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Antidiabetic Effect of *Nono* (A Nigerian Fermented Milk) on Alloxan-Induced Diabetic Rats

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Abstract: The effect of *Nono* (a Nigerian fermented milk) on diabetic situation in rats, induced by intraperitonial injection of alloxan monohydrate is reported. *Nono* administration for 4 weeks lowered the raised plasma glucose concentration of diabetic rats; restoring it to the non diabetic rat level. The low tissue protein and raised plasma protein concentrations; hyperlipidemia; and hypercholesterolemia consequential on diabetic induction were reversed to the normal level by the *Nono* administration. The reversals of these effects of diabetes may be due to the lowering of the plasma glucose concentration by *Nono* or direct activity of either the bacteria or milk components of *Nono* on these substances. The results indicate that consumption of *Nono* produced by wild strain lactic acid bacteria may be helpful in the management of diabetes.

Key words: *Nono* (a Nigerian fermented milk), alloxan monohydrate, anti-diabetic effect, plasma glucose, hyperglycemia, hypercholesterolemia, hyperlipidemia

INTRODUCTION

Interest in the health implications of fermented foods has continued to be on the increase. Aderiye and Laleye (2003) reported that in Nigeria, these foods are employed as alternative methods of treatment for diahorrea, dysentery and common stomach upsets even before affected individuals seek professional medical attention. Such Nigerian fermented foods include *Ogi* from maize, *Fufu*, *Gari* from cassava, *Nono* from milk and *Iru* from locust beans.

There are numerous reports of fermented milk products having probiotic effect. Most researched is the hypocholesterolemic effect. For example, Akalin *et al*. (1997) and Laleye *et al*. (2007) have reported the hypocholesterolemic effects of Lactic Acid Bacteria (LAB) fortified yoghourt in mice; and of *Nono*, a Nigerian fermented milk product in albino rats respectively. These hypocholesterolemic effects have been credited to the LAB or bifidobacteria component of fermented milk (Tabuchi *et al*., 2003a; Abd El-Gawad *et al*., 2005; Laleye *et al*., 2007).

The probiotic effect of fermented foods is being examined over a wider range of diseases. Laleye (2007) has suggested that LAB containing fermented foods such as *Ogi* and *Nono* can be employed in the management of chronic metabolic disorder such as diabetes mellitus. Tabuchi *et al*. (2003b) reported improved glucose tolerance in neonatally streptozotocin-induced diabetic rats when their foods were supplemented with *Lactobacillus* GG cells. Diabetes mellitus is a disease characterized by hyperglycemia resulting from defects in insulin secretion or action or both. This results in damage, dysfunction and failure of various organs especially the eyes, kidneys, heart and blood vessels. The management efforts include attempts at bringing down the hyperglycemia and consequently reverse the organ damage.

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Literature on the use of Nigerian fermented foods/beverages in the management of chronic metabolic disorder is scanty. It is to this end that this study investigated the effect of the consumption of *Nono* (a Nigerian fermented milk product) on alloxan-induced diabetes in albino rats. *Nono* is a Nigerian fermented milk beverage obtained from natural fermentation of fresh cow milk, which results in a curdled and highly soured product that is widely consumed in Nigeria and the West African sub region (Akinyanju, 1989; Savadogo *et al*., 2004).

MATERIALS AND METHODS

The *Nono* used in this study was obtained from a single local producer in rural Nigeria. This was to ensure reasonable consistency of the product. The LAB population of each sample purchased was assayed for by the pour plate method employing MRS agar with incubation in a microaerophilic condition at 37°C.

Weaned albino rats were obtained from the Department of Biochemistry, University of Jos, Nigeria. The rats were fed commercial feed and water *ad libitum*. Thirty-two rats with average body weight of 100 g were sorted at random into 4 groups. The four groups were treated as follows: Diabetes induced given normal saline; Diabetes induced given *Nono*; diabetes not induced given normal saline and diabetes not induced given *Nono*.

