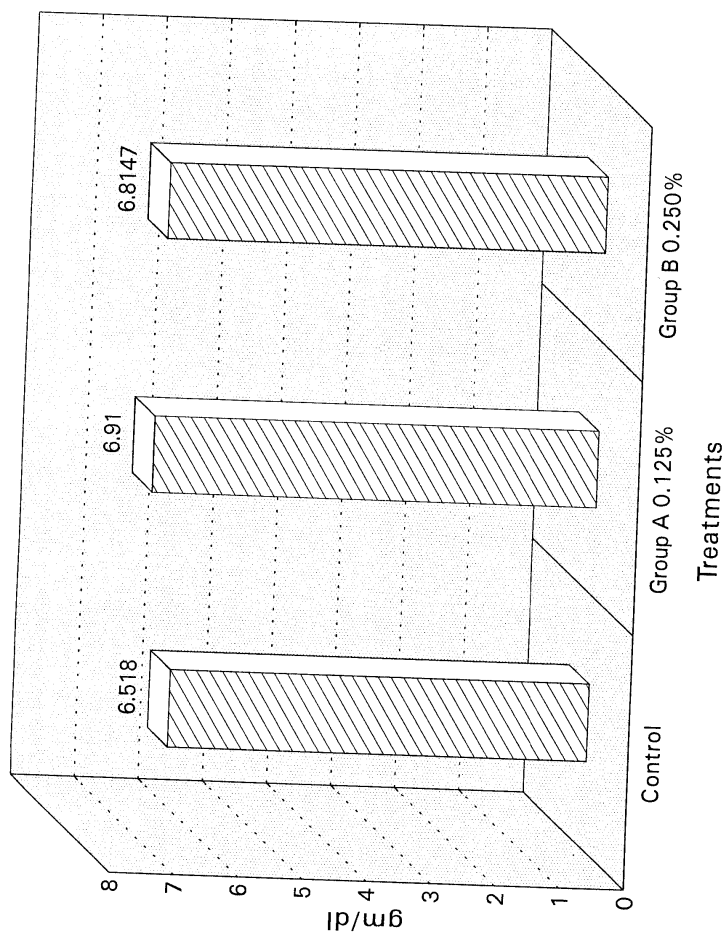
Fig. 5: Effect of different levels of saccharin on serum total proteins (gm/dl) in female rats

Table 10:   Analysis of variance of the effect of treatments and periods on the

serum total proteins.

Source of variation	Degree of freedom	Mean square	F value	Pr > F
Treatments	2	0.886	1.51	0.0156
Treatments x periods	6	0.0719	0.37	0.8952
Experiment error	48	0.195		

Table 11: The effect of daily oral administration of different levels of saccharin on serum albumin at the different periods in female rats (gm/dl) (values are expressed as means ± S.E)

Item		No. of rats	X ± S.E
a. Effect of treatments:			
Control		20	3.984 ± 0.155a
Group A 0.125%		20	3.884 ± 0.138a
Group B 0.25%		20	3.837 ± 0.196a
b. Effect of treatments and periods			
Control	2 weeks	5	3.646 ± 0.262a
	4 weeks	5	3.378 ± 0.447a
	6 weeks	5	3.512 ± 0.208a
	8 weeks	5	3.400 ± 0.169a
Group A (0.125%)	2 weeks	5	3.676 ± 0.184a
	4 weeks	5	3.328 ± 0.242a
	6 weeks	5	3.628 ± 0.166a
	8 weeks	5	2.904 ± 0.102a
Group B (0.25%)	2 weeks	5	3.675 ± 0.186a
	4 weeks	5	3.204 ± 0.155a
	6 weeks	5	3.262 ± 0.127a
	8 weeks	5	3.808 ± 0.165a

Different letter mean significant while the same letters mean non significant
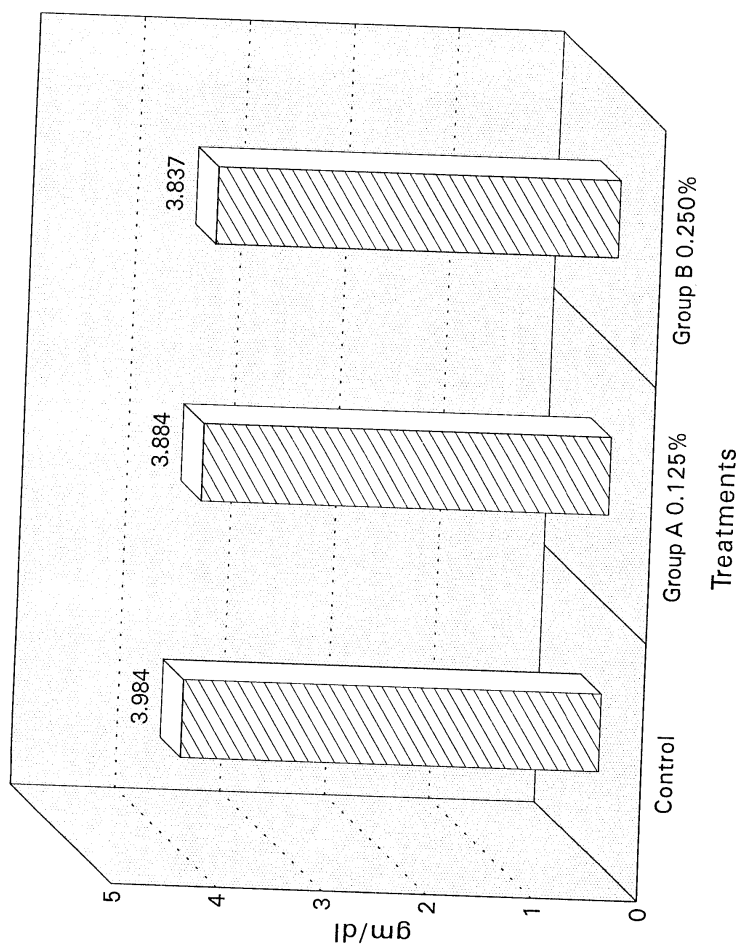
Fig. 6: Effect of different levels of saccharin on serum albumin (gm/dl) in female rats

Table 12:   Analysis of variance of the effect of treatments and periods on the

serum albumin.

