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Evaluation of the Activities of G6PDH and LDH in the Liver of Adult Wistar Rats following the administration of *Ocimum sanctum*

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ABSTRACT: The aim of this study was to investigate some of the effects of aqueous extract of *Ocimum sanctum* on glucose-6-phosphate dehydrogenase and lactate dehydrogenase in adult wistar rats (*Rattus norvergicus*). Thirty two adult wistar rats weighing between 150-230g used in this experiment were divided into four groups of eight (n=8) animals each. Each animal in Group I, II, III and control were administered respectively with 250mg/kg bw, 150mg/kg bw, 50mg/kg bw and 0 kg bw of the extract per day for a period of three weeks (21 days) through the oral route. At the end of this investigation, a dose-dependent increased activity of the enzymes were observed. . This observations affirms the earlier observed hepato-protective effect of *ocimum sanctum.* This experiment also suggests that carbohydrates are facilitated more along the hexose monophosphate pathway for G6PDH and the generation of lactate may serve as substrate for gluconeogenesis.

Keywords: *Ocimum sanctum,* glucose-6-phosphate dehydrogenase, lactate dehydrogenase

Introduction

Tulsi is described as sacred (Wealth of India, 1991) and a medicinal plant in ancient literature (Kirtikar and Basu, 1975). The name *Tulsi* is derived from '*Sanskrit'*, which means "matchless one" (Shosh and Tulasi, 1995). This plant belongs to the family *Labiatae*, characterized by square stem and specific aroma. The botanical name of *Tulsi* is *Ocimum sanctum* (Linn).It is abundantly found in India, Malaysia, Australia, West Africa and some of the Arab countries (Mondal *et al,* 2009). Several medicinal properties have been attributed to this plant (Gupta, 2002). Today people use different parts of Tulsi for treatment of various ailments based on traditional knowledge. Traditionally, leaves of Tulsi plant was used as demulcent, stimulant and expectorant. It was used to cure of upper respiratory tract infections, skin infections (Harsa *et al,* 2003) and earache. An infusion of its leaves had been used as an anti-sposmodic in gastric disorders of children.

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A concoction of root of Tulsi is still being used as a diaphroretic in malarial fever treatment. The seeds are useful in different ailments of genito-urinary system (Wealth of India, 1991). Tulsi is traditionally prescribed good for heart and also stimulates digestion, reducing breathing difficulties and cough (Ghosh and Tulasi, 1995). It has been used in treatment of snake-bite and scorpion-sting (Kirtikar and Basu, 1975). *Ocimum sanctum* has specific aromatic odour because of the presence of essential or volatile oil, mainly concentrated in the leaf. This aromatic volatile oil mainly contains phenols, terpenes and aldehydes. The oil extracted from the seeds is mainly composed of fatty acids. Besides the oil, the plant also contains alkaloids, glycosides, saponines and tannins. The leaves contain ascorbic acid and carotene as well (Wealth of India, 1991). Scientific evidences available on various medicinal aspects of tulsi include antimicrobial, adaptogenic, antidiabetic, hepato-protective, anti-inflammatory, anti-carcinogenic, radio-protective, immunomodulatory, neuro-protective, cardio-protective and mosquito repellant properties (Mondal et al, 2009). 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 (Zhang et al., 2000). 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. Thus, in this study we investigated the effect of the extract of *Ocimum sanctum* on glucose-6-phosphate dehydrogenase (G6PDH) and lactate dehydrogenase (LDH) levels in the liver. These are a very important enzymes in the metabolism of glucose.

Materials and Methods

Animals

Presumably healthy Thirty two adult wistar rats of both sexes were used for this experiment. The animals weighed between 150-230g. The animals were kept and housed in the animal holdings of the Department of Anatomy, University of Ilorin, Ilorin Kwara State, under standard laboratory conditions of temperature and light and humidity and were feed with rat pellets and water *ad libitum*. The rats were randomly divided into four groups.

Plant collection

Plant samples were collected from the Botanical Garden of the University of Ilorin, Ilorin, Kwara State, Nigeria. The sample was taken to the University Department of Plant Biology for authentication. The leaves were oven dried at a temperature of 40°C and pulverized with mortar and pestle and the powdered form which was completely dry was stored for further usage in deep freezing compartment. It was stored in a non- humid environment.

Extract preparation

Three hundred and fifty grams of powdered *Ocimum sanctum* was dissolved in 1500ml of distilled water. The solution was left to stand in a refrigerator for 48 hours. This was latter filtered using Whatman's umber one filter paper. The filtrate obtained was placed in a beaker to be evaporated in a water bath at a temperature of 60°C until a dark sticky residue was obtained. This was further oven dried and maintained in desiccators until a constant weight was obtained. The dried extract was stored in a tightly covered container in a refrigerator.

