IN FRUCTOSE INDUCED METABOLIC SYNDROME INWISTAR RATS

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ABSTRACT
Background: Metabolic syndrome otherwise referred to as insulin resistance is defined as a cluster of conditions that raises the risk of cardiovascular disease, stroke and diabetes and is characterized by abdominal obesity, insulin resistance, hypertension, and dyslipidaemia.
Aim: The study was aimed at investigating the modulation of renal indices and nitric oxide synthase activity in fructose induced metabolic syndrome in Wistar rats.
Methods: Twenty (20) Wistar rats were randomly divided into 2 groups of 10 rats each: Group 1: test group treated with 10% fructose solution and Group 2: control received tap water. Baseline body weight, body mass index, fasting blood glucose, fasting lipid profile, urea, creatinine and nitric oxide synthase activity were measured using standard techniques. The rats were treated for 56 days and then final body weight and body mass index were taken after which the ratswere anaesthetized and blood sample was obtained forbiochemical analysis.
Results: The result from the study indicated that, there was a significant ($p < 0.05$) difference in body weight, fasting lipid profile, Urea, Creatinine and Nitric oxide synthase activities in treatment group compared with control group. The initial and final FBG concentrations in the test group was found to be significant ($p<0.05$). Also there was a significant difference between initial and final body mass index in the test group but not in control group. There was no significant difference between initial and final FBS concentrations in the control group.
Conclusion: the study provides evidence that 10% fructose administration caused increase in body weight, BMI, fasting blood sugar concentration dyslipidaemia, Urea, Creatinine activity and decrease activity of nitric oxide synthase
Keywords: Fructose, Metabolic syndrome, Nitric oxide synthase, Renal indices

INTRODUCTION
Metabolic syndrome otherwise referred to as insulin resistance is defined as a cluster of conditions that raises the risks of cardiovascular disease, stroke and diabetes and is characterized by abdominal (central) obesity, insulin resistance, hypertension, and dyslipidaemia (Ford, 2005).Various definitions of metabolic syndrome have been coined by various organizations such as World Health Organization (WHO), International Diabetes Federation (IDF), and National Cholesterol of Adult Treatment Panel III (NCEP ATP III) (Alberti *et al.,* 2009). Later, these organizations combined and developed a new definition of metabolic syndrome known as "harmonized criteria" which included central obesity, elevated blood pressure, elevated triglyceride levels, low high-density lipoprotein (HDL), and elevated glucose levels (Alberti *et al*., 2009). Fructose, a simple monosaccharide that is being use as a sweetener in food and drinks (Le and Tappy, 2006). On an average, the consumption of fructose has increased to 16% from 1986 to 2007 (Tappy and Le, 2010). The increase in consumption of fructose is closely related to the incidence of obesity, this is due to fructose not being able to stimulate the secretion of insulin from pancreatic cells as a result absence of GLUT5 transporter from the pancreatic cells (Bray *et al*., 2004). Furthermore, the metabolism bypasses the

main pathway of glycolysis which converts glucose-6-phosphatase to fructose-1, 6-biphosphate by phosphofructokinase enzyme (Rutledge and Adeli, 2007). These two factors counteracted with that of glucose which stimulates the secretion of insulin from pancreatic cells thereby converts the glucose to glycogen. The metabolism of glucose also undergoes the rate limiting step in glycolysis pathway. There are speculations that excessive fructose consumption is a cause of dyslipidaemia(Basciano *et al.,* 2005, Malik *et al.,* 2015), insulin resistance, obesity (Elliott *et al.,* 2002) leading to metabolic syndrome, type 2 diabetes (Malik *et al.,* 2015) and cardiovascular disease (Rippe *et al.,* 2015). Nitric oxide synthases (NOS) are a family of enzymes catalysing the production of the meta-stable free radical nitric oxide (NO) and there are three isoforms of the NOS enzyme: endothelial (eNOS), neuronal (nNOS), and inducible (iNOS), each isoform has separate functions. Endothelial (eNOS) is the predominant vascular NO synthase isoform and is responsible for the majority of NO production in the vasculature (Moncada and Higgs, 2006). NO is an important regulator and mediator of numerous processes in the nervous, immune and cardiovascular systems. These include vascular smooth muscle relaxation, resulting in arterial vasodilation and increasing blood flow (Weller and Richard, 2013). NO is also a neurotransmitter and has been associated with neuronal activity and various functions such as avoidance learning, partially mediates macrophage cytotoxocity against microbes and tumour cells. Besides mediating normal functions, NO is mediated in pathophysiological states as diverse as septic shock, hypertension, stroke and neurodegenerative disorders (Davis *et al.,* 1997).

MATERIALS AND METHODS

Study Area

The study was carried out in the Department of Chemical pathology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto.