Source of variation	Degree of freedom	Mean square	F value	Pr > F
Treatments	2	0.1124	0.61	0.5453
Treatments x periods	6	0.2978	1.63	0.1602
Experiment error	48			

Table 13: The effect of daily oral administration of different levels of saccharin on serum globulins at the different periods in female rats (gm/dl) (values are expressed as means ± S.E)

Item		No. of rats	X ± S.E
a. Effect of treatments:			
Control		20	2.5340 ± 0.1670a
Group A 0.125%		20	3.0058 ± 0.1348a
Group B 0.25%		20	3.0102 ± 0.2300a
b. Effect of treatments and periods			
Control	2 weeks	5	2.707 ± 0.301a
	4 weeks	5	3.222 ± 0.217a
	6 weeks	5	2.838 ± 0.253a
	8 weeks	5	2.370 ± 0.205a
Group A	2 weeks	5	2.259 ± 0.143a
(0.125%)	4 weeks	5	3.542 ± 0.126a
	6 weeks	5	3.078 ± 0.2216a
	8 weeks	5	3.144 ± 0.207a
Group B	2 weeks	5	2.803 ± 0.310a
(0.25%)	4 weeks	5	3.026 ± 0.258a
	6 weeks	5	3.478 ± 0.286a
	8 weeks	5	3.094 ± 0.320a

Different letter mean significant while the same letters mean non significant

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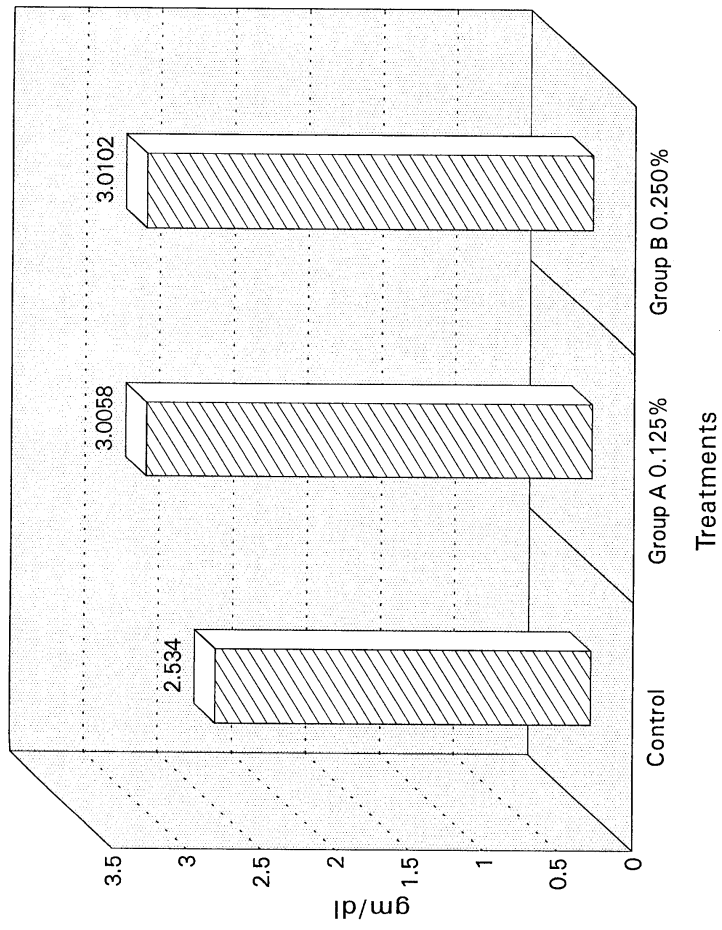
Fig. 7: Effect of different levels of saccharin on serum globulin (gm/dl) in female rats

Table 14:   Analysis of variance of the effect of treatments and periods on the serum globulins.

Source of variation	Degree of freedom	Mean square	F value	Pr > F
Treatments	2	1.495	1.96	0.0111
Treatments x periods	6	0.283	0.91	0.4760
Experiment error	48	0.3017		

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Table 15: The effect of daily oral administration of different levels of saccharin on serum A/G ration at the different periods in female rats (values are expressed as means ± S.E)

Item		No. of rats	X ± S.E
a. Effect of treatments:			
Control		20	1.895 ± 0.248a
Group A 0.125%		20	1.368 ± 0.123a
Group B 0.25%		20	1.573 ± 0.248a
b. Effect of treatments and periods			
Control	2 weeks	5	1.352 ± 1.169a
	4 weeks	5	1.070 ± 0.0925a
	6 weeks	5	1.304 ± 0.1918a
	8 weeks	5	1.864 ± 0.144a
Group A (0.125%)	2 weeks	5	1.558 ± 0.163a
	4 weeks	5	0.950 ± 0.094a
	6 weeks	5	1.144 ± 0.156a
	8 weeks	5	1.222 ± 0.1082a
Group B (0.25%)	2 weeks	5	1.128 ± 0.348a
	4 weeks	5	0.984 ± 0.1185a
	6 weeks	5	0.964 ± 0.157a
	8 weeks	5	1.296 ± 0.159a