Extract Administration

Thirty two adult Wistar rats weighing between 150-230g were used for this experiment. They were divided into four groups (I, II, III, and control) of eight (n=8) animals each. Each animal in Group I, II, III and control were administered respectively with 250mg/kg bw, 150mg/kg bw, 50mg/kg bw and 0 kg bw of the extract per day for a period of three weeks (21 days) through the oral route. The administration was conducted 9.00am every morning and once a day. The extract was administered at exactly 0700 hours for the entire duration of the research.

Animal sacrifice

The animals used were sacrificed via cervical dislocation. A midline incision on the anterior abdominal wall was made to expose the abdominal viscera. The liver was excised and used to prepare the homogenate for subsequent enzyme analysis.

Preparation of Liver Homogenate

A 10% homogenates of the tissues in chilled phosphate buffer was immediately prepared with Polter-Elvhjem homogenizer. The homogenate were centrifuge (5000rmp) for 10 minutes, the supernatants were immediately stored in the freezer (-20°C) and assayed within 48 hours. The enzymatic activities of LDH were estimated according to the procedure of Anosike and Ejio for using a commercially available kit (Randox Lab. Ltd.).

Statistical Analysis

Values were reported as mean Â± S.E.M and data were analyzed using student's t-test using the statistical software SPSS version 14 at confidence interval $p<0.05$ and Microsoft excel 2007.

Results

The results obtained from the present study showed a significant increase in the activities of both lactate dehydrogenase and glucose-6-phosphate dehydrogenase at $p<0.05$.The increases observed were dose-dependent. Figure 1 below shows a bar chart representing the activity of G6PDH in the liver. Figure 2 also shows a bar chart showing the activity of LDH in the liver.

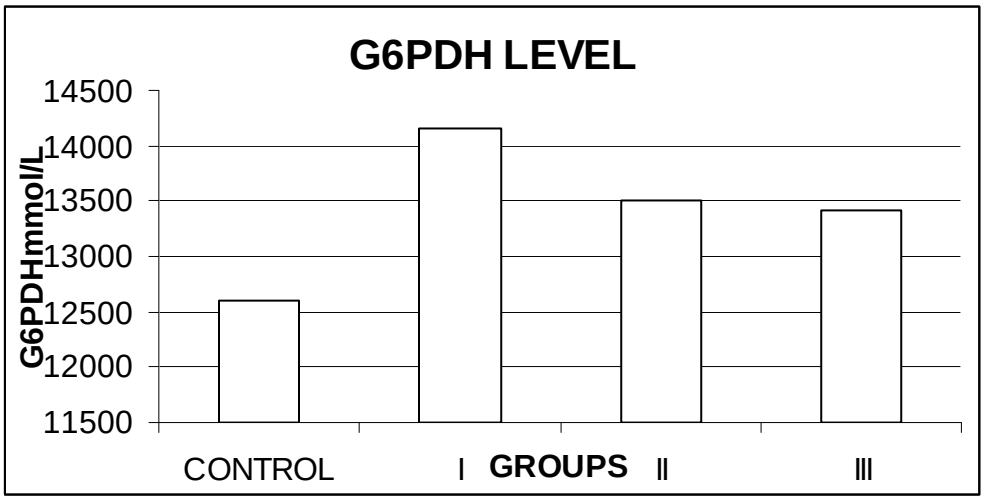

Figure 1: Bar chart showing the activities of G6PDH in the liver.

The activities of glucose-6-phosphate dehydrogenase (G6PDH) is increased in all the experimental groups when compared to the control group, which is shown in figure 1. Increase in the activity of the enzyme was observed to be dose-dependent. Increased activity of G6PDH results in the synthesis of ribose-5-phosphate (Kuo and Tang 1999). This is converted to 5-phosphoribosyl-1-pyrophosphate that acts as a donor of the ribose phosphate unit in the nucleotide biosynthesis (Kuo and Tang, 1999). G6PDH 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-phosphate gluconate in the pentose phosphate pathway (Beuthler et al., 1996; Kleitzein et al., 1994). This enzyme is highly

conserved during evolution and plays multiple roles in the cell. Enzymes are the biocatalyst that regulates the rates at which all physiological processes take place (Rodwell, 1993). 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 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 (Zhang et al., 2000).One of the uses of NADPH in the cell is to prevent oxidative stress. It reduces the coenzyme gluthathione, which converts reactive H_20_2 into H20, when absent, the H202 would be converted to hydroxyl free radicals which can attack the cell. This may explain the various reported protective effects.

