ISSN: 2377-6196
The American Journal of
SCIENCE AND MEDICAL RESEARCH, 1(2), 202-206

Research Article Open Access

Effect of *Hemidesmus indicus* leaf extract on Liver enzymes of Diabetic induced Wistar rats

Lingaiah[1], Estari Mamidala[2] and P. Nagaraja Rao[3]*

[1,3] Department of zoology, Osmania University, Hyderabad-500 007, Telangana State, India
[2]Department of zoology, Kakatiya University, Warangal-506 009, Telangana State, India.

**Email: lingaiahzoology@gmail.com*

Abstract

Many traditional plant treatments for diabetes mellitus are used throughout the world and few of the traditional plant treatments for diabetes have been shifted scientific scrutiny. The present study aimed to study the effect of *Hemidesmus indicus* plant leaf extract on the liver enzymes in alloxan induced diabetic Wister rats. *Hemedesmus indicus* plant was successively extracted with different organic solvents (acetone and chloroform) in increasing polarity order by using Soxhlet extractor. The extract was treated in diabetic rats and observed the levels of liver enzymes Glucose-6-Phosphatase, Lactate Dehydrogenase, Hexokinase, Glucose-6-Phosphate Dehydrogenase. The enzyme concentration was compared to diabetic control and standard drug treated rats. The Glucose-6-Phosphatase, Lactate Dehydrogenase levels were decreased and Hexokinase, Glucose-6-Phosphate Dehydrogenase levels were increased in extract treated rats when compared to control rats. This study concludes that, *Hemedesmus indicus* made a significant effect in regulating and controlling carbohydrate metabolic enzymes such as Hexokinase, Glucose-6-phosphatase, Glucose-6-phosphatae Dehydrogenase and Lactate Dehydrogenase very effectively.

Keywords: Diabetes, Ethnobotanical survey, Medicinal plants, Liver enzymes.

INTRODUCTION

Diabetes mellitus is the world's largest endocrine disorder[1]. It has been suggested that a total of 300 million people around the world will have diabetes by the year 2025 and the global cost of treating diabetes and its complications could reach US$ 1 trillion annually (Arky RA.1982). Diabetes mellitus is characterized by hyperglycemia together with biochemical alterations of glucose and lipid metabolism (Chakrabarti and Rajagopalan, 2002). Liver is an insulin dependent tissue, which plays a vital role in glucose and lipid homeostasis and is severely affected during diabetes (E. Renold et al,1954). Liver participates in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol, phospholipids and triglycerides.

How to Cite this Article:
Lingaiah, Estari Mamidala and P. Nagaraja Rao (2016). Effect of Hemidesmus indicus leaf extract on Liver enzymes of Diabetic induced Wistar rats. *The Ame J Sci & Med Res*, 1(2):202-206.. doi:10.17812/ajsmr1211.
Published online @ 17 March 2016

Decreased glycolysis, impeded glycogenesis and increased gluconeogenesis are some of the changes of glucose metabolism in the diabetic liver[5].*Hemidesmus indicus* (L.) R. Br. Known as Indian Sarsaparilla (English), Ananta, Gopasuta, Sariva (Sanskrit), Anantamul (Hindi) (Bopanna and Bhagyalakshmi, 1997). The plant has been used traditionally (Lingaiah,and Nagarajarao, 2013) for the treatment of blood disorders, lowdigestion, anorexia, diarrhea, asthma, fever, cough, itching, and skin diseases including leprosy (Bopanna and Bhagyalakshmi, 1997). Various effects of *Hemidesmus indicus* (L.),such as hypoglycemic (Lampronti et al, 2008), hypolipidemic (M. I. Alam, B. Auddy,1956) antioxidant, antithrombotic (Prabakan and Anandan, 2000) anti-inflammatory (Kotnis et al, 2004) antiulcerogenic (Mukherjee,1981), hepatoprotective (N. K. Mary, C. R. Achuthan, 2003), renoprotective (Okokon et al, 2012), and neutralization of viper venom (Oliver-BeverB,1986) have been reported. Many traditional plant treatments for diabetes mellitus are used throughout the world (Patil et al, 2013). Few of the traditional plant treatments for diabetes have been shifted scientific scrutiny. This study was thus initiated with the aim of evaluating the effects of an aqueous leaf extract of

Hemidesmus indicus. L. on the liver enzymes in alloxan induced diabetic wister rats.

