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*Full Length Research Paper*

# LDH and G-6PDH activities in the ovaries of adult female Wistar rats following the administration of aqueous extracts of neem (*Azadirachta indica*) leaves

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**The present study was designed to evaluate the effects of aqueous extracts of neem (*Azadirachta Indica)* leaves (which have been documented for its antifertility effect on experimental animals) on glucose-6-phosphate dehydrogenase (G-6PDH) and lactate dehydrogenase (LDH) levels in the ovaries of adult female wistar rats. Twenty four adult female wistar rats weighing 200 ± 10 g were divided into three groups A, B and C of eight animals each. Groups A and B were given 3 and 6 mg/kg body weight of extract respectively and the control group was given water orally for 21 days, at the end of which the animals were sacrificed and their ovaries assayed spectrophotometrically for the activities of G-6PDH and LDH. There was significant (p = 0.046) decrease in G-6-PDH and significant (p = 0.047) increase in LDH enzyme activities in the administered groups. The results indicate that extracts of neem which is widely consumed for a variety of ailments alters carbohydrate metabolism in the ovarian tissue.**

**Key words:** Neem, ovary, lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G6PDH).

## INTRODUCTION

Studies on the effects of plant extracts on fertility are comparatively few. From a public health perspective, the need for contraception has never been greater (Moore et al., 1996). Enzymes are the biocatalyst that regulates the rates at which all physiological processes take place (Rodwell, 1993). 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 (Zhang et al., 2000). The integrity of the cells as well as the entire anti-oxidant 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 (Zhang et al., 2000).

An alteration in G6PDH activity and therefore a change in NADPH integrity subject cells regulating ovarian activity to oxidative damage, which may account for some of the earlier reports that had been given on the antifertility effects of neem. NADPH and oxygen are very important in the 3 enzyme catalyzed steps required in the synthesis of estrogen in the ovary from precursors such as androgens: testosterone and androstendione (Siiteri and Ferbres, 1979). The present study was therefore designed to evaluate the effects of aqueous extracts of neem leaves on glucose-6-phosphate dehydrogenase (G-6PDH) and lactate dehydrogenase (LDH) levels in the ovaries of adult female wistar rats.

## MATERIALS AND METHODS

### Plant material and extract preparation

The leaves of *Azadirachta Indica* were collected within the premises

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

| Enzyme | Group A | Group B | Group C |
|---|---|---|---|
| LDH | 344.00±16.24 | 626.58±25.46* | 401.00±70.12* |
| G-6PDH | 335.28±17.83 | 100.00±1.91* | 185.86±5.46* |

Values are mean±SEM.
*Represents level of significance (LDH p = 0.047) (G-6PDH p = 0.046), n=8.

of the College of Medicine, University of Ilorin, Kwara State, Nigeria. Identification of the plant was carried out in the Department of Botany, University of Ilorin. A large quantity of leaves were collected, washed clean (with water), air-dried and ground to powder giving an approximate weight of 500 g, the powder was mixed with 4 L of distilled water and the mixture was left for 48 h, the mixture was filtered and the filtrate was concentrated in a water bath (maintained at 90 ± 0.2°C) to yield 10 g of a green solid extract. The solid extract was stored in a refrigerator for use.

#### Experimental animals

Twenty four female albino rats weighing (200 ± 10 g) 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 January and February 2008. The animals were randomly divided into three groups of eight animals each (Groups A, B and C) using the alternate selection method. Body weights of the animals were obtained using a weighing scale. Group A received 3 mg/kg bodyweight of the extract orally; Group B received 6 mg/kg bodyweight of the extract while Group C served as the control group and received water for 21 days. The animals were sacrificed by cervical dislocation and the ovaries were immediately blotted dry, weighed, immediately transferred to a 0.25 M sucrose solution, homogenized, 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 by the methods of Kind and King (1954) and King and Jagatheeson (1959) respectively. 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 13 at 95% confidence interval. A $p<0.05$ was considered statistically significant. p = 0.046 for G-6PDH while p = 0.047 for LDH.