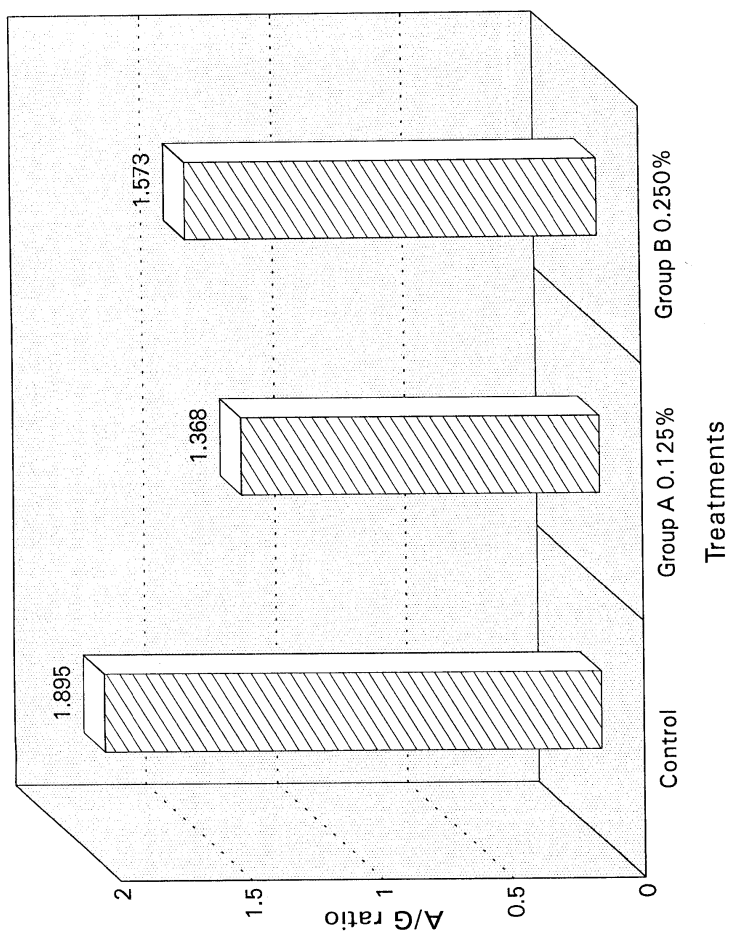Different letter mean significant while the same letters mean non significant

Fig. 8: Effect of different levels of saccharin on serum A/G ratio in female rats

Table 16: Analysis of variance of the effect of treatments and periods of the A/G ratio.

Source of variation	Degree of freedom	Mean square	F value	Pr > F
Treatments	2	1.409	5.21	0.0090
Treatments x periods	6	0.4569	1.69	0.1441
Experiment error	48	0.2705		

Table 17: The effect of daily oral administration of different levels of saccharin on serum urea at the different periods in female rats (mg/dl) (values are expressed as means $\pm$ S.E)

Item		No. of rats	X $\pm$ S.E
a. Effect of treatments:			
Control		20	44.894 $\pm$ 2.631a
Group A 0.125%		20	47.629 $\pm$ 1.744a
Group B 0.25%		20	41.794 $\pm$ 2.802a
b. Effect of treatments and periods			
Control	2 weeks	5	46.960 $\pm$ 4.659a
	4 weeks	5	42.714 $\pm$ 3.571a
	6 weeks	5	43.108 $\pm$ 1.827a
	8 weeks	5	48.796 $\pm$ 4.729a
Group A (0.125%)	2 weeks	5	48.026 $\pm$ 2.665a
	4 weeks	5	46.474 $\pm$ 3.159a
	6 weeks	5	47.786 $\pm$ 2.528a
	8 weeks	5	49.352 $\pm$ 3.347a
Group B (0.25%)	2 weeks	5	47.362 $\pm$ 5.960a
	4 weeks	5	42.658 $\pm$ 4.661a
	6 weeks	5	44.166 $\pm$ 3.844a
	8 weeks	5	50.992 $\pm$ 2.827a

Different letter mean significant while the same letters mean non significant

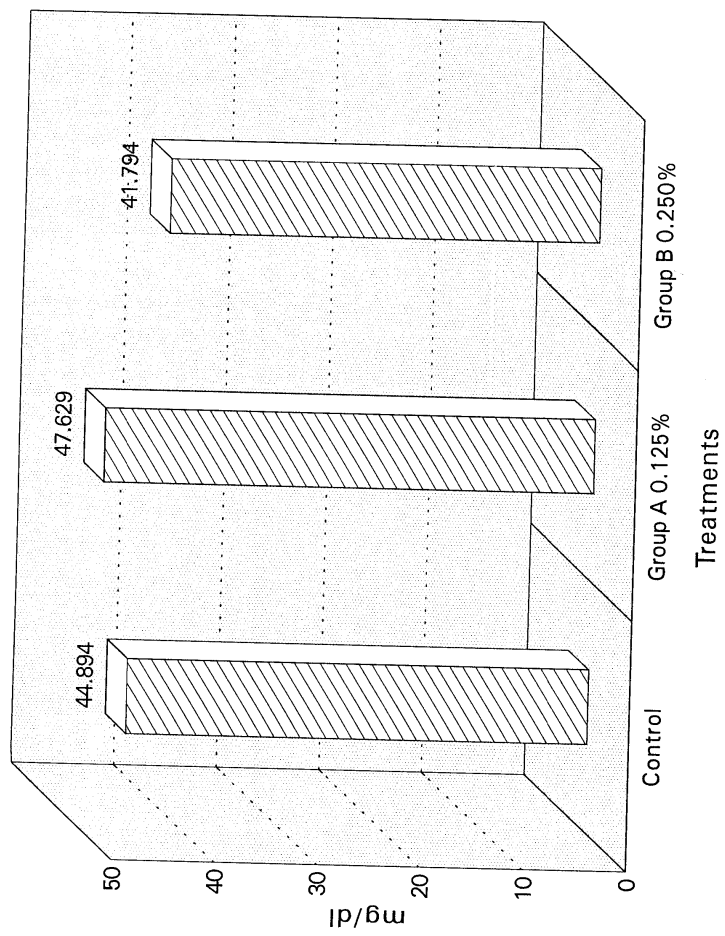Fig. 9: Effect of different levels of saccharin on serum urea (mg/dl) in female rats

Table 18:   Analysis of variance of the effect of treatments and periods on the serum urea.