MATERIALS AND METHODS

Chemicals:

Alloxan monohydrate and Glibenclamide are procured from Sigma, Bangalore. Wellion LUNA duo Glucometer and Blood gluco-strips procured from Med Trust-Glucoworld, Kerala. EDTA, Imidazole buffer (100 mM, pH 6.5), Ammonium molybdate (1%, w/v), Sodium citrate (2%,w/v), ice-cold sucrose (250 mM), NaCl (203.3 mmol), Pyruvate (9.76 mmol), $MgCl_2$ from Merk & Sigma, India.

Preparation of Extracts:

Selected *Hemedesmus indicus* plant was successively extracted with different organic solvents (acetone and chloroform) in increasing polarity order (Pathmanathan *etal.,* 2010) by using Soxhlet extractor. For each extraction,500grof powdered material was weighed accurately and placed in Soxhlet extraction chamber which was suspended above the flask containing 1000 ml of solvent. The flask was subjected to heat on heating element. This resulted in the evaporation of solvent which moves into the condenser where it will be converted into a liquid that trickles into the extraction chamber containing the plant material. The extraction chamber was designed so that when the solvent surrounding the sample exceeded at certain level it overflowed and trickled back down into the boiling flask. At the end of the extraction process, the flask containing the solvent extract was removed and excess solvent was evaporated by using rotary evaporator. The weight of the residue obtained in percent was calculated. Then the residue was dissolved in 25 ml of pure methanol and stored in an air tight glass vials at 40^0C until further use.

Extract yield % = W1/W2 X 100.

Where, W1 = Net Weight of powder in grams after extraction,
W2 = total Weight of powder in grams taken for extraction.

Test Animals:

Adult Wistar rats (150-155gr), 8-14 weeks aged were obtained from Animal house, University college of Pharmaceutical science, Kakatiya University, Warangal and were used for the study of antidiabetic antilipidemic activity of four selected plants. Rats were acclimatized for a period of 7 days before experimentation, housed in groups of seven polypropylene cages with lined soft wood as bedding (renewed every 24hrs),12hour day and 12 hour night cycle, relative humidity 50-60 and at temperature 22±3°c, rats were fed with pellet as diet and water. As per the ethical norms, conducted all the experiments and it was approved by institutional ethical committee.

Experimental design:

Animals selected were fasted overnight and then divided into four groups (n=4) as follows:

- Group-I: Normal Control rats (non-alloxanized) that was administered with standard feed and water.
- Group-II: Diabetic control rats (Untreated, alloxanized).
- Group III: Diabetic rats administered with Glybenclamide drug (600µg/kg/bw) as reference standard drug while.
- Group-IV: Diabetic rats administered with *Hemedesmus indicus* leaves extract (10 mg/bw).

Induction of experimental diabetes:

Diabetes was induced by intraperitoneal injection (single dose) of alloxan monohydrate (120 mg/ kg-b.w.) in (0.9% w/v) NaCl solution (normal saline) to overnight starved normal rats. Blood glucose level was checked by using one touch glucometer for primary diagnosis (Miyong et al, 2015) and diabetes was conformed after 72 hr of alloxanisation. Rats were selected for diabetic studies based on Fasting blood glucose levels (FBG)> 250 mg/dl. Treatment was continued for a period of 21 days following oral administration to the experimental animals by gastric intubation by using a force-feeding needle **(**Shaik Abdul Nabi *et al*., 2013).

