HISTOLOGICAL AND BIOCHEMICAL PARAMETERS EFFECT OF LIVER AFTER SUCROSE-INDUCED METABOLIC SYNDROME IN WISTAR RATS

ABSTRACT

Introduction: Metabolic syndrome (MetS) is a multiple factorial condition that draws a parallel to various risks such as dyslipidemia, hypertension, and hyperglycemia. As per the new International Diabetes Federation (IDF), for a person to be defined as having metabolic syndrome the person must have central obesity along with any two of the following four factors: Raised triglycerides (≥ 150 mg/dL), reduced HDL cholesterol (<40 mg/dL in males and<50 mg/dL in females), raised blood pressure (systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg) and raised fasting plasma glucose (FPG ≥ 100 mg/dL).

Sucrose is a common sugar, which is also known as "table sugar". It is a sweet-tasting carbohydrate that is present in almost everything we eat. It is a natural compound that gives us valuable energy as it is a quick and easy source of energy but sucrose tends to be harmful when it is over-consumed. Sucrose is consumed worldwide and it is known to have harmful effects on metabolic heath.

Aim: The aim of this study was to determine the histological and biochemical changes of liver in sucrose induced metabolic syndrome in Wistar rats.

Method: A total of 24 Wistar rats were randomly grouped into three groups A, B and C. 10g of sucrose was weighed and dissolved in 100 ml of distilled water to make the 10% sucrose solution and 20g was also weighed and dissolved in 100 ml of distilled water to make the 20% sucrose solution. Group A served as control and was administered orally with distilled water, group B was administered with the 10% sucrose solution orally and group C was administered with the 20% sucrose solution orally all for 6 weeks (42 days) The animals were anaesthetized using chloroform vapour in an enclosed transparent jar. Blood was collected through cardiac puncture and dispensed in to plain container for liver biochemical analysis and liver was carefully harvested and washed with normal saline as well as fixed in 10 % formol saline for histological preparation.

Results: There was increase in serum liver function parameters: total protein (TP), Albumin (ALB), Total bilirubin (TB), Aspartate transaminase (AST) and Alanine transaminase (ALT) but the increase was statistically insignificant. Malondialdehyde (MDA), Triglycerides (TG), High density lipoprotein- cholesterol (HDL-Chol) and Glucose parameters were also statistically insignificant. Histopathology examination of sections of the liver specimens revealed steatosis (deposits of fat on the liver) on the liver of both group B and C Wistar rats.

Conclusion: Histological examination of the liver specimens showed presence of fat cells deposition (steatosis) in all of the liver sections. Further studies would be needed to make a definitive conclusion as to whether the biochemical parameters can cause insignificant or significant changes.

Key Words: Include key words please

INTRODUCTION

Metabolic syndrome (MetS) is a multiple factorial condition that draws a parallel to various risks such as dyslipidemia, hypertension, and hyperglycemia. As per the new International Diabetes Federation (IDF), for a person to be defined as having metabolic syndrome the person (he or she) must have central obesity along with any two of the following four factors: Raised triglycerides (≥ 150 mg/dL), reduced high density lipoprotein (HDL) cholesterol (<40 mg/dL in males and<50 mg/dL in females), raised blood pressure (systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg) and raised fasting plasma glucose (FPG ≥ 100 mg/dL) [1]. According to previous reports by the IDF, about 20 % to 30 % of the world's population is currently suffering from metabolic syndrome. (MetS), which have been associated with an increase in age and BMI [2]. In MetS, there is a tendency to develop central obesity associated with an increase in circulatory free fatty acids [3]. This eventually leads to increase in blood pressure, insulin-resistance and hyperlipidemia [4]. Insulin resistance is often projected to be the major cause of Mets; however there are other factors like genetic variations in breaking down lipids in blood and age, which may contribute to its development [5]. Metabolic syndrome may give rise to a number of secondary complications which primarily include atherosclerosis and other cardiovascular disorders (reviewed in details by Reaven, [5]. The global prevalence of MetS differs depending upon geographic and sociodemographic factors, as well as the diagnostic criteria used. National Health and Nutrition Examination Survey data estimated that 35% of adults in the United States of America (USA), and as much as 50%of the over-60 years of age population, had a diagnosis of MetS (30.3% in men and 35.6% in women), based on the National Cholesterol Education Program Adult Treatment Panel III criteria, with recent trends suggesting a stable overall prevalence and a reduced prevalence in women [6]. In General, IDF has estimated that one-quarter of the world's adult population has metabolic syndrome.

