

## Effect of “Zobo” on alcohol-induced changes in body weight, blood glucose and lipid levels in experimental rabbits

I. ONYESOM<sup>1\*</sup>, D. ORIERO<sup>1</sup>, A.C. OKOLO and K.E. PENDER<sup>2</sup>

<sup>1</sup>Department of Medical Biochemistry, <sup>2</sup>Department of Physiology,  
Delta State University, Abraka (Nigeria)

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### ABSTRACT

Heavy consumption of alcohol has been observed to impair the mechanisms by which the body regulates blood glucose and lipid concentrations and these biochemical disturbances cause “Metabolic Syndrome”. The rising degree of this syndrome among alcoholics constitute threat to good health and life expectancy, yet there are no adequate measures to check the growing menace. Thus, this study attempts to investigate the effect of “zobo”, an indigenous herbal beverage, on alcohol-induced changes in feed consumption, body weight, blood glucose and lipid levels in experimental albino rabbits. Twenty (20) male albino rabbits with an initial mean weight of  $1.47 \pm 0.03$  kg and about 4 months old were purchased and divided into 4 groups with 5 animals each. Group A, the control group, received 1.10g normal saline/kg body weight, while group B animals were given 1.10g (30%) ethanol/kg body weight. Group C rabbits were administered with 1.10g (30%) ethanol + 0.36g “zobo”/kg body weight, but group D animals were treated with same amounts of “zobo” and normal saline in lieu of ethanol. All administrations were via oral route and each treatment was over a routine period of 20 weeks. Results show that at the end of the 20<sup>th</sup> week, ethanol administration significantly ( $P < 0.05$ ) reduced the amount of feed consumed, body weight, blood glucose and HDL-cholesterol by 19.3%, 17.0%, 60.9%, and 23.6% respectively, but triacylglycerol (TAG) and LDL-cholesterol contents were increased ( $P < 0.05$ ) by 49.2% and 46.7%. However, ethanol + “zobo” administration reduced feed consumed, body weight, blood glucose, and HDL-cholesterol by 4.8%, 10.2%, 20.6%, and 15.7%, respectively, but increased TAG (23.7%;  $P > 0.05$ ) and LDL-cholesterol (19.2%;  $P > 0.05$ ). Our study suggests that heavy consumption of alcohol could reduce appetite and complicate the metabolism of nutrients and weight maintenance, but “zobo”, seems to possess the potential of alleviating the biochemical features of ‘Metabolic Syndrome’ associated with chronic and excessive consumption of ethanol. “Zobo” should be further studied for possible therapeutic benefits in the management of alcohol-induced disorders.

**Key words:** “Zobo”, Alcohol, Glucose, HDL-cholesterol, LDL-cholesterol, Metabolic Syndrome.

### INTRODUCTION

Alcohol is willfully consumed for its mood-altering effects, and therefore plays the role of a psychoactive drug. It is also rich in energy, and in many societies alcoholic beverages are considered as part of basic food supply. Some alcoholics ingest as much as 50% of their total daily calories from alcohol, thereby neglecting important foods (Feinman and Lieber, 1992).

Heavy consumption of alcohol has been reported to elicit cardiovascular (Onyesom, *et al.*, 2006) and nutritional (Onyesom and Naiho, 2006) complications. Alcohol interferes with nutritional process by affecting digestion, metabolism of nutrients, and excretion of waste (Lieber, 1988). Excessive consumption of alcohol can impair the mechanisms by which the body controls blood glucose (Patel, 1989) and lipid (Lieber and Savolainen, 1984) levels, and these culminate in disease conditions.

Alcohol-induced diseases, though diseases of choice, have continue to grow in our society without any serious efforts to curb them. This growing disease trend obviously constitutes threat to good health and the strength of labour force. The use of traditional medicine and medicinal plants in most developing countries for the maintenance of good health has been widely observed (El nagggar, *et al.*, 2005; Usoh, *et al.*, 2005). Thus, research efforts are currently focusing on the protective biochemical complement of naturally occurring plant phytochemicals in promoting good health and alleviating disease conditions.