Enzyme Assays:

Glucose-6-Phosphatase (EC 3.1.3.9):

Glucose-6-phosphatase catalyzes the conversion of glucose-6-phosphate to glucose. The liver was homogenized in ice-cold sucrose (250 mM) solution. The enzyme activity was expressed as unit per gram per minute in tissue (Patil et al, 2011).

Lactate Dehydrogenase (EC 1.1.1.27):

Lactate dehydrogenase catalyzes the conversion of L-lactate to pyruvate with simultaneous reduction and oxidation of NAD to NADH. Change in absorbance with time as a result of converting NAD to NADH is directly proportional to LDH activity (Oliver, 1986).

Hexokinase (EC 2.7.1.1):

The hexokinase assay is based on the reduction of NAD^+ through a coupled reaction with glucose-6-phosphate dehydrogenase (Mukharjee, 1981).

Glucose-6-Phosphate Dehydrogenase (EC 1.1.1.49):

The measure of glucose-6-phosphate dehydrogenase activity is the rate of increase in absorbance. Addition of maleimide inhibits oxidation of reaction products by 6-phospho gluconolactone.One unit of enzyme activity is defined as that quantity which catalyses the reduction of 1 mM of NADP per minute.

RESULTS

Glucose 6-phosphatase is an enzyme that hydrolyzes glucose-6-phosphate, resulting in the creation of a phosphate group and free glucose. The liver glucose-6-phosphatase was increased (21.5 U/mg) in alloxan induced diabetic rats and decreased (13.36 U/mg) in standard control group while the level of this enzyme was moderate decrease (18.4 U/mg) in *Hemedesmus indicus* plant extract treated group animals.

Lactate Dehydrogenase concentration in plant extract treated rats was moderately decreased from 8.3 U/mg to 6.4 U/mg when compared to diabetic control rats. The levels of this enzyme in standard drug treated rats was 4.2 U/mg. Hexokinase concentration was moderately increased from 0.9 U/mg to 1.2 U/mg) in *Hemedesmus indicus* plant extract treated rats when compared to diabetic control rats.

DISCUSSION

The liver enzymes like Hexokinase, Glucose-6-phosphatase, Lactate Dehydrogenase and Glucose-6-phosphate dehydrogenase were studied to understand the impact of the selected plant extracts on carbohydrate metabolism to further confirm their role in diabetes management. These enzymes were selected by earlier researchers to study carbohydrate metabolism (Monica Nannipieri et.al 2005. Anna Ludovica Fracanza nietal., 2008). It was reported earlier that Alloxan induced diabetes caused lipid peroxide mediate tissue damage in the liver, kidney, and heart (Anuradha, Selvam, 1993).These changes can alter the properties and functions of the cell, resulting in either increased synthesis of some enzymes. (Goldberg et al., 1977).

Glucose-6-phosphatase generally catalyses an exchange reaction between glucose and Glucose-6-phosphate and this enzyme forms a phosphor enzyme intermediate (Hass, and Byrne, 1960), It is a mechanism that is also consistent with the multiple phosphate transferase activities (Van Schaftingen and Isabelle Gerin, 2002). Administration of *Boerhaviadiffusa* plant extracts decreased the glucose-6-phosphatase activity and increased plasma insulin level and also acted as an antioxidantin the test animals(Pari and Amarnath Satheesh, 2004). It was also reported earlier that *Enicostema littorale* plant extracts also decreased glucose 6-phosphatase activity (Maroo, Vasu, and Gupta. 2000. Vijayvargia, Kumar, and Gupta, 2003). In this study the levels of Glucose-6-Phosphatase was decreased (18.4u/mg) in *Hemedesmus indicus* plant extract treated group wistar rats. Therefore it is indicated that, the selected plant i.e *Hemedesmus indicus,* had decreased level of glucose 6-phosphatase activity in our experimental animals. Glucose 6-phosphatase is the enzyme of gluconegenetic pathway. This enzyme responsible for the addition of glucose into blood, it shows allosteric inhibition. Studies on the metabolism of C14-labeled fructose, glycerol, and pyruvate by rat liver slices have shown that livers from diabetic rats exhibit an increased production of glucose and a decreased production of glycogen from these substrates, as well as a diminished uptake of glucose (Renold, 1954). In Diabetics, the concentration of this enzyme in blood increases as the enzyme activity is maximum which results in the synthesis of excess glucose from glucose 6-phosphate and causes Hyperglycemia. The decreased levels of glucose-6-phosphate was observed in *Hemedesmus indicus* plant extract treated rats. Therefore, this study concludes that, *Hemedesmus indicus* plant extract may effect on insulin secretion. This suggested that the enzyme was elevated in diabetic rat liver (alloxan diabetes) due to conditions arising from the lack of insulin, and decrease enzyme levels when alloxan induced diabetic rats where treated with Glybenclamide drug, and *Hemedesmus indicus* Plant extract.