Source of variation	Degree of freedom	Mean square	F value	Pr > F
Treatments	2	172.178	2.10	0.1337
Treatments x periods	6	172.836	2.11	0.0697
Experiment error	48	82.0436		

Table 19: The effect of daily oral administration of different levels of saccharin
on serum creatinine at the different periods in female rats (mg/dl)
(values are expressed as means ± S.E)

Item		No. of rats	X ± S.E
a. Effect of treatments:			
Control		20	0.734 ± 0.088 a
Group A 0.125%		20	0.636 ± 0.0753a
Group B 0.25%		20	0.546 ± 0.0665a
b. Effect of treatments and periods			
Control	2 weeks	5	1.072 ± 0.383a
	4 weeks	5	0.624 ± 0.143a
	6 weeks	5	0.640 ± 0.290a
	8 weeks	5	0.600 ± 0.169a
Group A (0.125%)	2 weeks	5	0.760 ± 0.0746a
	4 weeks	5	0.648 ± 0.190a
	6 weeks	5	0.488 ± 0.0581a
	8 weeks	5	0.650 ± 0.2316a
Group B (0.25%)	2 weeks	5	0.664 ± 0.0612a
	4 weeks	5	0.400 ± 0.1547a
	6 weeks	5	0.720 ± 0.0424a
	8 weeks	5	0.400 ± 0.0977a

Different letter mean significant while the same letters mean non significant

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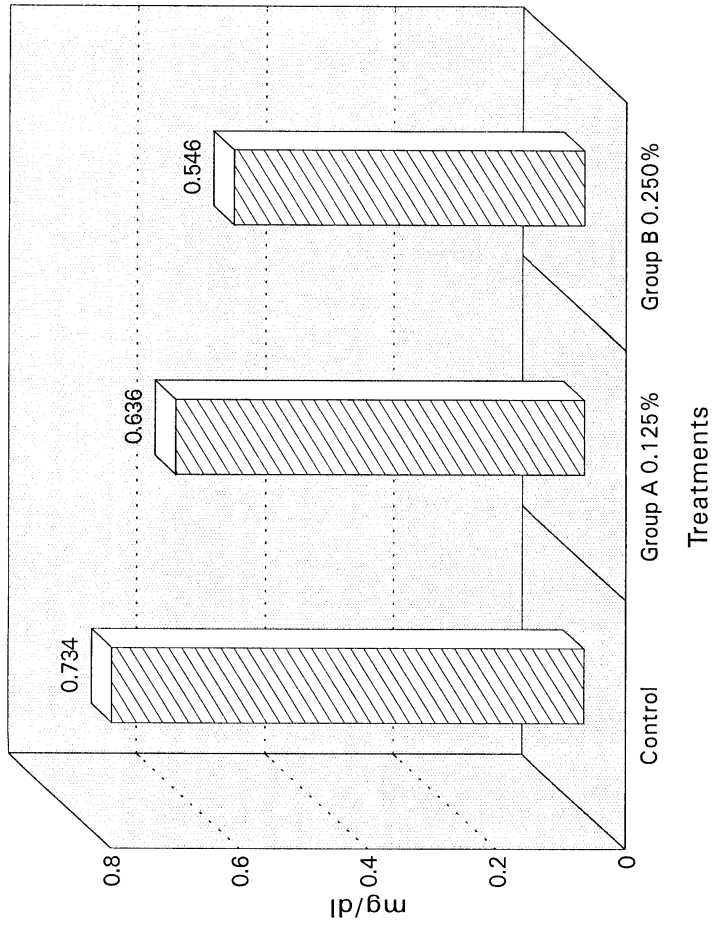
Fig. 10: Effect of different levels of saccharin on serum creatinine (mg/dl) in female rats

Table 20:   Analysis of variance of the effect of treatments and periods on the serum creatinine.

Source of variation	Degree of freedom	Mean square	F value	Pr > F
Treatments	2	0.1768	1.01	0.3710
Treatments x periods	6	0.1004	0.57	0.7483
Experiment error	48	0.1746		

Table 21:   The effect of daily oral administration of different levels of saccharin
            on serum uric acid at the different periods in female rats (mg/dl)
            (values are expressed as means ± S.E)

Item		No. of rats	X ± S.E
a. Effect of treatments:			
Control		20	2.963 ± 0.158a
Group A 0.125%		20	3.092 ± 0.268a
Group B 0.25%		20	2.606 ± 0.1389a
b. Effect of treatments and periods			
Control	2 weeks	5	2.956 ± 0.3349a
	4 weeks	5	2.940 ± 0.381a
	6 weeks	5	2.076 ± 0.426a
	8 weeks	5	2.880 ± 0.1838a
Group A (0.125%)	2 weeks	5	2.596 ± 0.250a
	4 weeks	5	3.062 ± 0.504a
	6 weeks	5	2.882 ± 0.175a
	8 weeks	5	2.804 ± 0.159a
Group B (0.25%)	2 weeks	5	2.992 ± 0.174a
	4 weeks	5	2.366 ± 0.245a
	6 weeks	5	2.562 ± 0.406a
	8 weeks	5	2.506 ± 0.210a

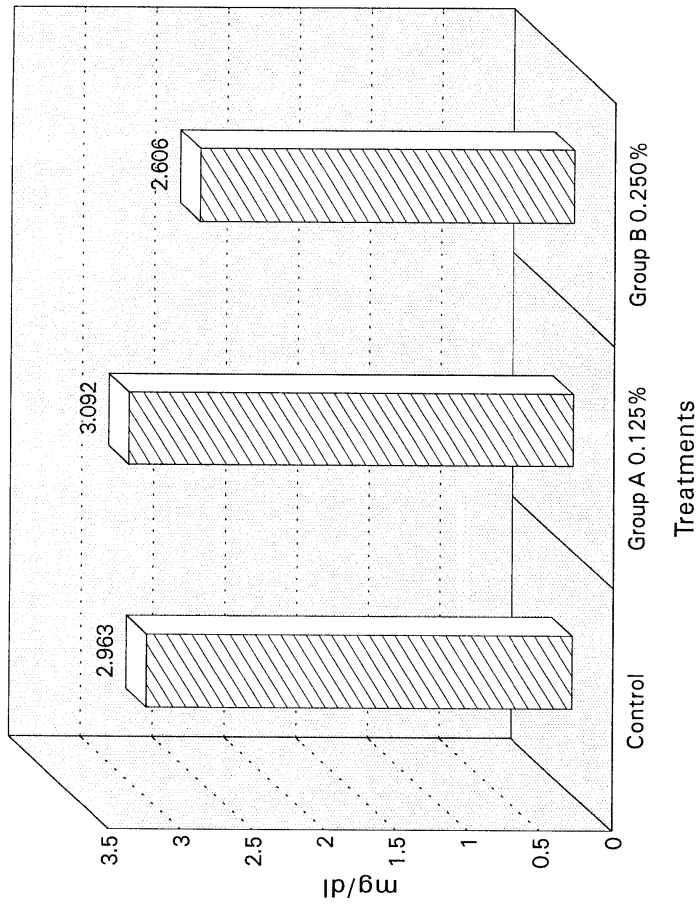Different letter mean significant while the same letters mean non significant