“Zobo”, the hot-water extract of the red flower of *Hibiscus sabdariffa* L, is commonly consumed as an indigenous beverage in Nigeria (Fasoyiro, 2005). It has been reported to serve as a herbal remedy for various disease conditions (Adegunloye, *et al.*, 1996; Hertong and Heskens, 1993), yet its influence on the biochemical features of “Metabolic Syndrome” – hyperglycaemia, insulin resistance, glucose intolerance, hyperlipidaemia, obesity, and secondary cardiovascular dysfunctions, induced by alcohol, has remained scare in literature.

Therefore, this research aims at studying the effect of “zobo” on alcohol-induced changes in feed consumption, body weight, blood glucose and lipid levels in experimental rabbits.

## MATERIALS AND METHODS

### Preparation of “Zobo” drink

One hundred grams (100g) of dried *Hibiscus sabdariffa* red petals was added to 500 ml of distilled water and boiled for 15min. The mixture was then filtered after allowing to cool and the filtrate obtained was decanted into sealed clean bottle and preserved at 4°C until required (Obi, *et al.*, 2005).

### Experimental animals

Twenty (20) male albino rabbits having initial mean weight of  $1.47 \pm 0.03$ kg (1.44 – 1.50 kg) and about 4 months old were purchased from a local farmer in Warri, Delta State, Nigeria. Animal care and handling complied with the guidelines of the National Institutes of Health (NRC, 1985).

The animals were housed singly in clean metal hutches and acclimatized on growers’ mash for 10 days. Thereafter, the rabbits were randomly separated into 4 experimental groups with 5 rabbits per group. Group A, the control group, orally received 1.10g normal saline / kg body weight, while group B animals were given 1.10g (30%) ethanol / kg body weight, orally. Group C rabbits were administered with 0.36g “zobo” / kg body weight after about 20 min of ingesting 1.10 (30%) ethanol / kg body weight, but group D were treated with same amount of “zobo” and normal saline in lieu of ethanol. Rabbits in the 4 different experimental groups, (A-D), were feed with constant amounts of growers’ mash and the various daily administrations (A = normal saline, B = ethanol, C = ethanol + “zobo”, and D = normal saline + “zobo”) for a period of 20 weeks. Clean drinking water was provided with no restricted access to the animals in each group.

Before feeding, the feeds were mixed with water in the ratio of 10:1 (v/v), so as to achieve a texture acceptable to the animals. The feed, growers’ mash, was composed and supplied by Bendel Feeds and Flour Mill(BFFM) Ltd., Ewu, Edo State, Nigeria. The choice of the large single daily dose of ethanol and the ratio of “zobo” to ethanol (1:3) were based on previous studies (Mordi, 2006). Chronic large doses of ethanol have been documented to significantly induce metabolic disorders when compared with either small or moderate dose (Onyesom and Oriero, 2005), hence the choice to study large dose.

### Measurement of feed consumption

Each animal in each group was presented with 50 g feed twice daily-morning (8.00am) and evening (6.00pm). The daily amount of feed consumed by each animal was obtained by subtracting the weight of feed remnant from the total weight of feed presented to each animal daily (Okolie, *et al.* 1994; Okolie and Osagie, 1999). Stale feed remnants were regularly discarded and the amount of feed consumed by members of each group was recorded daily. The daily records were then pooled in order to determine the mean weekly feed consumed. The weights of the rabbits were determined every 5 week for 20 weeks.

### Collection of blood samples

Fasting whole blood samples were collected from the rabbit's vein in the right ear under anaesthesia (50 mg pentobarbital/kg weight) in the morning after an overnight fast at 0 (before treatment commenced) and after the 5<sup>th</sup>, 10<sup>th</sup>, 15<sup>th</sup> and 20<sup>th</sup> week of treatment into sodium fluoride bottles. About 3.0ml of plasma was obtained from each whole blood sample after centrifuging at 1200 x g for 5 min at room temperature. The plasma was then decanted into bijoux bottles, stored frozen and analyzed within 48hr.