Reagents and Chemicals

Analytical graded chemicals and reagents commercially available (Randox Laboratories Ltd) were used for this research.

Experimental Animals

Twenty (20) male Wistar albino weighing between 100 to 216g (aged 8-10 weeks old) were purchased from the Animal House of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. The rats were housed in well aerated cages under hygienic conditions in the same animal house. They were allowed to acclimatise for a period of two (2) weeks. They were maintained in a clean metabolic cage-sand, placed in a well-ventilated room condition as described by Aniagu *et al*. (2005). The animals were fed pelletized growers feed (Vital®), obtained from Grand Cereal Soil Mills Limited, Jos, Nigeria. They were also allowed access to clean drinking water *ad libitum* throughout the experimental period. Cleaning of the animal cages was carried out daily, and on regular basis.

Ethical Considerations

All the experimental protocols were in accordance with the Institutional Animal Ethics Committee Guidelines as well as internationally accepted practices for use and care of laboratory animals as contained in United States Guidelines, and also in accordance with the recommendations of the International Association for the Study of Pain(IASP).

Induction of Metabolic syndrome

10% fructose solution was given to the experimental group as drinking water for a period of 8 weeks to induce metabolic syndrome.

Experimental Design

Twenty (20) rats were randomly divided into two (2) groups as follows:

Group 1: received 10% fructose solution as drinking water and standard rodent chow for 8 weeks and Group 2: Rats received tap water and standard rodent chow for 8 weeks. Each group consisted of equal number of rats n=10

Measurement of Body Weight

The body weight in gram (g) of each rat was measured before and after administering Fructose to the test group. This was done by placing each rat into a container and following calibration and zeroing the weighing balance, the weight of each was taken as described by Ochei and Kolhatkar (2000).

Measurement of Body Length

The body length in centimeters (cm) of the rats were measured prior to the commencement of the experiment (day 1) and on the day of sample collection (day 56). The length the rats were measured from the anus to the tip of the nose in centimetres.

Estimation of Body Mass Index

The body mass index of each rat was calculated using the following expression:

Body mass index (g/ cm^2) = Body weight (gram)/ body length (cm^2).

Blood Sample Collection and Processing

After eight (8) weeks of the experiment, the animals were fasted for 12 hours and blood was collected by cardiac puncture from chloroform anaesthetized rats into fluoride oxalate containers for glucose estimation and plane containers for other biochemical analysis. The samples collected were allowed to clot at room temperature and later centrifuged at 4000 rpm for 10 minutes. The serum and plasma of each sample was separated and transferred into cryo vial, caped and stored (frozen) at -20 until required for analysis

Determination of biochemical parameters

Biochemical analyses were performed at the Chemical Pathology Laboratory of UsmanuDanfodiyo University, Sokoto. The separated Plasmas and serums were used for the assays of Fasting blood glucose, Total cholestrol(Trinder, 1969), TriglycerideTrinder, (1969), High Density Lipoprotein(Trinder, 1969), Nitric Oxide Synthase (Schmidt, 1995), Urea (Rosenthal, 1955), Creatinine (Jaffe, 1886) and Low Density Lipoprotein wasFriedewald'sFormula (Friedewald*et al.,* 1972).

Statistical analyses

The results obtained from the study were expressed as mean ± standard deviation for the rats in each groups. Statistical differences were compared with independent student t-test, p values < 0.05 were considered to be significant. All statistical tests were carried out using statistical package for social science (SPSS) for windows, version 20.0 (SPSS Inc., Chicago, IL, USA).

RESULTS:

TABLE 1. The mean initial and final body weight and body mass index of the rats used in the study.

GROUP	Initial body weight (g)	Final body weight (g)	Initial BMI	Final BMI	P value
Control	162 ±0.01	115±0.02	0.54 ± 0.07	0.51 ± 0.05	$p<0.05$
Test	187±0.01	297±0.06	0.47± 0.05	0.66 ± 0.07	

Values are of mean ± standard error of mean.

TABLE 2. Plasma Urea concentration of both control and test rats.

Group	Initial FBG (mmol/l)	Final FBG (mmol/l)	Urea (mmol/L)	Creatinine (mg/dl)	p- value
Control (Group 1)	3.21 ± 0.12	3.27 ± 0.09	3.64±0.29	0.69±0.08	
Test (Group 2)	5.34 ± 0.58	7.69 ± 0.41	7.60±1.13	1.16±0.10	p< 0.05

±± Values are Mean ± Standard error of the mean

TABLE 3. Fasting Lipid profile concentrations of test and control rats

GROUPS	TC	HDL	LDL	VLDL	Triglyceride (mg/dl)	AIX (mg/dl)
CONTROL	143.71±21.56	63.57±3.40	75.00±16.00	15.83±1.70	79.14±8.5	2.3±6.34
TEST	252.54±15.14	54.71±5.22	167.86±17.89	22.23±1.27	111.14±6.3	4.61±2.90
P VALUE	S (p<0.05)	S (p<0.05)	S (p<0.05)	S (p<0.05)	(p<0.05)	S(p<0.05)

