Table sugar effect(s) on sperm parameters and antioxidant status in male Wistar rats

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Abstract
Sugar, available as sucrose, is a natural ingredient that has always been part of the human diet, providing cellular energy source. Its consumption has been linked to several metabolic diseases. This study is therefore designed to assess the effect of oral table or white sugar on sperm parameters and antioxidant status of adult male Wistar rats. Twenty adult male Wistar rats (100-180g) were randomized into 4 groups of 5 rats. Group 1 (Control) received distil water. Groups 2, 3 and 4 rats received 20mg, 30mg and 60mg of table sugar per Kg bw respectively. After 30days oral treatment, a testes (with its epididymis) was removed for sperm function test and blood was withdrawn from each rat through cardiac puncture for superoxide dismutase and glutathione peroxidase antioxidant enzyme assays under chlorofoam anaesthesia. Data obtained, expressed as means ± SEM, were analyzed using ANOVA. Result showed significant reduction in body weight and sperm parameters (count, normal motility/progressivity and normal morphology) in the treated groups compared with control. Sperm abnormal motility and abnormal morphology were significantly increased in the treated group compared with control. Antioxidant assays showed no significant change in the treated groups relative to control. These findings suggest that table sugar adversely impact sperm functions well below the RDA of table sugar and may therefore, promote male fertility.

Keywords: White/Table Sugar; Sperm Parameters; Superoxide Dismutase; Glutathione peroxidase

1. Introduction
Sugar, a globally consumed product, is the generic name for carbohydrates; carbohydrates are a rich source of energy in the body. It has been a constituent of human diets predating the 11th century when the consumption of sugar became well documented in Europe; with the earliest records been among the Chinese and Indians [1]. From marinara sauce to peanut butter, added sugar can be found in even the most unexpected products. Many people rely on quick, processed foods for meals and snacks. Since these products often contain added sugar, it makes up a large proportion of their daily calorie intake. The World Health Organization (WHO) recommends a daily intake of 10% or less of one’s total energy intake [2], although this recommendation has become a subject of controversy in recent studies [3]. Sugar consumption levels varies from country to country [4]. In the US, added sugars account for up to 17% of the total calorie intake of adults and up to 14% for children [5].

Sugars could be simple type or monosaccharides like glucose, fructose and galactose or may be found as compound sugars which are made up of two or more monosaccharides (monosaccharide complex) like sucrose, lactose and maltose. The term ‘White or table sugar’ most often is used to describe refined sucrose. In the body, the compound sugars are hydrolyzed into monosaccharides, which are readily oxidized in the glycolytic pathway to provide the needed energy source (as high energy phosphate compounds like adenosine triphosphate (ATP) for cellular use. Free sugars in the diet mostly come in the form of sucrose, which is digested into glucose and fructose on a 1:1 ratio in the gastrointestinal tract for absorption [6]. However, other sugars, such as sorbitol or mannose, can also be detected.

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Glycolysable sugars have both beneficial and detrimental effects on the working of the body system and on male fertility in particular. It has been shown consistently that people who drink sugary beverages, such as soda and juice, weigh more than people who don’t drink [7], tended to have more visceral fat which is usually associated with conditions like overweight/obesity [8], diabetes mellitus (DM), heart diseases [9,10,11] and atherosclerosis [12] among others. Men with uncontrolled high blood glucose (type 1 and type 2 DM) are at increased risk for infertility issues ranging from erectile dysfunction, delayed ejaculation, reversed ejaculation, reduced sperm quality and low testosterone levels [13].

Arising from these global effects of excessive sugar intake, the American heart association (AHA), advised that all Americans 2 years and older limit added sugars in the diet to less than 10% of total calories, as well recommending a drastic cut back on added sugar to help slow the obesity and heart disease epidemics. The association further suggested a stricter added-sugar limit (recommended daily allowance (RDA)) of no more than 100 calories per day (about 6 teaspoons or 24 grams) for most adult women and no more than 150 calories per day (about 9 teaspoons or 36 grams of sugar) for most men [14], translating to 510.4mg/kg body weight (bw) of added sugar per day for a 70kg man.

The mammalian spermatozoa respond to high glucose concentration in several ways. Glucose concentrations of about 5mmol/L produce much higher penetration rates than do fructose or mannose in human spermatozoa [15]. Moreover, glucose, but not fructose, produces a high fertility rate and capacitation-like changes in the chlorotetracycline fluorescence pattern of mouse spermatozoa subjected to in vitro capacitation [16]. Glucose supplementation in drinking water improves semen qualities, plasma total protein, cholesterol, and globulin on the one hand, and decreases abnormal and dead sperm in rabbit on the other. It equally improves semen and plasma constituents in the buck rabbit [17]. On the contrary, sugar-sweetened beverage consumption has been associated with lower sperm motility in otherwise healthy young men [18,19], while in experimental rats, sperm motility, concentration and viability were significantly reduced [20].