Fig. 11: Effect of different levels of sccharin on serum uric acid (mg/dl) in female rats

Table 22:   Analysis of variance of the effect of treatments and periods on the serum uric acid.

Source of variation	Degree of freedom	Mean square	F value	Pr > F
Treatments	2	3.0174	2.39	0.0035
Treatments x periods	6	2.473	2.24	0.003
Experiment error	48	0.4718		

DISCUSSION

A wide variety of tests are available for the investigation of the hepatobiliary system. Most of these tests measure parenchymal functions.

A.   Effect of Saccharine®on Liver Function Tests:

Serum Aminotransferases:

Serum aminotransferases have proved very useful for the diagnosis, differential diagnosis and monitoring of various hepatobiliary disorders. They are very sensitive screening tests for the presence of liver disease. Both ALT (SGPT) and AST (SGOT) are located in the cytosol of the liver cell. AST, in addition, is also found in the mitochondria.

1.   Serum aspartate aminotransferase activity:

Our results revealed that, the mean values of S.AS-T activity were 64.90 ± 5.055 I.U., 60.30 ± 5.760 I.U. and 66.30 ± 5.167 I.U. in control, group A and group B respectively (Table 1 and Fig. 1).

The present study showed that, the mean values of S.AS-T activity in the control group were 64.400 ± 6.836, 56.400 ± 5.791, 60.600 ± 4.429 and 58.650 ± 6.925 I.U. after the 2nd, 4th, 6th and 8th week from the beginning of the experiment, respectively. From the results presented here, it was found that, the mean values of S.AS-T activity in group A were 66.20 ± 6.643, 62.600 ± 5.334,

Discussion

57.600 ± 4.578 and 54.800 ±2.156 IU after the end of the same periods, respectively. Whereas, the mean values of S.AS-T activity in group B were 65.200 ± 8.644, 70.600 ± 7.556, 63.800 ± 6.630 and 66.20 ± 6.019 after the 2, 4, 6 and 8 weeks from the beginning of the experiment, respectively (Table 1).

Statistical analysis of the obtained data (ANOVA test) concerning the effect of treatments on AS-T activity, showed that, there were no significant changes between the mean values of control group, group A and group B, at the different periods of the experiment, respectively, (Table 2). Thitherto, no available literature for the effect of saccharin on the mean values of S.AS-T activity.

Surprisingly, our present data demonstrated that, no obvious effect of saccharin on S.AS-T activity in rats received saccharin.

2.   Serum alanine aminotransferase activity (S.AL-T):

Alanine aminotransferase enzyme (AL-T) is present in the cellular sap of hepatic tissue and released into plasma due to increased permeability of the cell membrane or breakdown of the liver cells following inflammation and necrosis (Cornelius and Kaneko, 1963 and Talwar *et al.*, 1989).

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The obtained results as summarized in Table (3) and illustrated in Figure (2) showed that, the mean values of S.AL-T activity were 23.60 ± 2.055, 26.50 ± 2.491 and 26.75 ± 2.658 IU in control, group A and group B, respectively.

Our results concerning the effect of treatments at the different periods showed that, the mean values of S.AL-T activity in control group were 24.00 ± 3.507, 20.600 ± 3.529, 26.400 ± 4.342 and 20.400 ± 1.363 IU after 2, 4, 6 and 8 weeks from the beginning of the experiment, respectively. Meanwhile the mean values of S.AL-T activity in rats received 0.125% saccharin solution (group A) at the same periods were 20.200 ± 2.009, 28.600 ± 6.297, 27.400 ± 5.801 and 23.800 ± 1.496 IU, respectively (Table 3).

In female rats received 0.25% saccharin solution orally (group B), the recorded mean values of S.Al-T activity were 22.000 ± 2.073, 25.800 ± 7.895, 28.200 ± 2.835 and 23.000 ± 1.974 IU after 2, 4, 6 and 8 weeks from the beginning of the experiment, respectively.

Statistical analysis of the obtained data (ANOVA test) concerning the effect of saccharin on S.AL-T activity revealed that, there were no significant changes induced by different levels of this substance compared with control group (Table 4).

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As previously mentioned in our review of literature that saccharin can stimulate cephalic phase insulin release (Berthoud *et al.,* 1981 and Woods and Bernstein, 1980).

Surprisingly, our present data demonstrated that, no obvious effect of saccharin on S.AL-T activity in rats received both concentrations of saccharin.

3.   Serum total bilirubin:

Bilirubin is formed from hemoglobin and other heme proteins. It is taken up by the liver cells, conjugated and excreted into the bile canaliculi.

The recorded mean values of serum total bilirubin in control and other two investigated groups were 0.222 ± 0.0266, 0.252 ± 0.0348 and 0.228 ± 0.0254 mg/dl, respectively (Table 5 and Fig. 3).