The word sucrose was coined in 1857 by the English chemist William Miller, from the French word sucre (sugar) and the generic chemical suffix for sugarose [7]. The abbreviated term Suc is often used for sucrose in scientific literature. The name saccharose was coined in 1860 by the French chemist Marcellin Berthelot. Saccharose is an obsolete word and for sugars in general, especially sucrose [8]. Sucrose is a natural sweetener most often called table sugar; there are three main sources of sucrose in the diet [9]. It has long been noticed that high-sugar intake may have adverse health effects. It has been iterated that in rodents, consumption of a high-sucrose diet leads to the development of obesity, insulin resistance, diabetes, dyslipidemia, fatty liver, and high blood pressure [10]. More than 50 years ago, it had already been suspected that consumption of refined sugar in humans may be linked to dyslipidemia and coronary heart disease [11]. Sucrose and fructose are not essential components of Man's feeding, and their consumption has remained low throughout the prehistory and middle age, Sucrose has a molecular formular $C_{12}H_{22}O_{11}$ [12].

In nature, sucrose is present in many plants, and in particular their roots, fruits and nectars, because it serves as away to store energy, primarily from photosynthesis [13]. Many mammals, birds, insects and bacteria accumulate and feed on the sucrose in plants and for some of them, it

is their main source [13]. Seen from human perspective, honeybees are especially important because the honey does accumulate sucrose and does produce honey, which is an important food stuff all over the world (John, 2008). The carbohydrates in honey itself primarily do consist of fructose and glucose with trace of amount of sucrose only [14].

3.0 METHODOLOGY

3.1 STUDY LOCATION

The study was conducted within the Department of Histopathology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University Sokoto and Histopathology Laboratory, Usmanu Danfodiyo University Teaching Hospital.

3.2STUDY DESIGN

The total of 24 Wistar rats were randomly grouped into three groups A, B and C. 10g of sucrose was weighed and dissolved in100 ml of distilled water to make the 10% solution of sucrose and 20g was also weighed and dissolved in 100 ml of distilled water to make the 20% sucrose solution. Group A served as control and was administered orally with distilled water, group B was administered with the 10% sucrose solution orally and group C was administered with the 20% sucrose solution orally all for 6 weeks (42 days) The animals were anaesthetized using chloroform vapour in an enclosed transparent jar. Blood was collected through cardiac puncture and dispensed in to plain container for liver biochemical analysis and liver was carefully harvested and washed with normal saline as well as fixed in 10 % formol saline for histological preparation.

3.3 EXPERIMENTAL ANIMALS

Twenty four (24) adult wistar rats with an average weight of 120 kg to 150 kg were purchased from the Department of Pharmacology and Toxicology, Faculty of pharmaceutical science, Ahmadu Bello University Zaria in Nigeria and were transported to Sokoto, where they there were kept in animals house, Faculty of Pharmaceutical Science, Usmanu Danfodiyo University Sokoto. The rats were housed in a metal cage with 12 hours dark/light cycle. They were fed with standard pellets (grower mesh) and pure water with different concentration of sucrose solution (10% and 20%). The animals were allowed to acclimatize for 2 weeks s before proceeding to the study. Before the commencement of the study, physical examination of the animals were carried out and were found to be in a very good state of health and were kept before the day of sacrifice.

3.3 PROCUREMENT OF SUCROSE

The pure granulated Sucrose was purchased from Gawon Nama Market along Usmanu Danfodiyo University Teaching Hospital, Wamako Local Government Sokoto State.

3.4 PREPARATION OF SUCROSE SOLUTION

The preparation of 10% solution was carried out by measuring, 10g of pure granulated sucrose with weighing balance and diluted in 100 ml of distilled water. So also the same applied to the 20% sucrose preparation, 20g of sucrose was weighed and diluted in 100 ml of distilled water.

3.5 ANIMALS SACRIFICE AND SAMPLE COLLECTION

The animals were weighed and sacrificed following mild anesthesia with chloroform inhalation in an enclosed transparent plastic jar. The blood samples for biochemical studies were collected in plain containers via cardiac puncture; the Liver was carefully harvested and washed with normal saline then fixed in 10% formol saline.

