International Journal of Biomedical and Health Sciences
Vol. 7, No. 3, September 30, 2011
Printed in Nigeria

0794-4748/2011 $5.00 + 0.00

http://www.asopah.org

IJBHS/2011015

Lactate dehydrogenase and glucose-6 phosphate dehydrogenase activities in the testes of adult male Wistar rats following the administration of ethanol

O B Alabi[1], T O Oyesomi [1*], O R Jimoh[1], M S Ajao[2], E ACaxton-Martins[1]

[1]Anatomy Department, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, P.M.B 1515 Ilorin, Nigeria
[2]School of Anatomical Sciences, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

(Received January 26, 2011; Accepted April 5, 2011)

ABSTRACT: The present study was designed to evaluate the effects of the administration of ethanol on glucose-6-phosphate dehydrogenase (G-6PDH) and lactate dehydrogenase (LDH) levels in the testes of adult male wistar rats. Sixteen (16) adult male wistar rats weighing between 180 – 200g were divided into three groups A, and B of eight animals each. In Group B each animal were given 1ml of 15% ethanol daily while control group (Group A) were given the same ml (1ml) of phosphate buffered saline orally for a period of 21 days, at the end of which the animals were sacrificed and their testis assayed spectrophotometrically for the activities of G-6PDH and LDH. There was a significant ($p<0.05$) increase in G-6-PDH and LDH enzyme activities in the administered groups. The results indicate that administration of ethanol which is widely consumed alters carbohydrate metabolism in the testicular tissue.

Keywords: Ethanol, testis, lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G-6PDH).

Introduction

Enzymes are the biocatalyst that regulates the rates at which all physiological processes take place [1]. NADPH is the principal intracellular reductant and its production is mainly dependent on glucose-6-phosphate dehydrogenase, hence inhibition of G6PDH activity decrease NADPH, a coenzyme that is essential for the protection against and repair of oxidative damage and also plays a very vital role in maintaining the proper 3-dimensional structure of proteins in the cell membrane. As the first and rate limiting enzyme in the pentose phosphate pathway, the role of G6PDH is important to the architecture of the cell [2]. The integrity of the cells as well as the entire antioxidant system and other processes requiring reduction rely on the adequate supply of NADPH. Alterations in G6PDH will therefore alter for the supply of energy to the cells [2].

*Author to whom all correspondence should be addressed.
E-mail; drstoyesomi@yahoo.com
Phone (Mobile); +234703298677, +2348051917326.

NADPH and oxygen are very important in the three enzyme catalyzed steps required in the synthesis of estrogen in the ovary from precursors such as androgens: testosterone and androstendione [3]. The present study was therefore designed to evaluate the effects of aqueous extracts of cashew stem bark on glucose-6-phosphate dehydrogenase (G-6PDH) and lactate dehydrogenase (LDH) levels in the testes of adult male wistar rats.

Materials and Methods

Experimental animals

Sixteen male adult wistar rats weighing between 180 – 200g were bred in the animal house of the Faculty of Basic Medical Sciences, University of Ilorin. The rats were housed and maintained under standard conditions, food and water was given *ad libitum*. All animals were handled in conformity with the rules and guidelines of the animal rights committee of the University of Ilorin. The study protocol was approved by the same committee. They were evaluated and judged presumably healthy, fit enough to use for the study. LDH and G-6PDH kits were bought from Randox lab. Ltd.UK. The study was conducted between May and June 2010. The animals were randomly divided into three groups of eight animals each (Groups A, and B) using the alternate selection method. Body weights of the animals were obtained using a weighing scale. Group B received 1ml of 15% ethanol while Group A served as the control group received 1ml phosphate buffered saline for 21 days. The animals were sacrificed by cervical dislocation and the testes were immediately weighed, immediately transferred to a 0.25 M sucrose solution, homogenized, and centrifuged at 5000 rpm for 10 min. The supernatants were immediately stored in the freezer (-20°C) and assayed within 48 h. LDH and G-6PDH activities were estimated [4, 5]. The enzyme activity was read spectrophotometrically.

Statistical analysis

Values were reported as mean ± S.E.M and data were analyzed using students t-test with the statistical software SPSS version 14 at 95% confidence interval. A $p<0.05$ was considered statistically significant. $p < 0.05$ for G-6PDH and LDH.

Results and Discussion

Table 1 presents the enzyme activity in the testis of rats treated with 1ml of 15% ethanol compared with untreated rats. The activity of glucose-6-phosphate dehydrogenase was significantly increased in the treated groups compared with the control group at ($p < 0.05$) level of significance (Figure 1). Lactate dehydrogenase levels increased significantly at $p < 0.05$ level of significance (Figure 2).