It is quite clear from the data presented here that, the mean values of serum total bilirubin after 2, 4, 6 and 8 weeks from the beginning of experiment were 0.219 ± 0.0782, 0.225 ± 0.0572, 0.249 ± 0.0261 and 0.195 ± 0.0558 mg/dl, respectively. (Table 5). Meanwhile, the corresponding values in female rats received 0.125% saccharin solution (group A) were 0.249 ± 0.0772, 0.193 ± 0.0456, 0.256 ± 0.0737 and 0.211 ± 0.0657 mg/dl. The recorded values in rats

received 0.25% saccharin (group B) were 0.216 ± 0.0580, 0.275 ± 0.114, 0.250 ± 0.080 and 0.251 ± 0.086 after the end of the previously mentioned periods.

Statistically, there were no significant changes in the mean values of serum total bilirubin in the treated groups compared with the control group (ANOVA test).

4. Serum direct bilirubin or conjugated bilirubin:

Conjugation of bilirubin is a process whereby water-insoluble unconjugated bilirubin is converted to a water-soluble derivative for excretion into the bile. Bilirubin is predominantly conjugated with glucuronic acid residues (Talwar *et al.*, 1989).

The recorded mean values of serum direct bilirubin in control, group A and group B were 0.05280 ± 0.0117, 0.0651 ± 0.0211 and 0.0668 ± 0.0195 mg/dl, respectively (Table 7, Fig. 4).

The mean values of serum direct bilirubin as recorded in control group after 2, 4, 6 and 8 weeks from the beginning of the experiment were 0.0424 ± 0.0178, 0.0574 ± 0.0282, 0.0574 ± 0.0201 and 0.0440 ± 0.0162 mg/dl, respectively.

The recorded mean values in group A were 0.0360 ± 0.0120, 0.0304 ± 0.0174, 0.0580 ± 0.0232 and 0.0302 ± 0.0192 mg/dl at the end of the 2nd, 4th, 6th and 8th weeks respectively. The mean values in group B were 0.0402 ± 0.0202, 0.0308 ± 0.0101, 0.0452 ± 0.0089 and 0.0382 ± 0.0180 mg/dl at the end of the previously mentioned periods.

Statistical analysis of the obtained data (ANOVA test) concerning the effect of treatments on serum direct bilirubin showed that, there were no significant changes between the treated groups and the control group (Table 8). To our best knowledge, no reports are available for the effect of saccharin on serum direct bilirubin. Our present data demonstrated that, there were no obvious effect of saccharin on serum direct bilirubin in rats received saccharin.

5.   Serum total proteins:

The hepatocytes synthesize numerous intracellular proteins and various export proteins. Among the latter are virtually all the plasma proteins (except gamma-globulins), out of which albumin is the most important.

The recorded mean values of serum total proteins in control, group A and group B were 6.518 ± 0.0982, 6.910 ± 0.0796 and 6.8147 ± 0.01020 gm/dl, respectively (Table 9 and Fig. 5).

The mean values of serum total proteins in control group were 6.354 $\pm$ 0.218, 6.600 $\pm$ 0.244, 0.350 $\pm$ 0.169 and 6.770 $\pm$ 0.124 gm/dl after 2, 4, 6 and 8 weeks from the beginning of the experiment, respectively (Table 9).

The mean values in group A after 2, 4, 6 and 8 weeks from the beginning of the experiment were 7.044 $\pm$ 0.129, 6.870 $\pm$ 0.178, 6.700 $\pm$ 0.165 and 7.028 $\pm$ 0.157 gm respectively. The mean values in group B were 6.778 $\pm$ 0.311, 6.970 $\pm$ 0.130, 6.740 $\pm$ 0.194 and 6.900 $\pm$ 0.251 gm/dl after 2, 4, 6 and 8 weeks, from the administration of saccharin, respectively.

Statistical analysis of the obtained data (ANOVA test) (Table 10) concerning the effect of treatments on serum total proteins showed that, there were no significant changes between the mean values of control, group A and group B.

Thitherto, no available literature for the effect of saccharin on the mean values of serum total proteins. Our present study showed that, no significant effect of saccharin on serum total proteins of experimental rats.

6. Serum albumin:

Albumin is a simple protein synthesized in the liver. It is concerned with the transport of many substances like some hormones, fatty acids, bilirubin, trace

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metals, tryptophan and other organic anions. In liver disease with significant damage to the hepatocytes, there is decreased synthesis of albumin causing hypoalbuminemia. A reduction of about 50% in the rate of synthesis, would result in only a 20% fall in serum albumin concentration.

The recorded mean values in control, group A and group B were 3.984 $\pm$ 0.155, 3.884 $\pm$ 0.138 and 3.837 $\pm$ 0.196 gm/dl, respectively (Table 11 and Fig. 6).

The mean values of serum albumin in control group were 3.646 $\pm$ 0.262, 3.378 $\pm$ 0.447, 3.512 $\pm$ 0.208 and 3.400 $\pm$ 0.102 gm/dl after 2, 4, 6 and 8 weeks from the beginning of the experiment respectively. The recorded mean values of serum albumin in group of rats received 0.125% saccharin solution (group A) were 3.676 $\pm$ 0.184, 3.328 $\pm$ 0.242, 3.628 $\pm$ 0.166 and 2.904 $\pm$ 0.102 gm/dl after 2, 4, 6 and 8 weeks from the beginning of the experiment, respectively.

The mean values in group of rats received 0.25% saccharin solution (group B) at the 2nd, 4th, 6th and 8th weeks were 3.675 $\pm$ 0.186, 3.204 $\pm$ 0.155, 3.262 $\pm$ 0.127 and 3.808 $\pm$ 0.165 gm/dl respectively, (Table 11). Statistical analysis of the obtained data (ANOVA test) concerning the effect of the saccharin on serum

albumin revealed that, there were no significant changes between the experimental groups (A and B) and control group throughout the experimental period (Table 12).

Thitherto, no available literature for the effect of saccharin on the mean values of serum albumin.

Surprisingly, our present data demonstrated that, no obvious effect of saccharin on serum albumin in rats received saccharin.

7.   **Serum globulins:**

Several types of alpha and beta globulins are synthesized in the hepatocytes. Chronic inflammatory diseases of the liver cause hyperglobulinemia.

