ORIGINAL ARTICLE

EFFECT OF WHITE SUGAR, BROWN SUGAR AND JAGGERY POWDER SYRUP ON BIOCHEMICAL AND HISTOPATHOLOGICAL MARKERS OF SPRAGUE-DAWLEY RATS

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Background: There is a common myth that white sugar produces more harm as compared to brown sugar and jaggery powder which may contain useful chemicals other than sucrose. This study was aimed to assess this common belief by evaluating different parameters in Sprague dawley rats. **Methods:** This 12-weeks study was carried out at Postgraduate Medical Institute, Lahore from Sep to Nov 2018. Sucrose content of white sugar, brown sugar and jaggery powder was determined and a 10% sucrose syrup was given to twenty male adult Sprague dawley rats divided into four groups. Group A: normal control (plain drinking water), group B: white sugar group (10% sucrose syrup), group C: brown sugar group and group D: jaggery powder group for 12 consecutive weeks. Body-weight, non-invasive blood pressure and biochemical tests were performed. Liver, pancreas and kidney sections prepared and examined under light microscope. **Results:** No significant differences were observed between body-weight, blood-pressure, serum insulin, lipid profile, and renal function tests. Blood sugar was higher in all experimental groups than normal control with no statistical differences in between. Liver enzymes especially AST and ALP were significantly deranged than normal control in brown sugar group. Liver weight was also significantly higher in brown and white sugar groups. Histology of liver, pancreas and kidney sections showed congestion and inflammatory changes in all experimental groups. **Conclusion:** Consecutive administration of all three types of sugar produced no significant differences except for liver weight and enzymes which were the most deranged in brown sugar group.

Keywords: Sugar, Jaggery powder, Histopathology, Liver, Pancreas, Kidney, Marker, Sprague-dawley

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INTRODUCTION

Hypertension remains a major risk factor for stroke, cardiovascular disease, renal disease, and death. It accounts for 10% of the total annual health budget in developed countries.[1] By 2025, the number of people living with hypertension is expected to reach 1.56 billion people.[2]

Dietary factors that increase blood pressure (BP) are of interest to public health authorities.[3,4]

The introduction of refined sugars into the food supply and the subsequent rise in sugar consumption has mirrored the increase in the prevalence of hypertension over the last century.[3]

Sucrose is the most frequently used, and admired component to obtain sweetness in human food preparation. It is a disaccharide containing two monosacharides fructose and glucose chemically linked together. Sucrose is used in various products as industrial sweetener such as drinks, confectionary, jams, jellies and preserves. It is primarily esteemed for its sweetness and serves as a vital source of energy, yielding 394 kcal/100 g of refined sugar.[5]

Increases in ingestion of sucrose have shown to elevate the blood pressure in experimental animals.[6] Furthermore, reduced consumption of sugar-sweetened beverages, as well as sugars in the form of sucrose, glucose and fructose, was found to be significantly associated with reduced BP in a prospective study.[7]

The mechanism by which sucrose increases the blood is by causing the stimulation of the sympathetic nervous system thus leading to an increase in Renin secretion which leads to renal sodium retention and vascular resistance. Other probable mechanism involved in the sucrose induce hypertension can be insulin resistance and hyperinsulinemia which has been shown to develop when normal rats eat a high sucrose diet.[8]

Added sugars perhaps matter more than dietary sodium for hypertension. Naturally occurring sugars are not harmful for health however added sugar is a problem and should be targeted more clearly in dietary guidelines to sustain cardio-metabolic and general health.

METHODOLOGY

This 12-weeks experiment was carried out in Postgraduate Medical Institute, Lahore, Pakistan from Sep to Nov 2018. Ethical approval was granted by the

Institutional Review Committee. Guidelines laid in Helsinki declaration by WMA in 2013 for animal research were followed strictly.[1]

Twenty adult male Sprague-dawley rats that weighed 150–200 grams were first acclimatized to the environment of the animal house for one week. They were kept at standard temperature (22±3 °C) and under 12 hours light-and-dark cycles. Standard rat chow was administered *ad libitum* to all rats.