## RESULTS AND DISCUSSION

Table 1 presents the enzyme activity in the ovaries of rats treated with *A. indica* leaf extract compared with untreated rats. The activity of glucose-6-phosphate dehydrogenase was significantly reduced in the treated groups compared with the control group at (p = 0.046) level of significance (Figure 1). Lactate dehydrogenase levels increased significantly at p = 0.047 level of significance (Figure 2).

Results obtained in the present study showed that the aqueous leaf extract of *A. indica* reduced the activity of G6PDH significantly at (p = 0.046) 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 non-oxidative synthesis of 5-carbon sugars. This pathway is an alternative to glycolysis. The primary functions of the pathway are according to (Beutler et al., 1996) 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. 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, (Zhang et al., 2000).

One of the uses of NADPH in the cell is to prevent oxidative stress. It reduces the coenzyme glutathione, which converts reactive H2O2 into H2O, when absent, the H2O2 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 + $2NADP^+$ + H2O → ribulose 5-phosphate + 2 NADPH + $2H^+$ + CO2. Glucose-6-phosphate dehydrogenase is the rate-controlling enzyme of this pathway (Yizhen et al., 2005). 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 pro-

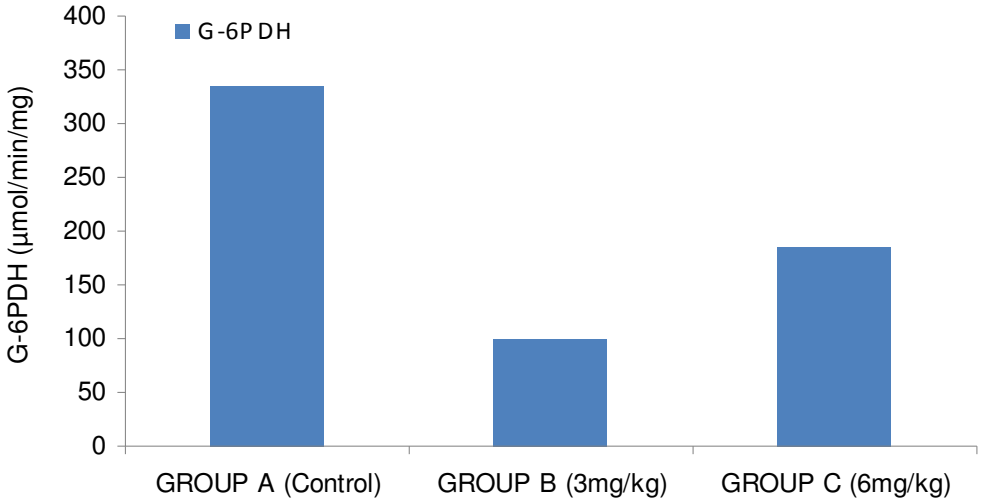


**Figure 1.** Activities of glucose-6-phosphate dehydrogenase (G-6PDH) in the ovaries of female wistar rats following the administration of aqueous extracts of neem (*Azadirachta indica*) leaves.

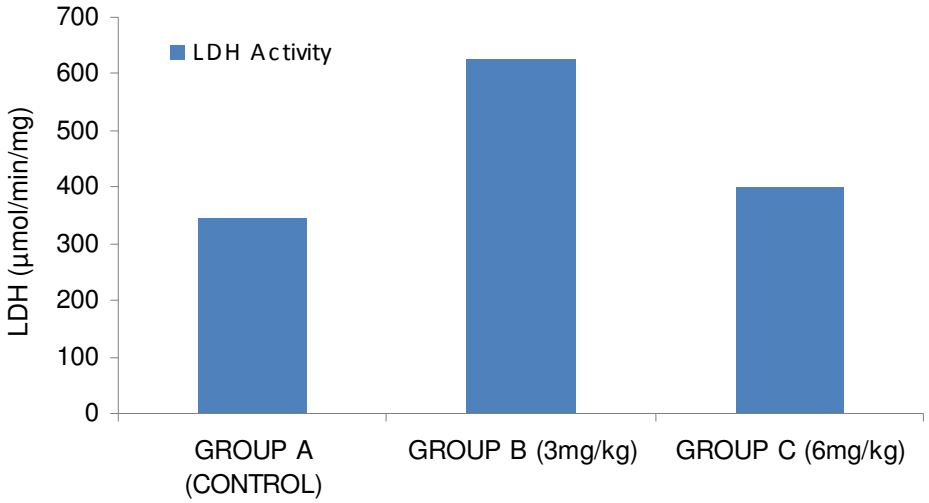


**Figure 2.** Activities of lactate dehydrogenase (LDH) in the ovaries of female wistar rats following the administration of aqueous extracts of neem (*Azadirachta indica*) leaves.