The recorded mean values of serum globulins in control, (group A) and (group B) were 2.534 ± 0.1670, 3.0058 ± 0.1348 and 3.0102 ± 0.2300 gm/dl (Table 13, Fig. 7).

The mean values of serum globulins in control group were 2.707 ± 0.301, 3.222 ± 0.217, 2.838 ± 0.253 and 2.370 ± 0.205 gm/dl at the 2nd, 4th, 6th and 8th weeks from the beginning of the experiment, respectively (Table 13). Meanwhile, the mean values of serum globulins in group A at the 2nd, 4th, 6th and

8th week were 2.259 ± 0.143, 3.542 ± 0.126, 3.078 ± 0.221 and 3.144 ± 0.207 gm/dl, respectively. The recorded mean values in group B were 2.803 ± 0.310, 3.026 ± 0.258, 3.478 ± 0.286 and 3.094 gm/dl after the end of the previously mentioned periods, respectively.

Statistical analysis for the obtained data (ANOVA test) about the effect of treatments on serum globulins revealed that there were no significant changes between experimental groups (A and B) compared to control group (Table 14).

8. **A/g ratio:**

The recorded data of A/g ratio as demonstrated in Table (15) and illustrated in Fig. (8) showed that the mean values were 1.895 ± 0.248, 1.368 ± 0.123 and 1.573 ± 0.248 in control, group A and group B, respectively.

The mean values of A/g ratio in control group were 1.352 ± 1.169, 1.070 ± 0.0925, 1.304 ± 0.1918 and 1.864 ± 0.144 at the 2nd, 4th, 6th and 8th week from the beginning of the experiment respectively, (Table 15). While the mean values of A/g ratio in group (A) at 2, 4, 6 and 8 weeks were 1.558 ± 0.163, 0.950 ± 0.094, 1.144 ± 0.156 and 1.222 ± 0.1082, respectively.

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The recorded mean values in group (B) were 2.128 ± 0.348, 0.984 ± 0.157 and 1.296 ± 0.159 after the end of the same mentioned periods, respectively.

Statistical analysis of the obtained data (ANOVA test) about the effect of treatments on A/g ratio revealed that there were no significant changes in experimental groups compared to control group (Table 16).

B.   Effect of Saccharin® on Kidney Function Tests

1.   Serum urea:

The obtained mean values of serum urea in control group, group (A) and group (B) were 44.894 ± 2.631, 47.629 ± 1.744 and 41.794 ± 2.802 mg/dl, respectively (Table 17 and Fig. 9).

The recorded mean values in control group were 46.960 241 4.659, 42.714 ± 3.571, 43.108 ± 1.827 and 48.796 at the 2nd, 4th, 6th and 8th week from the beginning of the experiment, respectively.

The recorded mean values at the 2nd, 4th, 6th and 8th week in the group (A) were 48.026 ± 2.665, 46.474 ± 3.159, 47.786 ± 2.528 and 49.352 ± 3.347, whereas in group (B) were 47.362 ± 5.960, 42.658 ± 4.661, 44.166 ± 3.844 and 50.992 ± 2.827, respectively.

Statistical analysis of the obtained data (ANOVA test) showed that both treatment levels resulted in non significant changes in serum urea (Table 18).

The literature was poor in the effect of saccharin on serum urea.

2.   Serum creatinine:

The data presented in Table (19) and illustrated in Fig. (10) revealed that the mean values of serum creatinine in control, group (A) and group (B) were 0.734 ± 0.088, 0.636 ± 0.0753 and 0.546 ± 0.0665 mg/dl respectively.

At the 2nd, 4th, 6th and 8th week post treatment, the mean values of serum creatinine were:

a.   Control group: 1.072 ± 0.383, 0.624 ± 0.143, 0.640 ± 0.290 and 0.600 ± 0.169 mg/dl.

b.   Group (A) 0.125% saccharin solution: 0.760 ± 0.074, 0.648 ± 0.190, 0.488 ± 0.0581 and 0.650 ± 0.2316 mg/dl.

c.   Group (B) 0.25% saccharin solution: 0.664 ± 0.0612, 0.400 ± 0.1547, 0.720 ± 0.0424 and 0.400 ± 0.0977 mg/dl.

Statistical analysis of the obtained data (ANOVA test) showed that oral administration of saccharin resulted in non significant changes in serum creatinine (Table 20).

No reports are available in the literature for the effect of saccharin on serum creatinine.

3.   **Serum uric acid:**

The obtained results revealed that, the serum uric acid in control group, group (A) and group (B) were 2.963 ± 0.158, 3.092 ± 0.268 and 2.606 ± 0.138 mg/dl, respectively (Table 21 and Fig. 11).

The present study showed that, the mean values of serum uric acid (mg/dl) in control group were 2.956 ± 0.334, 2.94 ± 0.0381, 2.076 ± 0.426 and 2.880 ± 0.1838. In group (A) were 2.596 ± 0.250, 3.062 ± 0.504, 2.882 ± 0.175 and 2.804 ± 0.159 while in group (B) serum levels of uric acid were 2.992 ± 0.174, 2.366 ± 0.245, 2.562 ± 0.406 and 2.506 ± 0.210 mg/dl after the 2nd, 4th, 6th and 8th week post treatment (Table 21).

Statistical analysis of the obtained data (ANOVA test) showed that both treatment levels resulted in non significant change in serum uric acid (Table 22).

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Thitherto, no available literature for the effect of saccharin on serum uric acid.

Our present data demonstrated that, no obvious effect of saccharin on serum uric acid in rats received saccharin solution.

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SUMMARY AND CONCLUSION

This work was carried out to study the effect of prolonged daily oral administration of saccharin for 8 weeks on some liver and kidney functions in female albino rats.