Rats were then randomly divided into four groups ($n=5$) using lottery method. A 10% sucrose syrup was prepared based on sucrose content found in three types of sugars (99% for white sugar, 91% for brown sugar and 75% for jaggery powder) analyzed at Pakistan Council of Scientific and Industrial Research Laboratories, Lahore. Therefore, Group A was given plain drinking water being normal control, group B was given 10.10 grams white sugar dissolved in 100 ml drinking water, group C was given 11.00 grams brown sugar dissolved in 100 ml drinking water, and group D was given 13.33 grams jaggery powder dissolved in 100 ml drinking water for 12 consecutive weeks.

Body weight and blood pressure of all rats was measured for 12 consecutive weeks. An inflatable cuff around rat tail was applied and blood pressure was measured non-invasively using computer-based data recording system PowerLab® model: M-ML 856, Australia. Three reading were taken from each rat per week and an average reading was calculated for that week.

After the completion of 12 weeks, rats were anaesthetized with chloroform and blood sample was drawn through cardiac puncture. The blood was allowed to clot and then centrifuged at 4,000 rpm for 10 minutes for serum separation. Serum glucose, serum insulin, liver function tests (Serum total bilirubin, ALT, AST and ALP), serum urea and creatinine and lipid profile (triglycerides, total cholesterol, LDL and HDL) were determined using commercially available kits made by CELM diagnosis, São Paulo, Brazil.

All rats were then euthanized, and their liver, kidneys and pancreas were dissected out, weighed and stored in 10% NBF. Tissues were processed and cut into 5 µm thick section placed on glass slides. These slides were stained with haematoxylin and eosin and analysed using 10× and 40× magnifications.

SPSS-25 was used for statistical analysis. Shapiro-Wilk test was used to check normality of the data. The quantitative variables found to be normally distributed were expressed as Mean±SD, while qualitative and non-normally distributed quantitative variables were expressed as median and interquartile ratio. Analysis of variance (ANOVA) and post-hoc Tukey test were used determine differences among and between groups in case of normally distributed data, while Kruskal-Wallis ANOVA and Mann-Whitney U tests were applied to see difference between non-normally distributed variables; and $p\leq0.05$ was considered statistically significant.

RESULTS

The body weight, non-invasive blood pressure and lipid profile of all rats had no statistically significant difference at the end of study (Table-1).

Serum glucose and serum insulin in all experimental groups were non-significantly different at the end of study. It was, however, numerically higher than normal control group in all experimental groups at the end of study (Table-2).

Apparently, brown sugar group had the most deranged liver enzymes. Statistically significant difference was found in ALP and AST of rats. Upon post-hoc Tukey test, brown sugar group had significantly higher ALP ($p=0.037$) than normal control while the difference among experimental groups was non-significant. Similarly, AST was significantly higher in brown sugar group ($p=0.004$) than jaggery powder group. Rest of the tests had non-significant differences (Table-3).

Liver weight was significantly different among all groups. It was highest in white sugar and brown sugar ($p=0.007$ and 0.006) followed by jaggery powder group ($p=0.049$).

Though serum urea was significantly different among all groups, it was numerically highest in normal control group which makes this finding unremarkable. Serum creatinine and uric acid had no statistical differences (Table-4).

Table-1: Body weight, blood pressure, and lipid profile of rats in normal control and experimental groups (n=5) (Mean±SD)

Parameter at 12 weeks	Normal Control	White Sugar	Brown Sugar	Jaggery Powder	*p*
Body weight (Gm)	271.2±9	282±25	270.2±8	293±14	0.673
Blood pressure (mmHg) at week 6	103.9±11	112.8±12	107±6	99.4±9	0.237
Blood pressure (mmHg) at week 12	104.5±6.8	109.8±9.5	112.7±13	103.3±10	0.283
Cholesterol (mg/dL)	78±13	90±12	67.2±16	72±6	0.064
Triglycerides (mg/dL)	61±4	68.6±16	58±4	54.8±9	0.183
HDL (mg/dL)	26±9	31±10	20±3	20±5	0.113
LDL (mg/dL)	39.4±12	45±12	35±16	41±2	0.671

Table-2: Serum glucose and serum insulin of rats in normal control and experimental groups

Parameter at 12 weeks	Normal Control	White Sugar	Brown Sugar	Jaggery Powder	*p*
	Mean±SD				
Serum glucose (mg/dL)	108.8±23	248.4±109	269.8±121	227.60±3	0.078
Serum insulin (mIU/L)	107±53	78±65	67±76	93±31	0.724