Values are of mean ± Standard Error of mean

TC = Total Cholesterol (mg/dl), HDL= High Density Lipoprotein (mg/dl), VLDL = Very Low Density Lipoprotein(mg/dl), LDL = Low Density Lipoprotein (mg/dl)

TABLE 4. Plasma nitric oxide synthase (NOS) activities in both test and control rats

GROUP	NOS (µIU/L)p- value	
Control	39.41±3.1	p>0.05
Test	31.83±3.8	

±± Values are of mean ± Standard Error of the Mean

DISCUSSION

The study examined the effect of 10% fructose in drinking water, which is equivalent to a diet containing 48-57% (by calories) fructose on metabolic characteristics in Wister rats. Results from the study showed that fructose enriched diet could induce dyslipidemia in wistar rats, increases fasting blood glucose, body weight, body mass index, Nitric oxide synthase and urea and creatinine. This findings are similar to studies by Mohammad *et al.* (2010), Maged *et al*. (2011) and Kholoud *et al*. (2012). There was significant difference (p<0.05) in mean TG, LDL, VLDL HDL and TC concentration between the test and control groups. This could be as a result of fructose being converted to dihydroxyacetonephosphate (DHAP) and glyceraldehyde-3-phosphate by fructokinase and aldolase B which then bypasses the rate-limiting step catalysed by phosphofructokinase. The DHAP is converted to glycerol-3-phosphate which provides the glycerol backbone for the triglyceride molecule resulting in triglyceride synthesis once liver glycogen is replenished (Rutledge and Adeli, 2007). The triglycerides formed are incorporated into very low density lipoproteins (VLDL) which can then lead to chain reactions in other lipoproteins and lipids such as LDL (Arner, 2011).

The results from the study revealed that mean fasting blood glucose levels in the test group was significantly higher (p<0.05) than values in the control. This findings are similar to studies by Kholoud *et al.* (2012), Maged *et al.* (2011)and Mustafa *et al.* (2010). This could be due to the accumulation of non-esterified free fatty acids which may reduce insulin sensitivity by increasing the intra myo-cellular lipid content (Virkamaki *et al*., 2001). In addition, over time, increased non-esterified fatty acid concentrations may have deleterious effect on β-cell function (Bergman and Ader, 2000). However these findings were not consistent with that of Norshalizah *et al.* (2014) and Mohammad *et al.* (2010) which showed no difference in fasting blood glucose between the fructose fed and the control group.

Differences between serum urea and creatinine concentrations in the test and control groups were statistically significant (p<0.05). Our current study showed that fructose administration in test group showed a significant increase of serum urea and creatinine concentration as compared to control group. These findings come in agreement with the data that suggest that the increase of fructose consumption is one of the factor that contributes to the occurrence of metabolic syndrome and consequently to the rise in incidence of chronic renal disease (Nakagawa *et al.,* 2005).

In the current study, administration of fructose in drinking water caused an increase in body weight and BMI of rats compared with the controls. Our finding is consistent with that by Norshalizah *et al*. (2014) and not consistent with that by Mohammad *et al.* (2010). These changes may be due to the lack of pancreatic β-cell stimulation by fructose to produce insulin which is involved in the regulation of body adiposity via its action in the CNS to inhibit food intake and increase energy expenditure resulting in weight gain and obesity (Bray e*t al*., 2004). Also, fructose alters leptin and gherlin concentrations leading to increase energy intake and thereby contributing to the weight gain and obesity (Johnson *et al*., 2010).

Differences between nitric oxide synthase activity in the test and control groups were statistically significant (p<0.05).This may be due to mitochondria dysfunction, accumulation of lipid metabolite, and increased NOS induced by nutrient overflow into cells due to the excess fructose consumption prompting electrons transfer to oxygen without ATP production, which potentially leads to oxidative damage within mitochondria and oxidation of LDL particles in the endothelium (Liu *et al*., 2009). Also, uric acid formed due to excess fructose consumption produces ROS (Riegersperger *et al.,* 2011) which might eventually cause endothelial dysfunction and impairs endothelial nitric oxide synthase (eNOS) activity and NO production (Muniyappa and Sowers, 2013).

CONCLUSION

In conclusion, the study provides evidence that 10% fructose administration caused increase in body weight, BMI, fasting blood sugar concentration dyslipidemia, Urea, Creatinine activity and decrease activity of nitric oxide synthase which are the characteristics of metabolic syndrome as described by the WHO.

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