3.6 LABORAT0RY ANALYSIS

The serum sample for liver function test, was used for the determination of serum activities of transaminases (AST and ALT) which was carried out using colorimetric method of Reitman and Frankel [15]. Total protein was determined using the Biuret method modified by Henry *et al* [16]. The concentration of albumin was determined as described by Grant and Kacchman. [17]. Albumin was determined by the method that was modified by Doumas *et al.* [18]. Cholesterol level was estimated using the method that was modified by Lopes-Virella *et al.* [19]. All measurements were done using Spectronic 21 spectrophotometer (Bausch and Lomb, NY).
The organ was brought out of fixative and examined macroscopically on cutting bench. A representative part of the kidney was cut and placed in a pre-labelled cassette. The tissues were dehydrated, cleared and impregnated using automatic tissue processor (Leica TPO1020 model), after which they were embedded using embedding center (Leica EG1160 model). Section of the embedded tissue blocks were cut at 3µm using rotary microtome (Leica RM2125RT) and then floated out on labeled glass slides. The cut sections were allowed to dry on hot plate for 15 minutes and stained in haematoxylin and eosin stains. Stained sections of the specimens were examined microscopically using x10 and x40 objectives lenses. Photomicrograph of kidney tissue sections were taken and presented alongside with the control sections.

3.9 DATA ANALYSIS

The results generated were analyzed using Graph pad in Stat Prism software. Normally testing was done and the data spread were found to be normally distributed. One-way analysis of variance was adopted as a parametric tool for mean comparison between the study groups. P value less than 0.05 was considered statistically significant.

4.0 RESULTS

The result of biochemical and histological analysis of the liver specimens in sucrose induced metabolic syndrome in Wistar rats with group A (control) administered with distilled water, group B administered with 10% sucrose distilled water (SDW) and group C administered with 20% sucrose distilled water (SDW) showed that the biochemical parameters of liver function tests were statistically insignificant while the histological sections of the liver showed presence of fats (steatosis).

The liver function parameters including: total protein (TP), Albumin (ALB), Total bilirubin (TB), Aspartate transaminase (AST) and Alanine transaminase (ALT) were statistically insignificant when compared with control. (Table 1).

Malondialdehyde (MDA), Triglycerides (TG), High density lipoprotein- cholesterol (HDL-Chol) and Glucose parameters were also statistically insignificant when compared with the control group. (Table 2).

Liver tissue sections in group A-Control, administered with distilled water and group B administered with 10% Sucrose distilled water SDW) showed normal histology with normal central vein in both group A and group B while in group B there was presence of fat deposits or fat cells deposition inclusions known as steatosis (Plate 1).

Liver tissue sections in group A- Control, administered with distilled water and group C administered with 20% Sucrose distilled water SDW showed normal histology with normal

central vein in both group A and group C while in group C there was presence of abundant fat cells deposits or lipid inclusions known as steatosis (Plate 2).

Table 1: Selected liver function test biochemical parameters of the Wistar rats.

Parameter	**20% SDW**	**10% SDW**	**Control**	**F-value**	**p-value**
TP (g/dL)	6.49± 0.73	7.00± 0.99	6.88± 0.89	0.739	0.489
ALB (g/dL)	3.26± 0.29	3.68± 0.69	3.60± 0.60	1.249	0307
TB (Mg/dL)	0.44± 0.05	0.44±0.05	0.44±0.05	0.038	0.963
AST (U/L)	155.50± 56.38	211.50±49.03	188.5± 53.32	2.200	0.136
ALT (U/L)	71.25±26.31	65.38± 22.99	64.50± 16.74	0.215	0.808

Values are expressed as mean ± SD. P >0.05 was considered statistically insignificant.

Legend: SDW= Sucrose Distilled Water. ALB= Albumin. TB= Total Bilirubin. AST= Aspartate Transaminase. ALT= Alanine transaminase. g/dl= gram per deciliter. Mg/dl= milligram per deciliter. U/L= unit per liter.

Table 2: Malondialdehyde MDA, Triglycerides TG, High Density Lipoprotein-Cholesterol HDL-Chol and Glucose Biochemical Parameters of the Wistar rats.

Parameter	**20% SDW**	**10% SDW**	**Control**	**F-value**	**p-value**
MDA (mmol/ml)	136.32± 49.03	125.23± 44.18	97.15±42.60	1.585	0.229
TG (mmol/L)	1.77± 0.82	1.41± 0.52	1.20± 0.14	2.118	0.145
HDL-Chol (mmol/L)	1.49± 0.32	1.46±0.35	1.74±0.61	0.928	0.411
Glucose (mmol/L)	4.13± 0.72	4.09±0.76	4.06±1.05	0.11	0.989

Values are expressed as mean ± SD. P >0.05 was considered statistically insignificant.