Table-3: Liver function tests of rats in normal control and experimental groups (n=5), (Mean±SD)

Parameter at 12 weeks	Normal Control	White Sugar	Brown Sugar	Jaggery Powder	*p*
ALP (mg/dL)	188±42	220±22	260±50*	252±30	0.032
ALT (mg/dL)	48±9.9	48±4.3	123±101	50±5.3	0.084
AST (mg/dL)	193±45	198±67	306.1±31^^	102±2	0.007
Bilirubin (mg/dL)	0.36±0.134	0.36±0.15	0.44±0.11	0.38±0.08	0.707
Liver weight in grams	7.3±0.52	9.5±1.04**	9.56±0.91**	8.9±1.02*	0.004

*p=0.037 as compared to normal control, ^^p=0.004 as compared to jaggery group

Table-4: Serum urea, creatinine and uric acid in normal control and experimental groups (n=5), (Mean±SD)

Parameter at 12 weeks	Normal Control	White Sugar	Brown Sugar	Jaggery Powder	*p*
Urea (mg/dL)	62±6	36.8±4	40±6	40±6	0.000*
Creatinine (mg/dL)	0.7±0.1	0.64±0.11	0.62±0.04	0.58±0.08	0.149
Uric acid (mg/dL)	1.84±0.4	2.08±0.73	3.6±1.24	3.2±1.9	0.094

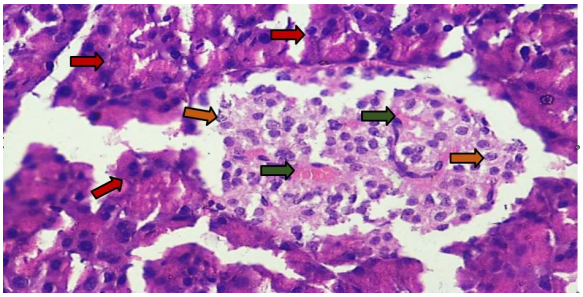

Figure-1a: Pancreatic section of White sugar group. H&E staining 40× magnification

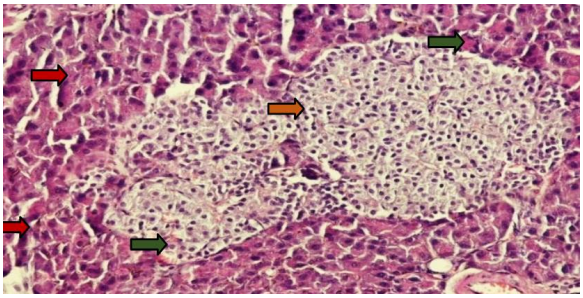

Figure-1b: Pancreatic section of brown sugar group. H&E staining 40× magnification

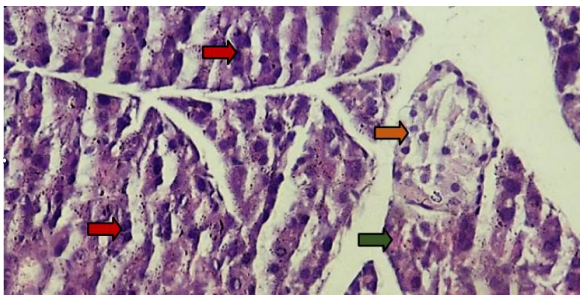

Figure-1c: Pancreatic section of jaggery treated group. H&E staining 40× magnification

Photomicrographs of pancreatic section from all experimental groups showed degenerated cells in islets of Langerhans (brown arrows), congested blood vessels (dark green arrows) and disrupted serous acini (maroon arrows). The changes were the most severe in jaggery powder group and least in brown sugar treated group.

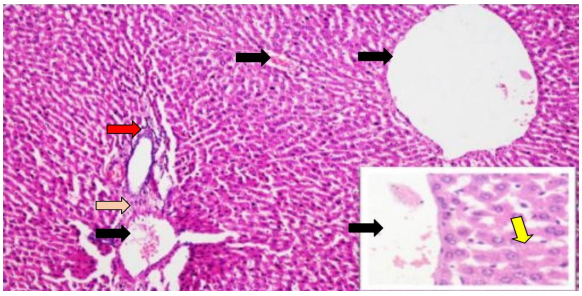

Figure-2a: Liver section of white sugar treated group. H&E staining. 10× and 40× (inbox)