### Analysis of plasma samples

Plasma glucose concentration was determined by the glucose oxidase method (Barham and Trinder, 1972). Plasma triacylglycerol (TAG) was quantified by the enzymatic endpoint colorimetric method (Searcy, 1969). Plasma total cholesterol level (Allain, *et al.*, 1974) and HDLcholesterol fraction (Burstin and Mortin, 1969) were measured by the enzymatic colorimetric method, but the cholesterol content in LDL was mathematically estimated (Friedwald, *et al.*, 1972).

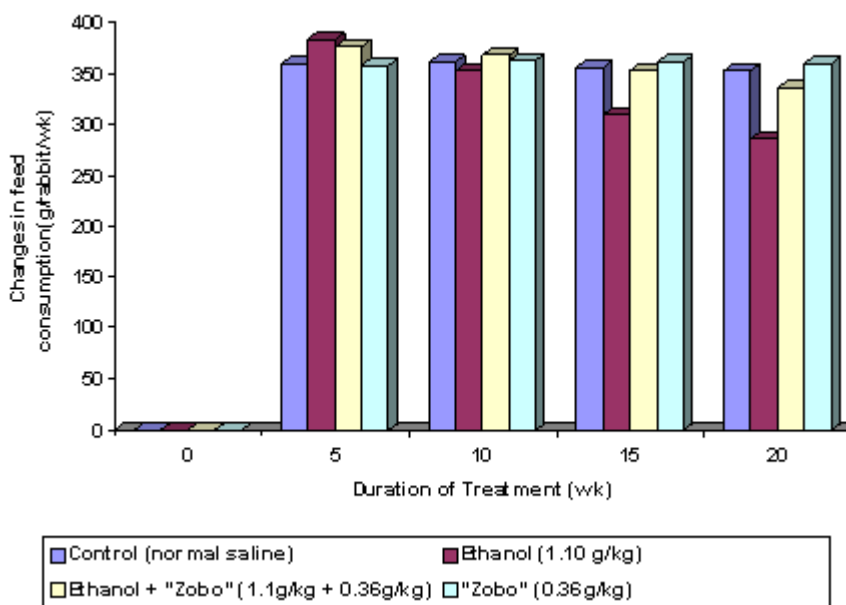
### Statistics

Statistical significance among groups were assessed using analysis of variance (ANOVA), while the Student's *t*-test, assuming unequal variances, was used to assess the significance of the results between groups.

## RESULTS

The results obtained from the investigation into the influence of "zobo" drink on ethanol-induced changes in feed consumption, body weight, blood glucose, triacylglycerol (TAG), HDL – cholesterol, and LDL-cholesterol levels in albino rabbits are respectively shown on Fig. 1-6.

Fig. 1, the changes in feed consumed, shows that at the end of the 20<sup>th</sup> week, ethanol (1.10g/kg) administration significantly ( $P < 0.05$ ) reduced the amount of feed consumed by 19.3% when compared with the control –matched value. Changes in feed consumption induced by either ethanol + "zobo" (4.8% decrease) or "zobo" alone (2.0% increase) were however, not significantly different ( $P > 0.05$ ).



**Fig. 1: Changes in feed consumption induced by ethanol and ethanol + "zobo" co-administration in rabbits**

Changes in body weight (Fig. 2), had similar trend to the changes in feed consumption. The basal (0 week) body weight for the control animals reduced by 4.1% ( $P>0.05$ ) at the end of the 20<sup>th</sup> week exposure period, but the percentage decreases for the groups given ethanol alone, and ethanol + "zobo"

were 17.0% ( $P<0.05$ ) and 10.2% ( $P>0.05$ ) respectively, while those given "zobo", only reduced by 1.4% ( $P>0.05$ ).

Fig. 3 shows that the administered dose of ethanol (1.10g/kg) significantly reduced ( $P<0.05$ ) blood