Table 1; LDH and G-6PDH activities in the control and experimental groups

Enzyme	Group A	Group C
LDH	36.37±1.23	47.67±1.96*
G-6PDH	72.32±1.00	81.22±0.67*

Values are mean±SEM.
*Represents level of significance (LDH $p < 0.05$) (G-6PDH $p < 0.05$), n=8.

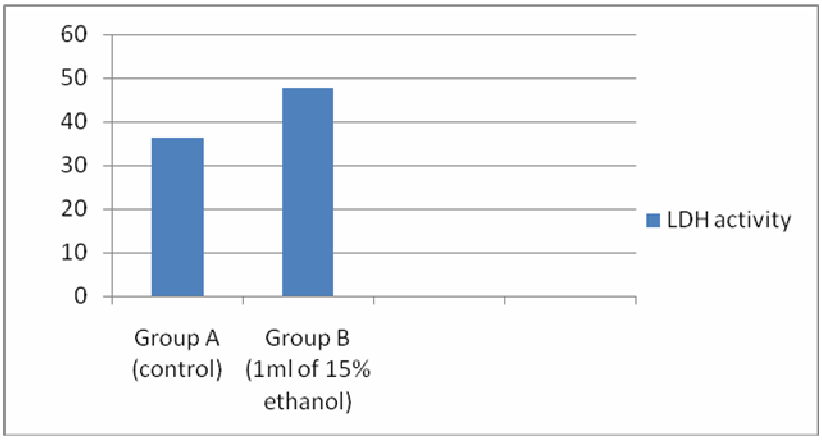

Figure 1. Activities of lactate dehydrogenase (LDH) in the testis of male wistar rats following the administration of ethanol (µmol/mim/mg)

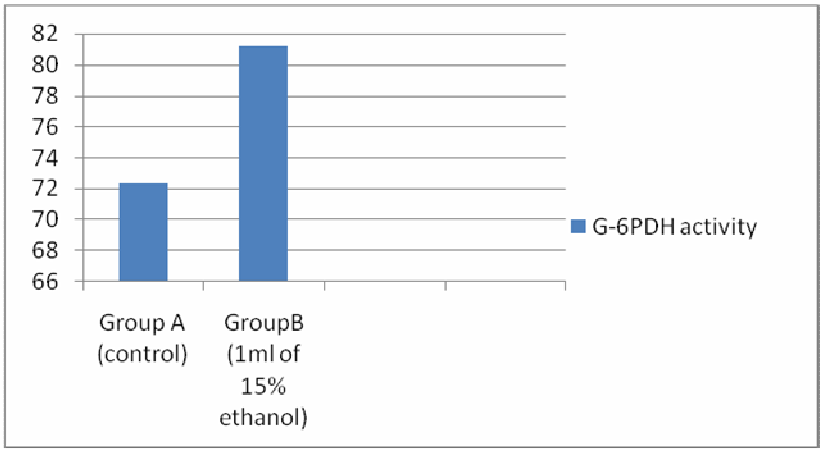

Figure 2. Activities of glucose-6-phosphate dehydrogenase (G-6PDH) in the testis of male wistar rats following the administration of ethanol (µmol/mim/mg)

Results obtained in the present study showed that the administration of 1ml of 15% ethanol increased the activity of G6PDH significantly. The pentose phosphate pathway (phosphogluconate pathway or hexose monophosphate shunt [HMP shunt]) is a cytosolic process that serves to generate NADPH and the synthesis of pentose (5-carbon) sugars. There are two distinct phases in the pathway. The first is the oxidative phase, in which NADPH is generated, and the second is the nonoxidative synthesis of 5-carbon sugars. This pathway is an alternative to glycolysis. The primary functions of the pathway are to generate reducing equivalents, in the form of NADPH, for reductive biosynthesis reactions within cells, and to provide the cell with ribose-5-phosphate (R5P) for the synthesis of the nucleotides and nucleic acids [6]. Although not a significant function of the pentose phosphate pathway (PPP), the pathway can operate to metabolize dietary pentose sugars derived from the digestion of nucleic acids as well as to rearrange the carbon skeletons of dietary carbohydrates into glycolytic-gluconeogenic intermediates located exclusively in the cytoplasm. The pathway is one of the three main ways with which the body creates molecules with reducing power, accounting for approximately 60% of NADPH production in man [2].

One of the uses of NADPH in the cell is to prevent oxidative stress. It reduces the coenzyme glutathione, which converts reactive H_2O_2 into H_2O, when absent, the H_2O_2 would be converted to hydroxyl free radicals, which can attack the cell. In the oxidative phase, two molecules of NADP+ are reduced to NADPH, utilizing the energy from

the conversion of glucose-6-phosphate into ribulose 5-phosphate. The overall reaction for this process is: Glucose 6-phosphate + 2NADP+ + H_2O ----------→ ribulose 5-phosphate + 2 NADPH + 2H+ + CO2. Glucose- 6-phosphate dehydrogenase is the rate-controlling enzyme of this pathway [7]. It is allosterically stimulated by NADP+. The ratio of NADPH:NADP+ is normally about 100:1 in liver cytosol. This makes the cytosol a highly-reducing environment. Formation of NADP+ by a NADPH-utilizing pathway, thus, stimulates production of more NADPH.