This study therefore, was designed to investigate the effect of table (added) sugar administration on sperm parameters and antioxidant status of adult male Wistar rats.

2. Materials and Method

2.1. Experimental design

Sixteen (16) healthy male albino Wistar rats weighing 100-180g were purchased from the animal house of College of Health Sciences, Benue State University, Makurdi for the study and randomized into four experimental groups 1, 2, 3 and 4, represented by wooden cages. The animals were acclimatized for fourteen days under standard laboratory condition, under an environmental temperature of 23±2°C; humidity of 55±15% and 12 hours light/dark cycles. The rats were freely exposed to growers chow and water ad libitum. Institutional ethical approval was obtained from the Research and Ethics committee of College of Health Sciences, Benue State University, Makurdi. Care of the animals was consistent with international animal care regulations [21].

2.2. Treatment Administration

White (table) sugar was procured as fine, granulated Dangote® sugar with 99.9% purity from a reputed retail outlet in Makurdi. Given that the LD₅₀ (Rat) of table sugar is 29.7g/kg (MSDS, 2011) and the RDA is 510mg/kg bw [14], the treatment was designed to use 4 percent of the RDA as baseline treatment and thereafter the doses were increased by a factor 1.5 and 3 (or 6 and 12 percent of RDA) respectively in the subsequent two groups as shown below:

- Group I (Control) – received distil water
- Group II – treated with 20mg/kg bw of white sugar daily
- Group III – treated with 30mg/kg bw of white sugar daily
- Group IV – treated with 60mg/kg bw of white sugar daily

All rats were treated orally for 30 days. Weights of the animals were taken before and after treatment.

2.3. Sample collection and analysis

After an overnight fast, on day 31, the animals were observed for general physical activity, mobility and thereafter anesthetized with inhalational chloroform. Five milliliters of blood sample was collected through cardiac puncture into sterile plain tubes and labeled. Each blood sample collected was subjected to centrifugation at 3200g for 15 minutes and the supernatant serum was collected for antioxidants assay. A testis and corresponding epididymis were harvested by a careful scrotal incision for semen analysis.
Semen analysis was performed by harvesting semen from the caudal region of the epididymis by milking in 0.1 mL 0.95% saline solution and examining same for sperm count, motility and morphology using standardized methods in accordance with the WHO semen analysis guideline [22,23,24].

2.3.1. Biochemical assay

Ellmans procedure [25] was used to estimate glutathione peroxidase (GPx) levels in the serum. The principle of this procedure is based on the fact that GPx catalyses the following reaction:

\[
\text{Se-GPx} + 2\text{GSH} + \text{H}_2\text{O}_2 \rightarrow \text{GSSH} + 2\text{H}_2\text{O}
\]

0.4 ml of phosphate buffer, 0.2 ml of EDTA, 0.1 ml of sodium azide, 0.2 ml of reduced glutathione and 0.1ml of H₂O₂ were added to two test tubes labeled as test and control. To the test, 0.2 ml of sample was and to the control 0.2 ml of water was added. The contents were mixed well and incubated at 37°C for 10 minutes. The reaction was arrested with the addition of 0.5 ml of 10% TCA. To determine the glutathione content, 1.0 ml of supernatant was removed by centrifugation, to that 3.0 ml of phosphate buffer and 0.5 ml of Ellman’s reagent was added. The colour developed was read at 412nm. Standards in the range of 40-200μg was taken and treated in the similar manner. Glutathione was measured by its reaction with DTNB to give a compound that absorbs at 412 nm.

Assay of superoxide dismutase (SOD) was done using Kakkar’s procedure [26] which is underscored by the principle that SOD uses the photochemical reduction of riboflavin as oxygen generating system and catalyses the inhibition of Nitro Blue Tetrizolium (NBT) reduction, the extent of which can be assayed spectrophotometrically.