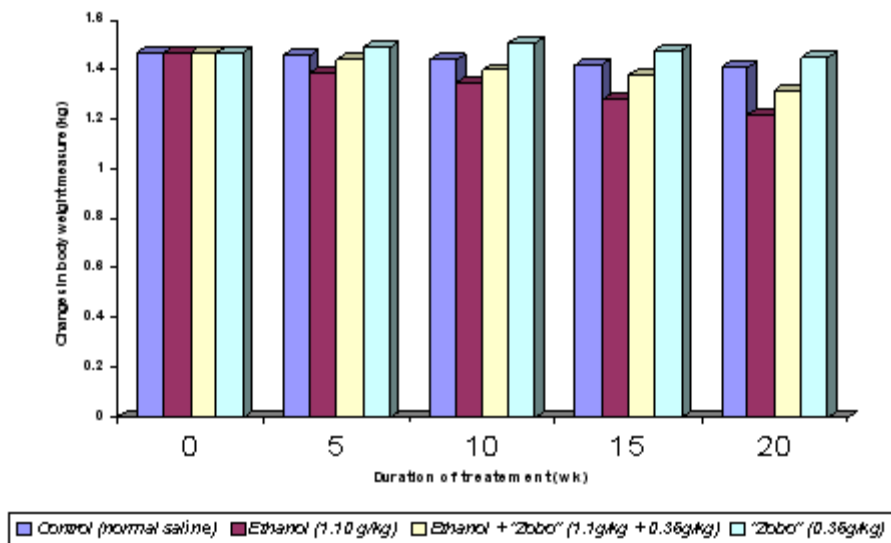


Fig. 2: Changes in body weight induced by ethanol and ethanol+ "zobo" co-administration in rabbits

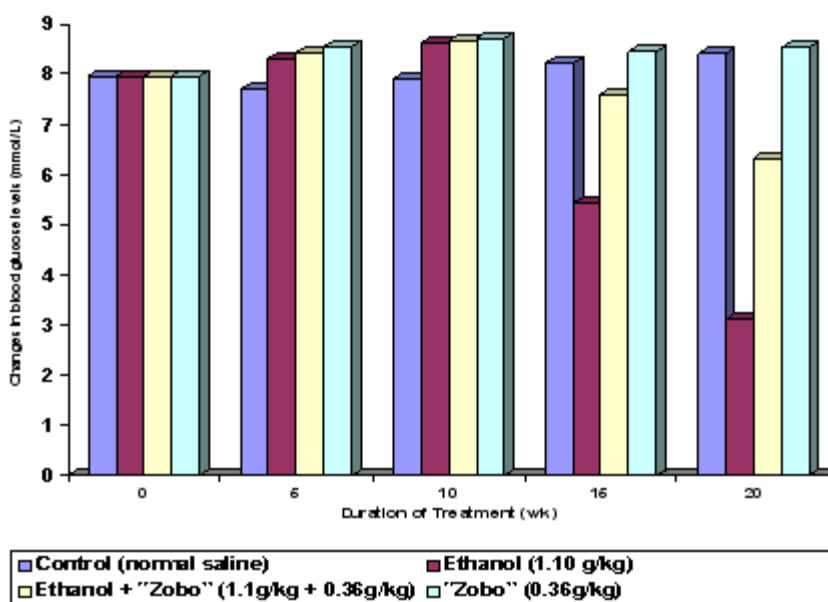
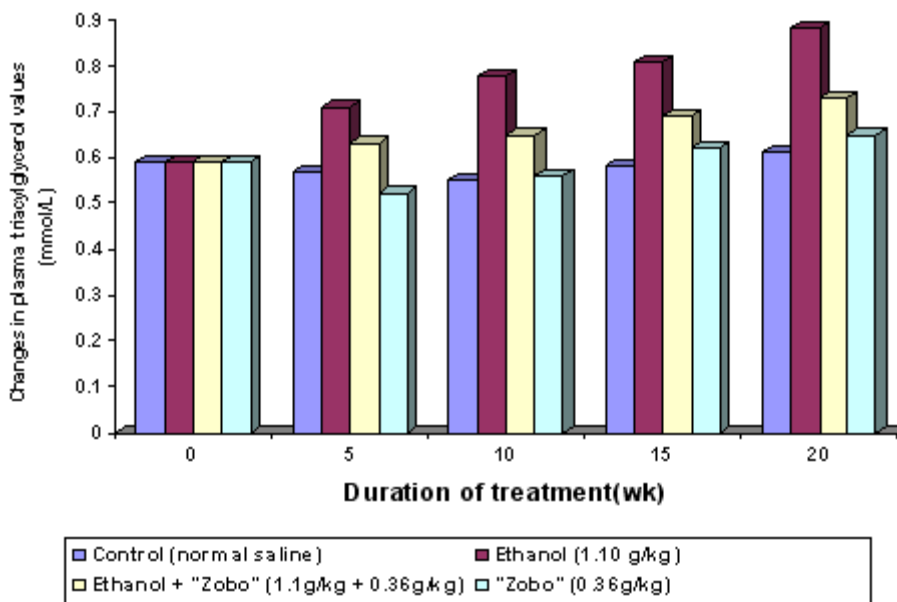
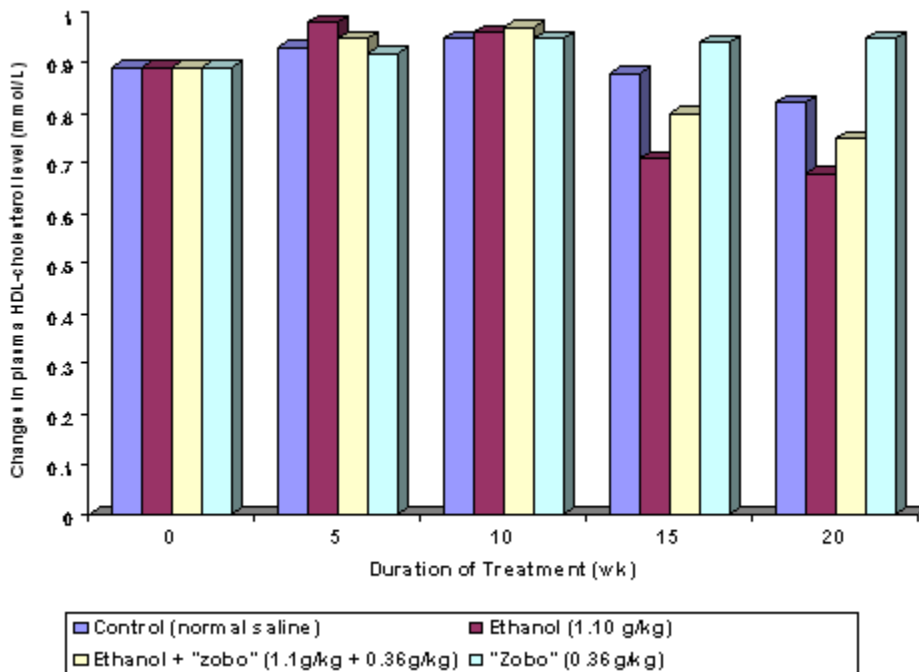