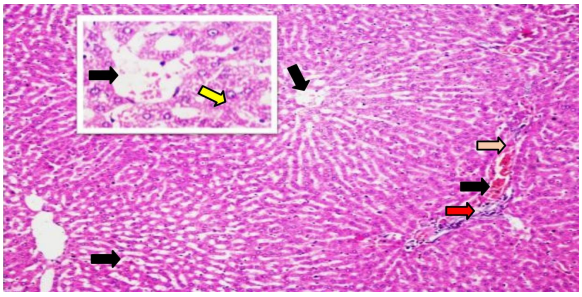

Figure-2b: Liver section of brown sugar treated group. H&E staining. 10× and 40× (inbox)

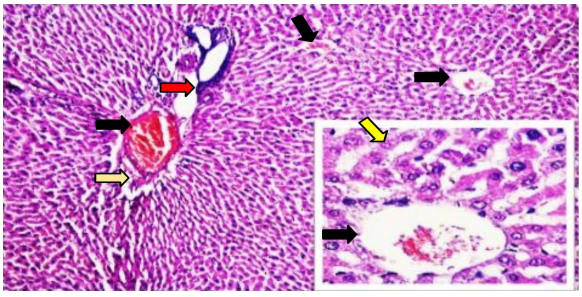

Figure-2c: Liver section of jaggery powder treated group. H&E staining. 10× and 40× (inbox)

Photomicrographs of liver sections under 40x zoom showed central vein, sinusoidal, and portal venous congestion (black arrows), degenerated hepatocytes with vacuolization (yellow arrows), perivenular fibrosis (peach arrows) and inflammatory cell infiltration (red arrows) as seen in Fig 2a, b and c. The changes were most marked in brown sugar group and were the least in white sugar treated group.

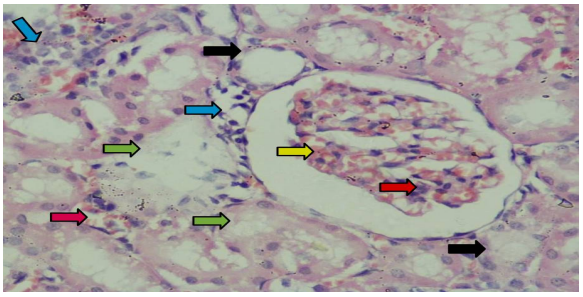

Figure-3a: Renal section in white sugar treated group. H&E staining 40× magnification

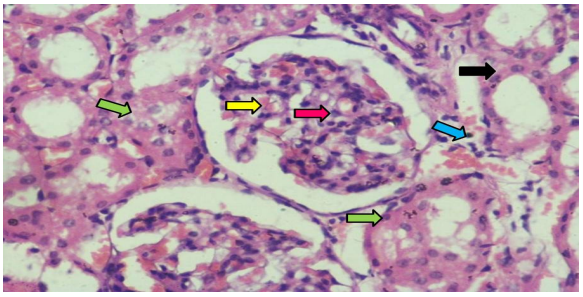

Figure-3b: Renal section in brown sugar treated group. H&E staining. 40× magnification

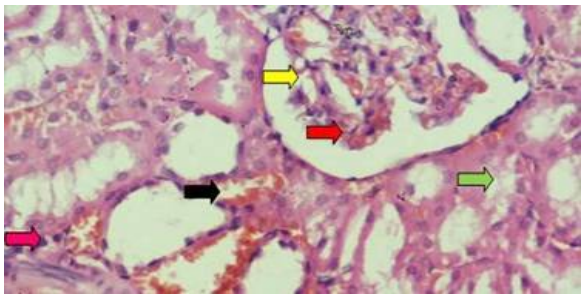

Figure-3c: Renal section in jaggery powder treated group. H&E staining. 40× magnification

Photomicrographs of a kidney sections showed degenerated (Red arrow) & congested glomerulus (Yellow arrow), highly vacuolated proximal (Light green arrow) & distal convoluted (Black arrow) tubules, stromal inflammatory cell infiltrate (Light blue arrow) & blood vessel congestion (Pink arrow). The changes were the most marked in jaggery powder group and the least in brown sugar group.