Diabetes was induced in the rats by intraperitonial injection of alloxan monohydrate dissolved in distilled water at a concentration of 100 mg kg^{-1} body weight. Diabetic situation was confirmed established before experimentation progressed. Administration of *Nono* was by oral intubation at 1 mL per rat thrice daily for 4 weeks. Groups not receiving *Nono* received 1 mL of normal saline per rat to equilibrate the stress of the treatment.

The method of Usman and Hosono (2000) was employed to collect blood samples from the tip of the tail of each rat which was used to determine the plasma glucose on a weekly basis. Plasma glucose concentration was determined by the method of Trinder (1969). At the end of the fourth week of *nono* administration the rats were sacrificed and the organs (kidney, heart and liver) were removed and preserved for the determination of total protein, lipid and cholesterol concentrations. Total protein in the serum and tissue homogenates was determined by the method of Mao and Tucci (1991). Total lipid and cholesterol in plasma and tissue homogenates were determined enzymatically with a commercial kit (Biosystem, SA.) The standard error of means of the different values was determined.

RESULTS AND DISCUSSION

Successful establishment of diabetes raised plasma glucose concentration to over 250 mg dL^{-1} from 100 mg dL^{-1} after induction by alloxan in rats (Fig. 1). This is expected (Mitchell, 2005). Diabetic rats fed with *Nono* had their plasma glucose concentration restored to the level of the control rats in which diabetes were not induced by the fourth week of feeding with *Nono*; while the plasma glucose concentration in non-diabetic rats suffered a slight reduction when fed with *Nono* (Fig. 1). This confirms the work of Tabuchi *et al.* (2003b), which reported hypoglycemic effects in rats fed with cells of lactic acid bacteria. It is thus conceivable that the *Nono* effect may be a function of its lactic acid bacteria component which was recorded at an average of 2.04×10^{6} mL^{-1} (range = $1.16\text{-}4.0\times10^{6}$).

The diabetic rats had significantly lower tissue protein and a concomitant raised plasma protein level relative to the non diabetic rats. This again is expected (William, 1999). *Nono* by the 4th week of feeding restored the protein level to the level of non diabetic rats (Table 1).

Hyperlipidemia and hypercholesterolemia are characteristic features of diabetes (Baggio and Drucker, 2006). Lipids and cholesterol have been reported to also accumulate in the tissues of the heart, liver and kidney. Table 2 and 3 show increased concentration of lipids and cholesterol in the

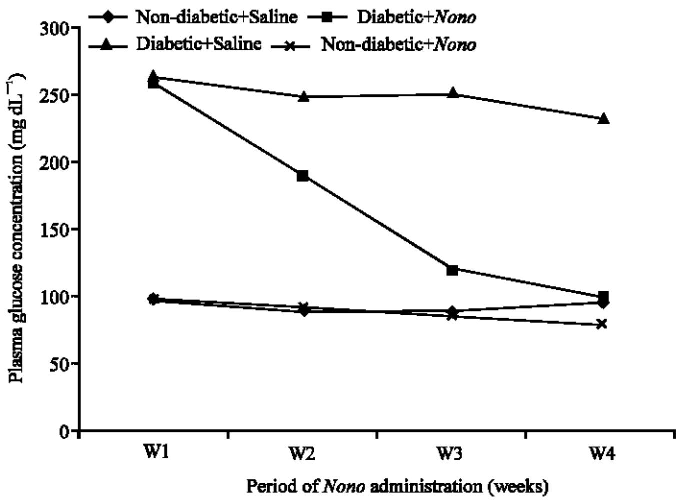

Fig. 1: Plasma glucose concentration in diabetic rats fed with *Nono*

Table 1: Total protein concentration (mg g^{-1}) in selected organs and plasma (mg dL^{-1}) in diabetic rats administered fermented milk (*Nono*) at 4 weeks of feeding