Fig. 3: Changes in blood glucose induced by ethanol and ethanol + "zobo" co-administration in rabbits



**Fig. 4: Changes in plasma triacylglycerol induced by ethanol and ethanol + "zobo" co- administration in rabbits**



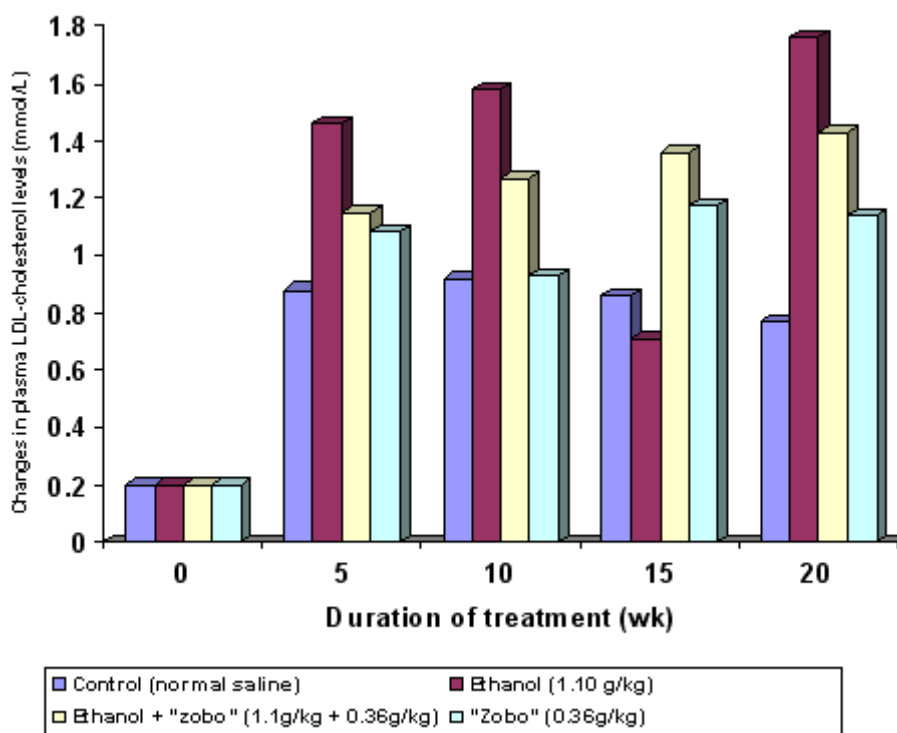
**Fig. 5: Changes in plasma HDL-cholesterol induced by ethanol and ethanol + "zobo" co-administration in rabbits**