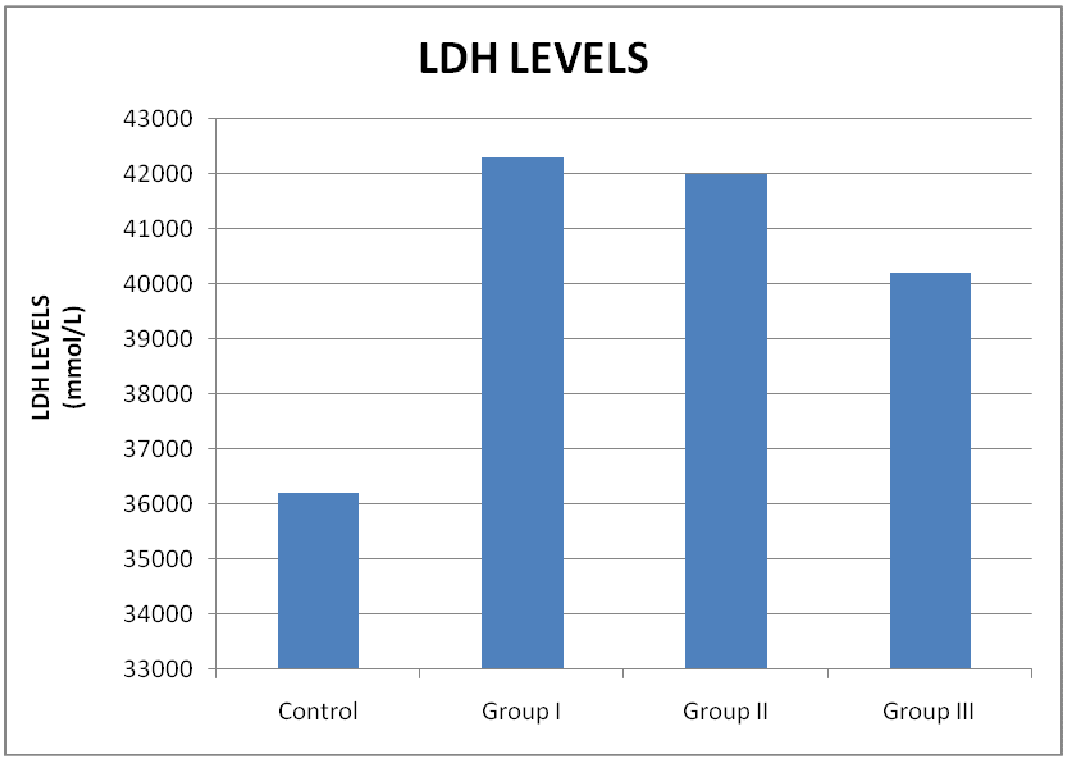

Figure 2: Bar chart showing the activities of LDH in the liver.

Ocimum sanctum may thus mediate the up regulation of this G6PDH with increased production of ribose-5-phosphate which enhances DNA synthesis, and thus increased cell proliferation of the hepatocytes. Until recently, the role of this house keeping 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 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 is upregulated by oxidants through a mechanism acting mainly on the rate of transcription of this gene (Salvemini et al, 1999). *Tulsi* offered liver protection against various experimentally induced damages. *Tulsi* extract treated group showed no mortality while control group showed 60% mortality in carbon tetrachloride induced liver damage in rats (Bhargava and Singh, 1981). The ethanolic extract of *Tulsi* can protect the liver damage from anti-tubercular drugs in experimental rats (Ubaid *et al*, 2003). The ethanolic extract of *Tulsi* treatment prior to paracetamol induced liver damage, have shown to protect the liver. This has been evident by significantly enhanced levels of serum enzymes (aspartate, aminotransferase, alkaline and acid phosphatase) and liver glutathione in experimental rats (Chattopadhyay *et al*, 1992).

There as been significantly enhanced levels of liver glutathione observed in experimental rats treated with *ocimum sanctum* (Chattopadhyay *et al,* 1992). As it is known that glutathione is essential for the detoxification of reactive free radicals and lipid hyperperoxides (Halliwell and Gutteridge, 1989). The NADPH which may be produced can reduce glutathione. These beneficial activities can thus promote cellular integrity of hepatocytes and other tissues of the body. Besides the up regulation of G6PDH that was observed in the liver in this experiment, it can be suggested that the administration of *O. sanctum,* carbohydrates are facilitated more along the hexose monophosphate pathway.

Figure 2 showed a significant increase in the activities of lactate dehydrogenase which was also observed to be dose-dependent. This enzyme facilitates the conversion of lactate to pyruvate (medicine.net).These findings suggest that the administration of *O. sanctum* up regulates the glycolytic enzyme lactate dehydrogenase in the liver of the animals used for this investigation. 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).This suggests that pyruvate generated from enhanced glycolysis in the animals used in this investigation was reduced to lactate and that the later served as a substrate for gluconeogenesis, therefore contributing to glucose output of the liver.

Conclusion

Based on the observations from this study, it can be concluded that the aqueous leaf extract *Ocimum sanctum* alters carbohydrate metabolism. This observations also affirms the earlier observed hepato-protective effect of *Ocimum sanctum.*

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