The incubation medium contained a final volume of 3.0 ml, 50 mM potassium phosphate buffer (pH 7.8), 45 M methionine, 5.3 mM riboflavin, 84 M NBT and 20 M potassium cyanide. The amount of homogenate added to this medium was kept below one unit of enzyme to ensure sufficient accuracy. The tubes were placed in an aluminium foil- lined box maintained at 25°C and equipped with 15W fluorescent lamps. Reduced NBT was measured spectrophotometrically at 600 nm after exposure to light for 10 minutes. The maximum reduction was evaluated in the absence of the enzyme. One unit of enzyme activity was defined as the amount of enzyme giving a 50% inhibition of the reduction of NBT. The values were calculated as units/mg protein

2.4. Statistical analysis

Result obtained, expressed as Mean ± standard error of mean (SEM), were entered into Microsoft® office excel spreadsheet (2019) and analyzed using graph pad prism version 8 software (International business machine (IBM®) Cooperation). One-way analysis of variance (ANOVA) was employed to test the existence of any significant differences between and within group means. Student t-test was used to determine levels of significant variation in means at probability level of 0.05. Where significant differences exist among means, Post Hoc test was thereafter applied to the significant mean differences to define specific group with mean differences.

3. Results

The results obtained in the study are presented in table 1 below.

**Table 1** Effect of oral sugar treatments total body weight and semen parameters in the adult Wistar rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean Body weight and Semen/Sperm parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body weight (kg)</td>
</tr>
<tr>
<td>(Control)</td>
<td>141±12.9</td>
</tr>
<tr>
<td>(20 mg/kg Sugar)</td>
<td>154±9.3*</td>
</tr>
</tbody>
</table>
3.1.1. Effect of Sugar on Body Weight

Table 1 shows the effect of white sugar treatments on the total body weight and semen/sperm parameters in this study. Relative to the Control group mean weight of 141±12.9 kg/bw, significant (P < 0.05) increase in body weight in groups 2 and 3 (154±9.3 and 199±10.9 respectively) were observed, while group 4 showed no weight change that was statistically significant. This implies that table sugar, as added sugar, potentially increases body weight.

3.1.2. Effect Of Sugar on Sperm Parameters

Sperm count showed significant (P < 0.05) dose dependent decreases across all treatment groups compared to the Control value of 40±0.97 x10^6 cells/ml, representing about 0.3, 0.58 and 0.95 fold changes respectively.

Normal sperm motility as well as sperm progressivity showed no statistical differences between groups 1, 2 and 3. There was however, significant (P < 0.05) decreases these parameters in group 4 at 5.1 ± 2.9% and 1.0 ± 0.0% relative to the Control values of 79 ± 7.7% and 3.8 ± 0.25% respectively; translating to reduction of 0.93 and 0.74 folds respectively. Similarly, there was no statistical difference observed in abnormal motility values in groups 1, 2 and 3 relative to the Control group (21 ± 7.7%). In group 4 however, the abnormal motility was 95 ± 2.9% (4.5-fold) which indicate a significant increase compared to the Control group value.

Regarding sperm morphology, the normal morphology of groups 3 and 4 showed significant difference (reduced) at 62 ± 1.8% and 31 ± 8.2% respectively compared to that of the Control group (91 ± 3.2%). This indicates 1.5- and 2.9-fold reductions respectively. On the other hand, a dose dependent significant increases of about 1.7, 1.9 and 7.8 folds were observed in abnormal sperm morphology of groups 2, 3 and 4 respectively compared to Control group (8.9 ± 3.2%). These findings imply that table sugar negatively impact on sperm functions on a dose dependent basis, especially from 30mg/kg bw upwards.

3.1.3. Effect of Sugar on Antioxidant Status

The effect of white sugar oral administration on superoxide dismutase and glutathione peroxidase in the Wistar is presented in figures 1 and 2 respectively.

![Superoxide Dismutase](image)

**Figure 1** Effect of Table Sugar at different doses (20mg/kg, 30mg/kg and 60mg/kg) on superoxide dismutase in adult male Wistar rats.
Effect of Table Sugar at different doses (20mg/kg, 30mg/kg and 60mg/kg) on glutathione peroxidase of adult male Wistar rats.

The biochemical assay of antioxidant response to the treatments used in this study showed that there were no observed differences in mean serum levels of superoxide dismutase and glutathione peroxidase levels across all treatment groups when compared to the Control group (20.47 ±0.95 u/mg), implying that table sugar intake has no significant effect on antioxidant activities in the Wistar rats in doses of up to 60mg/kg bw.

4. Discussion

Sugar or carbohydrate in general has been important source of chemical energy for cells. Glucose as the most preferred form of substrate for glycolysis in cells, yielding high energy phosphate compounds like adenosine tri-phosphate (ATP) for cellular use. However, high intake of this important energy source has been attributed to adverse outcomes in the body [8,9,10,27,28]. Thus, this study was designed to study the effect of table sugar, at low fractions of RDA, on sperm parameters and antioxidant status in the Wistar rats.