Treatments	Liver	Kidney	Heart	Plasma
Non diabetic+Saline	265±7.84^a	120±4.62^a	58±3.84^a	72±4.62^a
Diabetic+*Nono*	258±6.85^a	118±3.73^a	50±4.33^a	76±4.66^a
Diabetic+Saline	172±5.36^b	96±3.86^b	42±3.64^b	146±7.52^b
Non diabetic+*Nono*	272±7.66^a	132±7.66^a	68±3.64^a	68±4.82^a

Each value is the mean of determinations from the eight rats in each treatment group±SEM. Values with different notations are significantly different ($p \le 0.05$)

Table 2: Total lipid concentration (mg g^{-1}) in selected organs and plasma (mg dL^{-1}) of alloxan-induced diabetic rats administered fermented milk (*Nono*) at 4 weeks of feeding

Treatments	Liver	Kidney	Heart	Plasma
Non diabetic+Saline	280±5.32^a	55±4.62^a	37±3.42^a	160±6.28^a
Diabetic+nono	292±6.43^a	58±3.72^a	40±4.22^a	164±5.82^a
Diabetic+Saline	348±6.54^b	72±3.46^b	53±3.26^b	386±7.32^b
Non diabetic+*Nono*	278±6.43^a	53±3.88^a	38±4.26^a	156±4.36^a

Each value is the mean of determinations from the eight rats in each treatment group±SEM. Values with different notations are significantly different ($p \le 0.05$)

Table 3: Cholesterol concentration (mg g^{-1}) in selected organs and plasma (mg dL^{-1}) of alloxan-induced diabetic rats administered fermented milk (*Nono*) at 4 weeks of feeding

Treatments	Liver	Kidney	Heart	Plasma
Non diabetic+Saline	160±5.64^a	42±4.62^a	28±3.64^a	102±5.64^a
Diabetic+*Nono*	166±5.86^a	45±4.82^a	32±4.44^a	108±4.62^a
Diabetic+Saline	198±5.88^b	58±5.22^b	39±3.65^b	210±6.34^b
Non diabetic+*Nono*	160±4.86^a	40±5.33^a	26±4.32^a	100±5.11^a

Each value is the mean of determination from the eight rats in each treatment group±SEM. Values with different notations are significantly different ($p \le 0.05$)

organ tissues and the plasma of the diabetic animals. However, administration of *Nono* had significant hypolipidemic and hypocholesterolemic effect in both the plasma and the organs of diabetic rats by the 4th week of feeding.

The alterations in the protein, lipid and cholesterol concentrations in the organ tissues and the plasma are consequential on plasma hyperglycemic situation of diabetes. A control of the blood sugar is likely to result in normalization of the concentration of these other compounds. This apparently is what happened with the significant reduction in the plasma glucose concentration of the diabetic rats fed with *Nono*. It has been suggested that the chronic presence of LAB in the intestine through daily consumption of bacteria as in this study is needed for a long term effect on metabolism (St-Onge *et al*., 2000). Increase in the population of LAB in the intestine as a result of the administration of *Nono* may result in increased demands for glucose for normal energy requirement of these organisms to run their metabolic activities. This may lead to a decrease in the glucose concentration released into the serum and the various organs of the animals.

The hypocholesterolemic effect observed may also be as a result of the increased excretion of bile acids due to deconjugation activities of the LAB in *Nono* to produce free bile acids. Xiao *et al*. (2003) explained that serum cholesterol level decreases when a component suppresses resorption of bile acid. *Nono* is produced through natural fermentation without use of pure starter culture except fortuitous inoculation from previous fermentations (Akinyanju, 1989; Savadogo *et al*., 2004). Thus, this research indicates that wild strains of LAB in fermented milk may be capable of reversing diabetic situation. Further work is required to elucidate the mechanism of diabetic control by lactic acid bacteria containing foods.

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