Legend: SDW= Sucrose Distilled Water. MDA= Malondialdehyde. TG =Triglycerides. HDL-Chol= High density lipoprotein-cholesterol. Mmol/ml= millimoles per milliliter. Mmol/L= millimoles per liter.

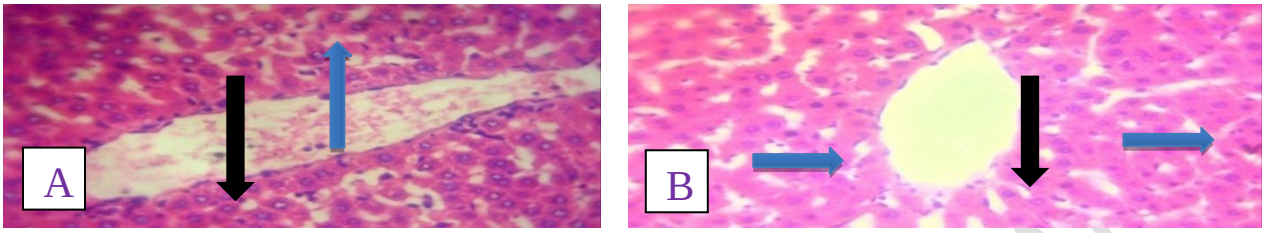

Plate 1: Photomicrograph of Liver tissue sectionsgroup A (control) and group B (10% SDW). H and E (x400).

Legend: Normal central (Black arrow), showing vein in both group A and group B Green arrow= showing fat deposits (Steatosis).

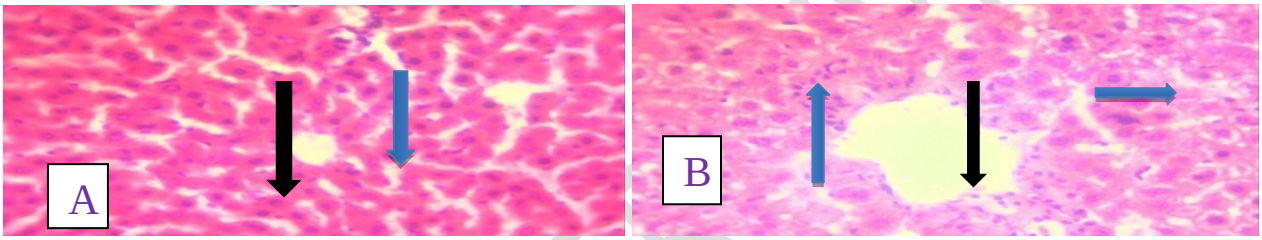

Plate 2: Photomicrograph of Liver tissue sections stained with Haematoxylin and Eosin (x400).

Legend: Black arrow= showing normal central vein in both group A and group C. Green arrow= showing fat deposits (Steatosis).

5.0 DISCUSSION

The present study evaluated the biochemical and histological changes of liver in sucrose induced metabolic syndrome in Wistar rats using 10% and 20% of sucrose which indicated some changes when compared with control.

The result obtained from some liver function parameters which included total protein (TP), Albumin (ALB), Total bilirubin (TB), Aspartate transaminase (AST) and Alanine transaminase (ALT) all were statistically insignificant when compared with the control group (Table 1). These findings were in contrast with the work reported by Aguilera *et al*, [20]. The reason could be due the differences in the diet used for induction of metabolic syndrome

Malondialdehyde (MDA), Triglycerides (TG), High density lipoprotein- cholesterol (HDL-Chol) and Glucose parameters were also statistically insignificant when compared with the control group with a P value > 0.05 (Table 2). These findings were not in agreement with the research of Ghezzi *et al*. [21].

The histological findings obtained from the liver section (plate 1), comparing group A which was the control group that was administered with distilled water and group B with 10% sucrose distilled water (SDW) and group C with 20% sucrose distilled water (SDW), the liver section

appeared normal with normal histology in group A the control group section, while in group B and group C, there was presence of fats (steatosis). These findings were in agreement with research report of Julie *et al*. [22] and Ana *et al* [23].

5.1 CONCLUSION

The liver histological finding showed presence of fat cells deposition (steatosis) in all liver sections. Further studies would be needed to make a definitive conclusion as to whether insignificant changes or significant changes in liver function biochemical parameters could be caused by sucrose induced metabolic syndrome..

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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