The total body weight of the animals in our study increased with increase in dosage of sugar administered in the first two treatment groups, but not in group that received 60mg/kg bw of the treatment. This is in conformity with findings of study conducted elsewhere [29,30]. This is due to the fact that sucrose is digested into glucose and fructose on a 1:1 ratio in the gastrointestinal tract for absorption. The metabolism of glucose is tightly regulated by insulin and hepatic glycolytic needs [11], with almost all the dietary glucose metabolized in peripheral tissues, leaving little for storage as fat in the liver, posing reduced risk of developing chronic diseases, such as insulin resistance and type 2 diabetes mellitus (T2DM). The metabolism of fructose, on the hand, is not regulated by insulin and hepatic energy needs, as the conversion to fructose 1-phosphate bypasses the key regulatory enzyme phosphofructose kinase-1, making fructose to be mostly metabolized and stored by the liver, with little metabolism in peripheral tissues. This has the potential to induce lipogenesis, resulting in hepatic fat accumulation [31,32], as well as increased gluconeogenesis with the attendant weight gain and metabolic disorders (27).

There was no significant weight gain in group 4 in our study, a finding supported by a similar study [33]. This may be attributable to change in dietary habit as the rats may so be addicted to the sugar intake that they abhorred the intake of the rat chow feed as evidenced by the unusual left over of feeds in group 4. Sucrose has high satiating effect with increases sense of fullness due to its sweetness. This a consequence of the is in line with the glucostatic hypothesis of food regulation where central glucoreceptive elements respond to rise in blood glucose concentration [34].

In this study, it was also observed that sperm count was reduced in the treatment groups in response to the treatment on dose dependent basis, while depression of normal sperm motility and progressivity occurred in group 4 only. Reduction in normal morphology was observed in groups 3 and 4. On the other hand, there was increase in abnormal
motility occurred in group 4 and increase in abnormal morphology in groups 2, 3 and 4. These indicates that table sugar at 20mg, 30mg and especially at 60mg per kg bw negatively affects sperm parameters by depressing sperm indices of count, normal motility (including progressivity) and normal morphology while increasing abnormal motility and abnormal morphology. This has negative implication for male fertility. On the antioxidant status under study, the doses of white sugar used have no significant effect.

The health of spermatozoa is one of the critical and essential contributory factors to male fertility and a successful reproductive function in man. Among the indices commonly used to assess male reproductive functions is assessment of sperm parameters (or semen analysis) which include count, morphology and qualitative motility [20,35]. The more qualitative these parameters are, the more fertile the male is adjudged to be.

Glycosylable sugars have been shown to be beneficial for sperm acrosomal reaction, capacitation and fertilization capacity [36,37]. As observed in this study, sugars have significant detrimental effects on sperm characteristics of count, motility and morphology [35,38,39,40]. Consumption of diet with high levels of sugar and saturated fats disrupt testicular metabolism and compromises spermatogenic processes in rats, usually associated with testicular mitochondrial dysfunction and oxidative stress, consequently resulting in sperm defects [41,42], and in the female rats, high fructose intake has profound effect on pregnancy outcome and fertility in female rats. It appears that the mechanism of action for the induction of abnormal sperm parameters by low dose sugar consumption is not through the activation of redox system in sperm cells as given by the author above as well as other authors [43,44,45].

In a study, it was observed Auger and colleagues [37] that high sucrose consumption resulted in decreased testicular, seminiferous tubule and seminal vesicle weights as well as serum testosterone concentration without affecting the concentration of the gonadotropin. Seminal vesicular secretion is important for sperm motility and stability of sperm DNA integrity [46]. Testosterone is physiologically associated with initiation and sustenance of qualitative spermatogenesis. This androgen is produced by the Leydig cells in the testes. Reduced production of testosterone by the testes consequently hampers spermatogenesis [47]. Since sucrose intake did not alter gonadotropin concentration, it may appear that sucrose acted locally to depress androgen production in the testes.

5. Conclusion

From the findings of the current study, we conclude that sucrose, as added sugar at doses well below the RDA, impairs sperm functions by depressing normal sperm parameters while increasing the abnormal parameters probably through suppression of seminal secretion and Leydig cell actions in the testes.

Recommendation

Arising from this study, we hereby recommend that the established advice on reduction of daily intake of table/sugar be sustained, there is need to replicate this study elsewhere with the aim of consolidating of refuting the existing RDA of table sugar.

Compliance of ethical standard

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Disclosure of Conflict of interests

We the authors hereby declare that this original work has not been previously published, nor is it before another journal for consideration

Statement of ethical approval

Institutional ethical approval was obtained from the College of Health Sciences, Benue State University Research and Ethical Committee for the conduct of the study on experimental animals on certificate no. CREC/RS/001 of 10th June, 2023.
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