

Investigation of Some the Activities of G6PDH and LDH in the Liver of Adult Male Wistar Rats Following the Administration of Aqueous Extract 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 male wistar rats (*Rattus norvegicus*). Thirty two adult male wistar rats weighing between 150-230 g 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 250, 150, 50 mg/kg bw of the extract per day for a period of 3 weeks (21 days) through the oral route while the last group was administered phosphate buffered saline as the control. At the end of this investigation, a dose-dependent increased activity of the enzymes was observed. This observation 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: Enzymes, gluconeogenesis, glucose-6-phosphatedehydrogenase, hepato-protective, lactatedehydrogenase, phosphate buffered saline, substrate

INTRODUCTION

Tulsi is described as sacred (Anonymous, 1991) and a medicinal plant in ancient literature (Kirtikar and Basu, 1975). The name *Tulsi* is derived from 'Sanskrit', which means "matchless one" (Delpret *et al.*, 2012). 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). Plants are one of the most important sources of medications (Atack, 1989).

Lots of herbal or medicinal properties has been linked to this plant, in Ayurveda and Siddha but also in Greek, Roman and Unani system of medicine (Gupta *et al.*, 2002).

In traditional medicine the Indian medicinal plants have been used in successful management of various diseases like bronchial asthma, chronic fever, cold cough, malaria dysentery, convulsions, diabetes, diarrhea, arthritis, emetic syndrome, skin diseases, Insect bites etc. and in treatment of gastric, hepatic, cardiovascular and immunological disorders (Sen, 2006).

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 for the 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-spasmodic in gastric disorders of children. A concoction of root of *Tulsi* is still being used as a diaphoretic in malarial fever treatment. The seeds are useful in different ailments of genito-urinary system (Anonymous, 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 (Anonymous, 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 repellent 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 on the other hand, catalyzes the interconversion of pyruvate and lactate with the concomitant interconversion of NADH and NAD⁺ (Rodwell, 1993).

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 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 150-230 g. 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. This study was carried out at the Department of Anatomy, University of Ilorin Kwara state Nigeria. It was conducted in the month of March, 2011

Plant collection: Plant samples were collected from the Botanical Garden of the University of Ilorin, Ilorin Kwara State. 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 was stored for usage at room temperature.

Extract preparation: Three hundred and fifty (350 g) of powdered *Ocimum sanctum* was dissolved in 1500 mL of Distilled water. The solution was left to stand in a refrigerator for 48 h. This was latter filtered using Whatman's number 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 150-230 g were used for this experiment. They were divided into four groups (I, II, III and IV (control)) of eight (n = 8) animals each. Each animal in Group I, II, III and control were administered respectively with 250, 150 and 50 mg/kg bw and 0 kg bw of the extract per day for a period of 3 weeks (21 days) through the oral route at 0800 h throughout the duration of this 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 (5000 rpm) for 10 min, the supernatants were immediately stored in the freezer (-20C) and assayed within 48 h. 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 $\bar{X} \pm S.E.M$ 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 AND DISCUSSION

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 increase observed was dose-dependent (Fig. 1 and 2).

The activities of Glucose-6-Phosphate Dehydrogenase (G6PDH) are increased in all the experimental groups when compared to the control group, which is shown in Fig. 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-

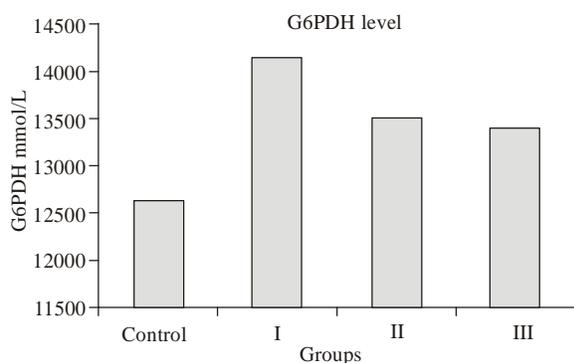


Fig. 1: A bar chart representing the activity of G6PDH in the liver G6PDH levels in mmol/L

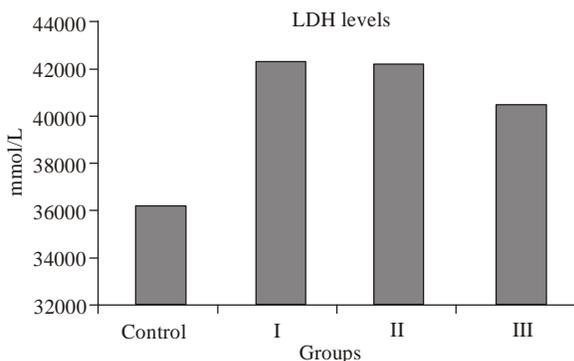


Fig. 2: A bar chart showing the activity of LDH in the liver LDH levels in mmol/L

phosphate gluconate in the pentose phosphate pathway (Rodwell, 1993). 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 glutathione, which converts reactive H₂O₂ into H₂O, when absent, the H₂O₂ would be converted to

hydroxyl free radicals which can attack the cell. This may explain the various reported protective effects.

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 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 has 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, 1999). 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

Among all herbs in the world *Ocimum sanctum* is considered as the queen of herbs due to its greater medicinal properties.

Therefore keeping the various medicinal benefits insight, investigations are called to be attempted in the purification of its components alongside its characterization interms of chemical nature and biopharmacological activities.

In conclusion, data from the present study shows that treatment with the aqueous leaf extract of *ocimum sanctum* in adult male wistar rats at the doses mentioned possesses no deleterious effect, using the above enzymes as markers.

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