glucose by 60.9% at the end of the 20-week administration time when compared with the basal (0 week) value. The 20<sup>th</sup> week mean blood glucose value for the ethanol + "zobo" (1.10g/kg +0.36g/kg) was 20.6% ( $P>0.05$ ) lower than the basal (0 week) level.

Changes in blood TAG and lipoprotein (HDL and LDL) cholesterol values (Fig. 4-6) show that ethanol progressively increases blood TAG and LDL-cholesterol, but reduces HDL-cholesterol from

the 15<sup>th</sup> week of exposing the experimental animals to ethanol consumption.

Our results suggest that chronic ethanol consumption by albino rabbits at a dose regarded large (1.10 g/kg), could reduce appetite and complicate the metabolism of nutrients and weight maintenance. Albeit, "zobo" appears to possess the potential of alleviating the biochemical complications associated with chronic and excessive consumption of ethanol.



**Fig. 6: Changes in plasma LDL-cholesterol induced by ethanol and ethanol + "zobo" co-administration in rabbits**

## DISCUSSION

A significant inverse relation between alcohol intake and body mass index has been reported (Colditz, *et al.*, 1991). The data on body weight changes obtained from this study (Fig. 2) appear to support this earlier observation. In addition, ethanol consumption seems to disturb nutrient (feed) intake and bioavailability which in turn

affect the metabolism of blood glucose and lipids. The changes in blood glucose induced by ethanol correlates positively with the changes in the rabbits' feed consumption rate (Figs 1 and 2). When there is no enough food to supply energy, stored sugar is depleted, and the products of alcohol metabolism inhibit the formation of glucose from other substances especially amino acids (Patel, 1989). It therefore, appears that alcohol – induced

reduction in feed intake could produce secondary hypoglycaemia, and this could be complicated by inappropriate insulin response, increased conversion of glucose to  $\mu$ -glycerophosphate and hypothalamic-pituitary-adrenal insufficiency, previously reported to be caused by chronic excessive alcohol consumption (Bunout, 1999). Consequently, lipid, which spare carbohydrate could then be mobilized to meet energy demand and this may cause complications in lipid metabolism. Alcohol metabolism has been reported to increase plasma TAG (Hodge, *et al.*, 1993; Onyesom, 2005), and this present study appears to support these previous reports. The NADH + H<sup>+</sup> generated during the oxidation of alcohol stimulates fatty acid synthesis which induces TAG synthesis due to impairment in the carnitine acyltransferase transport mechanism, though, increased hepatic

synthesis of prebetalipoprotein, changes in ATP availability, increased hepatic secretion of VLDL, and reduced clearance of chylomicron remnants have been observed (Contaldo, *et al.*, 1989).

Alcohol-induced reduction in feed consumption rate could introduce nutritional deficits which may complicate nutritional and metabolic states, and disturb body function and maintenance. However, "zobo", whose phytochemical contents have been shown to ameliorate free radical activities and associated lipid peroxidation and cell damage (Obi, *et al.*, 2005) also possesses the potential of alleviating the biochemical features of Metabolic Syndrome induced by alcohol, our study suggests. "Zobo" should be further investigated for medicinal benefits in managing alcohol related ills.

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