LDH is a functional enzyme in anaerobic glycolysis and it catalyses and helps in the conversion of pyruvate to lactate which is subsequently converted to glucose in glycogenic flux.**(**Xue-bing 2015**).** Lactate Dehydrogenase is an enzyme that catalyzes the conversion of lactate to pyruvate. This is an important step in energy production in cells. Many cells in the body contain Lactate Dehydrogenase enzyme. Increased LDH activity in diabetes has been reported, in diabetic patients earlier by Ramachandran et al., (2003) and Zappacosta et al., (1995). The results of the present study indicated that, LDH activity was significantly increased in diabetic induced wistar rats than in the control wistar rats. Hence LDH activity was higher in patients with diabetes than those in normal subjects (Prasenjit Manna, 2014) and it is due to excessive accumulation of pyruvate. This excessive pyruvate is converted to lactate for which LDH is needed. Hence the activity of LDH may be increased due to absence of insulin availability in diabetes (Chang and Schneider, 1972). In diabetic brain also LDH activity was increased serum activity of LDH have been observed in alloxan diabetic animals.

It is clear from these results that oxidative stress increased LDH activity in diabetic rats. *Annonasqamosa* plant extract has aporphine alkaloids; glycoside and squamoline compounds cause significant inhibition of LDH activity and decrease the LDH activity in diabetic rats and has restored LDH activity, probably as a result of the regulation of NAD^+/NADH ratio following stimulation of oxidation of NADH. Normal LDH activity is indicative of improved channeling of (pyruvate) glucose for mitochondria oxidation. The protective effect is due to prevention of any leakages of these marker enzymes (Kannan et al., 2006). It was reported that the reversal of LDH activity in diabetic rats with the treatment with *Murray koenigii* and *Ocimum sanctum* (Kannan et al., 2006). In our present investigation it was recorded that Lactate Dehydrogenase was decreased in *Hemedesmus indicus* plant extract treated group. Hence the present results suggest that they can effect LDH activity and the most effective plant extract that brought LDH near to

normal was *Hemedesmus indicus* plant extract. Earlier studies with the mangiferin (a plant product) and glibenclamide treated diabetic rats, LDH activity was reversed to near normal levels. This may be regulated by NAD^+/NADH ratio as suggested by earlier researchers (Kandasamy et al., 2009).