duction of reduced form of cytosolic coenzyme (NADPH) by controlling the step from glucose-6-phosphate to 6-phosphogluconate in the pentose phosphate pathway (Beutler et al., 1996; Kletzien et al., 1994). 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 (Yizhen et al., 2005). 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 (Salvemini et al., 1999)

Lactate dehydrogenase levels increased significantly ($p = 0.047$) in the groups administered with neem 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 $NAD^+$, (Rodwell, 1993). 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 (Butt et al., 2002). 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

(Rodwell, 1993). When cells die, their LDH is released and finds its way into the blood (Rodwell, 1993). Normal LDH levels vary with age, being higher in childhood due to bone growth (Butt et al., 2002). Analysis of LDH has not been standardized and normal ranges vary greatly between laboratories (Rodwell, 1993).

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 false-positively high levels of LDH due to erythrocyte damage (Butt et al., 2002). Tissue breakdown elevates levels of LDH, and therefore a measure of it indicates e.g. hemolysis (Butt et al., 2002). A high rate of destroyed cells indicates an elevated LDH activity and since there was significant alteration in LDH activity, it is inferred that the extract caused ovarian tissue breakdown.

Based on the observations from this study, it can be concluded that the aqueous leaf extract of neem (*A. indica*) alters carbohydrate metabolism. Furthermore, the extract altered the activity of G6PDH and LDH in the ovary which may account for some of the earlier reports that had been given on the effect of this extract on the architecture of the cells of the ovary.

**REFERENCES**

Beutler E, Vulliamy T, Luzzatto L (1996). Hematologically important mutations: glucose-6-phosphate dehydrogenase. Blood Cells Mol. Dis. 22(1): 49-76.

Butt AA, Michaels S, Greer D, Clark R, Kissinger P, Martin DH (2002). Serum LDH level as a clue to the diagnosis of histoplasmosis. AIDS Read 12(7): 317–21. PMID 12161854.

Kind PRN, King EJ (1954). Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J. Clin. Pathol. 7: 322.

King J, Jagatheeson KA (1959). A method for the determination of tartrate-labile, prostatic acid phosphatase in serum. J. Clin. Path. 12: 85.

Kletzien RF, Harris PK, Foellmi LA (1994). Glucose-6-phosphate dehydrogenase: a “housekeeping” enzyme subject to tissue-specific regulation by hormones, nutrients and oxidant stress. FASEB J. 8(2): 174-181.

Moore PS Adler NE, Kegdes SM (1996). Adolescents and the contraceptive pill; the impact of beliefs on intentions and use Obstet Gynecol 88(Suppl): 485-565.

Rodwell V (1993). Enzymes; General Properties. Harpers Biochemistry (24th Edition), Prentice-Hall Inc. p. 64.

Salvemini F, Franze M, Iervolino A, Filosa S, Salzano S, Ursini MV (1999). Enhanced glutathione levels of oxido-resistance mediated by increased glucose-6-phosphate dehydrogenase expression. J. Biol. Chem. 274(5): 2750-2757.

Siiteri PK, Ferbres F (1979). Ovarian hormones : Synthesis, Circulation and Mechanisms of action. In Endocrinology, Vol 3. DeGroot LJ (editor).

Yizhen XU, Osborrne BW, Stanton RC (2005). 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. 289: F1040-F1047.

Zhang Z, Apse K, Pang J and Stanton RC. (2000). High glucose inhibits glucose-6-phosphate via cAMP in aortic endothelial cells. J. Med. Genet. 16: 431-434.