For performing the present study 60 mature female healthy albino rats were used. Rats were divided into 3 equal groups each of 20. The first group received basal diet formulated according to NRC recommendation and was considered as a control group. The second group received basal diet and 0.125% saccharin in drinking water (group A). The third group received basal diet and 0.25% saccharin in drinking water. Random samples of 5 rats each were chosen from control and treated groups for collection of blood samples after 2, 4, 6 and 8 weeks from the beginning of the experiment. After overnight fasting, the rats were anaesthetized by inhalation anaesthesia using diethyle ether (Stone, 1954). Blood was drawn from the medial canthus of the eye by using heparinized hematocrit tube into a clean and dried centrifuge tube and left to clot at room temperature for one hour, then centrifuged at 3000 r.p.m. for 15 minutes to separte out serum samples. After collection of blood samples, these rats were excluded from the experiment. The clear non hemolyzed serum samples were separated and kept frozen at -20°C until analyzed. The collected sera were subjected for determination of serum transaminases, total bilirubins, direct bilirubin, total serum proteins, albumin, globulins, A/g ratio, urea, creatinine and uric acid.

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The obtained results showed that all the estimated parameters in serum of female rats treated with different levels of saccharin orally were within the normal ranges throughout the experimental periods in comparison with the control values. It could be concluded that prolonged oral administration of saccharin at a level of 0.125% and 0.25% for 8 weeks induced insignificant changes in serum levels of serum transaminases, total bilirubins, direct bilirubin, total serum proteins, albumin, globulins, A/g ratio, urea, creatinine and uric acid.

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دراسة بيوكيميائية عن بديل السكر (السكارين) فى إناث الفئران

إضطرابات الأيض الغذائى عديدة منها مرض البول السكرى الذى يصيب قطـاع كبير من الناس ذكور وإناث وأيضا يصيب الأطفال ونظرا لطبيعة هذا المرض الـذى يتمـيز بإرتفـاع نسبة سكر الدم وذلك لنقص معدل إفراز هرمون الأنسولين من غدة البنكرياس كان لزاما علي المرضي أن يقللوا من تناول كميات كبيرة من المواد السكرية رغم إحتياجهم لها. لذلك أتجهت أنظار العلماء فى العالم إلى البحث عن بدائل للمواد السكرية تستعمل كبديـل للسـكر فـى الحياة اليومية لهؤلاء المرضى ومن هذه البدائل السكارين ومواد أخرى منتشرة فى الأسواق.

وقد إستهدف هذا البحث دراسـة تـأثير السكارين كبديـل للسـكر علـى بعض القياسـات الخاصة بوظائف الكبد والكلى فى مصل إناث الفئران.

وقد تم إجراء هذا البحث على ٦٠ ستون فأرة إنثى بالغة من نوع الألبينو وذلك لثبـوت تناول الإناث السكارين بصورة أكبر من الذكور. تم شراء هذه الفئران من كلية الطب البيطـرى بالزقازيق. تراوحت أوزانها من ١٣٠-١٨٠ جرام للفأرة الواحدة.

وقد قسمت الفئران إلى ثلاث مجاميع عشرون لكل مجموعة كما يلى:

١-   مجموعة ضابطة (كنترول) قدم لها عليقة عادية وماء طبيعى.
٢-   مجموعة (أ) قدم لها محلول ٠,١٢٥٪ سكارين مع العليقة العادية لمدة ٨ أسابيع.
٣-   مجموعة (ب) قدم لها محلول ٠,٢٥٪ سكارين مع العليقة العادية لمدة ٨ أسابيع.

جمع العينات:

وقد أخذت عينة عشوائية مكونة من ٥ فئران من كل مجموعة من المجموعات الثلاثـة وذلك للحصول على عينات دم منها بعد تخدير الفئران بالإثير وذلك بعد نهايـة الأسبـوع الثـانى من إعطاء السكارين وتكرر ذلك بعد نهاية الأسبوع الرابع والسادس والثامن مـن بداية إعطـاء السكارين وفى كل مرة تستبعد فئران العينات العشوائية من التجربة بعد الحصول علـى عينـات الدم وترقيمها. وقد تم فصل المصل من عينات الدم وحفظه فى الثلاجة عنـد-٢٠°م وذلك بعد ترقيمة.

وقد تم دراسة تأثير أعطاء السكارين يوميا لمدة ٨ أسابيع على بعض القياسات الخاصة بوظائف الكبد والكلى فى مصل إناث الفئران كما يلى:

أ ـ   **وظائف الكبد:**

١ –   تقدير نشاط إنزيم الأسبارتات أمينوترانسفيريز فى المصل.

٢ –   تقدير نشاط إنزيم الألانين أمينوترانسفيريز فى المصل.

٣ –   تقدير البيلوربين الكلى فى المصل.

٤ –   تقدير البيلوربين المرتبط فى المصل.

٥ –   تقدير البروتينات الكلية فى المصل.

٦ –   تقدير الألبيومين فى المصل.

٧ –   تقدير الجلوبيولين فى المصل.

٨ –   تقدير نسبة الألبيومين: الجلوبيولين.

ب ـ   **وظائف الكلى:**

١ –   تقدير البولينا فى المصل.

٢ –   تقدير الكرياتينين.

٣ –   تقدير حمض البوليك.

أسفرت نتائج الدراسة على ما يلى:

وقد تبين من هذه الدراسة أن إعطاء السكارين يوميا بالفم لمدة ٨ أسابيع بمعدل ٠,١٢٥٪ و ٠,٢٥٪ فى مياه الشرب لإناث الفئران لا يسبب تغيرات معنوية فى معدلات الترانس أمينياز والبيلوربين الكلى والبيلوربين المرتبط والبروتينات الكلية والألبيومين والجلوبيولين ونسبة الألبيومين: الجلوبيولين والبولينا والكرياتينين وحمض البوليك فى المصل.

تحت إشراف

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دراسة بيوكيميائية عن بديل السكر (السكارين)
فى إناث الفئران

رسالة مقدمة من
ط.ب/محمد مصطفى يوسف الكدن
بكالوريوس الطب البيطرى جامعة الزقازيق ـ ١٩٨٦

للحصول على
درجة الماجستير
فى
العلوم الطبية البيطرية
(الكيمياء الحيوية والإكلينيكية)

إلى
كلية الطب البيطري
جامعة الاسكندرية

١٩٩٥