G-6PDH is a cytoplasmic enzyme that affects the production of reduced form of cytosolic coenzyme (NADPH) by controlling the step from glucose-6-phosphate to 6- phosphogluconate in the pentose phosphate pathway [6, 8]. This enzyme is highly conserved during evolution and plays multiple roles in the cell. Until recently, the role of this housekeeping enzyme in the cell response to the oxidative stress was limited to human erythrocytes that lack any other NADPH producing route [7]. However, recent observations have shown that the G6PDH also plays a protective role against reactive oxygen species in eukaryotic cells that possess alternative routes for the production of NADPH and that G6PDH expression is upregulated by oxidants through a mechanism acting mainly on the rate of transcription of this gene [9]. Lactate dehydrogenase levels increased significantly ($p < 0.05$) in the group administered with 1ml of 15% ethanol compared to the control group. Lactate dehydrogenase is an enzyme that catalyzes the inter-conversion of pyruvate and lactate with concomitant inter-conversion of NADH and NAD^+ [1] . At high concentration of lactate, the enzyme exhibit feedback inhibition and the rate of conversion of pyruvate to lactate is decreased.

This is an important step in energy production in cells [10]. Many different types of cells in the body contain LDH and some of the organs relatively rich in this enzyme are the heart, kidney, liver, and muscle [1]. When cells die, their LDH is released and finds its way into the blood [1]. Normal LDH levels vary with age, being higher in childhood due to bone growth [10]. Analysis of LDH has not been standardized and normal ranges vary greatly between laboratories [1].

In medicine, LDH is often used as a marker of tissue breakdown as LDH is abundant in red blood cells and can function as a marker for hemolysis. A blood sample that has been handled incorrectly can show falsepositively high levels of LDH due to erythrocyte damage [10]. Tissue breakdown elevates levels of LDH, and therefore a measure of it indicates e.g. hemolysis [10]. A high rate of destroyed cells indicates an elevated LDH activity and since there was significant alteration in LDH activity, it is inferred that ethanol caused testicular tissue breakdown.

Based on the observations from this study, it can be concluded that the administration of ethanol alters carbohydrate metabolism. Furthermore, the extract altered the activity of G6PDH and LDH in the testis which may account for some of the earlier reports that had been given on the effect of ethanol on the architecture of the cells of the testis.

References

1. **Rodwell V.** Enzymes; General Properties. Harpers Biochemistry (24th Edition), Prentice-Hall Inc. 1993; p. 64.
2. **Zhang Z, Apse K, Pang J and Stanton RC.**. High glucose inhibits glucose-6-phosphate via cAMP in aortic endothelial cells. J. Med.Genet. 2000.16: 431-434.
3. **Siiteri PK, Ferbres F.** Ovarian hormones: Synthesis, Circulation and Mechanisms of action. In Endocrinology, 1979; Vol 3. DeGroot LJ (editor).
4. **Kind PRN, King EJ**. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J. Clin. Pathol. 1954; 7: 322.
5. **King J, Jagatheeson KA**. A method for the determination of tartrate-labile, prostatic acid phosphatase in serum. J. Clin. Path. 1959; 12:85.
6. **Beutler E, Vulliamy T, Luzzatto L**: Hematologically important mutations: glucose-6-phosphate dehydrogenase. Blood Cells Mol. Dis. 1996; 22(1): 49-76.
7. **Yizhen XU, Osborrne BW, Stanton RC**. Diabetes causes inhibition of glucose-6-phosphate dehydrogenase via activation of PKA, which contributes to oxidative stress rat kidney cortex. Am. J. Physiolol. Renal Physiol. 2005; 289: F1040-F1047.
8. **Kletzien RF, Harris PK, Foellmi LA**. Glucose-6-phosphate dehydrogenase: a "housekeeping" enzyme subject to tissue-specificmregulation by hormones, nutrients and oxidant stress. FASEB J. 8(2) 1994:174-181.
9. **Salvemini F, Franze M, Iervolino A, Filosa S, Salzano S, Ursini MV**. Enhanced glutathione levels of oxido-resistance mediated by increased glucose-6-phosphate dehydrogenase expression. J. Biol.Chem. 1999; 274(5): 2750-2757.
10. **Butt AA, Michaels S, Greer D, Clark R, Kissinger P, Martin DH**. Serum LDH level as a clue to the diagnosis of histoplasmosis. AIDS Read 2002;12(7): 317–21. PMID 12161854.