DISCUSSION

In the present study we aimed to determine the effects of 10% sucrose syrups made from white sugar, brown sugar and jaggery powder on body weight, blood glucose levels, fasting insulin levels, blood pressure, lipid profile and tissue parenchyma of liver, pancreas, and kidneys.

We reported in the present study that the body weight of the rats did increase over time but was not statistically different among the study groups at any point in the study. This finding was consistent with the results reported by a study in which when feed high sucrose 40% diet to rats for 16 weeks.[9]

The fasting blood glucose levels were raised at the end of 12 weeks in the present study but they did not reach statistical significance yet. The same finding was reported by Souza Cruz *et al* when using a higher 40% sucrose diet.[10] Amin *et al* reported that rats given high sucrose diet had significantly larger visceral fat pads and hypertriglyceridemia, however, neither plasma glucose nor insulin levels were significantly higher, while hyperglycaemia and insulin resistance occur after 20 weeks of feeding high sucrose diet.[11]

The weight of the liver in the present study was significantly higher in the rats given sucrose syrup as compared to the control group that is likely due to an increase in liver fat deposits. In the present study we reported a non-significant difference in blood pressure of the rats at end of 12 weeks by using a non-invasive rat tail cuff monitor. This is consistent with findings of a study[9] who recorded blood pressure of sucrose fed rats by insertion of intra-aortic catheter (telemetric method). They reported that there was no statistical difference in the mean 24-hour blood pressure taken on monthly basis for 4 consecutive months. They further added that there was a consistent increase in hourly systolic blood pressure of rats when measured within an hour of providing food. So they suggested that sucrose intake could increase blood pressure during the immediate postprandial period.

In the present study we observed the hepatotoxic effect of jaggery powder and brown sugar evident by a significantly raised serum AST, ALP levels in these groups. The mean Serum ALT levels were highest in brown sugar and jaggery powder syrup groups as compared to the other two groups but difference did not reach statistical significance (p>0,05). The serum fasting cholesterol and triglycerides were not statistically different among the study groups. A study[12] done in 2016 observed that there was no significant difference between serum AST and ALT levels of adult Wister rats given fresh cane brown sugar juice for 4 consecutive weeks while they also reported no significant difference between fasting serum cholesterol and triglycerides which is consistent with the finding of the present study. Another study in 2020 reported that the there was no significant difference in the lipid profile of adult Sprague-dawley rats when given low doses of white and brown sugar but the difference became significant as compared to control when high doses of these two natural sugar sweeteners were given for 12 consecutive weeks.[13]

In the present study the maximum liver parenchymal damage was observed in the brown sugar group when compared to control group. A similar finding was reported by Corona-Pérez *et al* in their study. They reported that the rats given sucrose had morphological alterations in their liver parenchymal cells. The reason being that the sugar ingestion initializes structural changes, including decrease in hepatocyte number and an increased hepatocyte size.

These characteristics are linked to hepatocyte ballooning, which is a common feature of the injured liver.[14] They also reported that there was higher vacuolation in the group given sucrose, which is linked to an increase in cellular deposits and cellular damage.[15] They reported the presence of collagen in the hepatocytes of sucrose-fed animals, and mild fibrosis mainly in the perisinusoidal region and pericentral zone, which relates to Non-Alcoholic Fatty Liver Disease.[16] The higher levels of liver enzymes called transaminases reported in this study also suggest a disruption of the liver function.[17] A study showed comparable effects after 8 weeks of sucrose supplementation, classifying their study results as of moderate-grade fibrosis. In contrast to this, another study[18] found no evidence of liver fibrosis even after giving sucrose supplements for 20 weeks, despite reporting higher lipid content.

In the present study we observed that the maximum histo-pathological changes indicative of pancreatic inflammation were present in the group given jaggery powder syrup when compared to the control group. Same was reported in another study.[9]

CONCLUSION

The rat pancreas and kidneys showed adverse inflammatory histopathological changes maximally in rats taking jaggery powder followed by white sugar. The adverse effects on liver cells were maximally seen on rats given brown sugar. Further work is recommended with larger sample size and different chemical constituents found in jaggery powder and brown sugars so that these adverse changes may be attributed to the proper chemical substance.

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HNL: Concept, study design, data collection, manuscript writing, approval
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RMY: Concept, data collection, final approval
SRC: Data collection, manuscript writing, final approval
AHS: Study design, final approval
SC: Data collection, final approval

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