Hexokinase is universally present in all cells types in all organs (Jacek,2015).The hexokinase involved in the phosphorylation of glucose in glycolysis i.e. catalyses the activation of glucose to glucose 6-phosphate and plays a central role in the maintenance of glucose homeostasis (Magataand Koga, 1998, Vestergaard, 1999). Impairment of hexokinase activity suggests the impaired oxidation of glucose via glycolysis leading to its accumulation in blood resulting in hyperglycemia. Insulin influences the intracellular utilization of glucose in a number of ways. Insulin increases hepatic glycolysis by increasing the activity and amount of several key enzymes including glucokinase and phosphofructokinase. In the liver, this enzyme is an important regulator of glucose storage and disposal (Newgard et al.,1999). Hexokinase was significantly reduced in the liver of diabetic rats, which is due to decreased utilization of glucose leading to increased blood glucose levels and also may be due to insulin deficiency (insulin stimulates and activates hexokinase) (Vestergaard, 1999). Treatment of diabetic induced wistar rats with *T. arjuna*elevated the activity of hexokinase. *T.arjuna*may stimulate insulin secretion which may activate hexokinase, thereby increasing utilization of glucose leading to decreased blood glucose levels (Krishnakumari et al.,2006). *Boerhavia diffusa* caused an increase in hexokinase activity and decrease in glucose-6-phosphatase and also fructose bis-phosphatase activity, increase in plasma insulin level and showed property of antioxidant property (Pari et al., 2004). *Enicostema littorale* medicinal plant again observed to increase hexokinase activity, decrease glucose 6-phosphatase and fructose1,6-bisphosphatase activity and dose dependent hypoglycemic activity (Gupta et al.,2003). Hexokinase in *Hemedesmus indicus* plant extract treated group was 1.2 U/mg, Hence *Hemedesmus indicus* plant extract is effective as it increased the level of Hexokinase. Similar results were earlier obtained by many workers (Anders Knutsson, 2003). In Diabetics, the activity of Hexokinase is decrease because of insulin deficiency glucose is not transported to extra-hepatic tissue. The levels of this enzyme in *Hemedesmus indicus* plant extract treated rats was increased. Hence it is concluded that, the *Hemedesmus indicus* plant extract may effect on insulin secretion.

Glucos-6-Phosphate dehydrogenase (G-6-PD) is an enzyme of the pentose phosphate pathway that is responsible for the generation of NADP, which is required in detoxifying oxygen derived free radicals (Tain et al.,1998.Salvemini et al.,1999) G-6-PDH, the first and rate limiting enzyme of the pentose phosphate pathway, and it has long been regarded as important in the biosynthesis of sugar moiety of nucleic acid (Luzzatto and Metha,1995) and determines the amount of NADPH by controlling the metabolism of glucose via pentose phosphate pathway. It has been usually known that G-6-PDH was a typical “housekeeping” enzyme that was regulated solely by the ratio of NADPH and NADP (Kletzien et al.,1994. Tian et al.,1999). The production of NADPH required for the regeneration of glutathione in the mitochondria and is critical for scavenging the mitochondrial ROS through glutathione reductase and glutathione peroxidase system (Jo et al.,2001). G-6PDH may have directly reduced the basis ROS formation and as a consequence, increased the cellular concentration of glutathione (Salvemini et al.,1999). Thus, G-6-PDH plays a critical role in cell growth by providing NADPH in erythrocytes and regeneration of reduced glutathione (Tian et al.,1998), which prevent the hemoglobin denaturation and preserves the integrity of red blood cell membrane sulphydril groups. At the same time, it plays an important role in detoxification of hydrogen peroxide and oxygen radicals in and on the red blood cells (Weksler,1978). G-6-PDH enzyme is extra mitochondrial in location and highly specific for NADP as an electron acceptor (Meizer et al.,1997; Nadana Saravanan.,2007). In this study, the concentration of this enzyme was increased in *Hemedesmus indicus* plant extract treated rats. Hence it is hypothesized that, the *Hemedesmus indicus* plant extract may affect on insulin secretion and that effect on Pentose phosphate pathway.

Based on our experiments, it is concluded that the administration of plant extracts such as *Hemedesmusindicus*made a significant effect in regulating and controlling carbohydrate metabolic enzymes such as Hexokinase, Glucose-6-phosphatase, Glucose-6-phosphatae Dehydrogenase and Lactate Dehydrogenase very effectively.

Acknowledgement:

The authors thanks to Department of Zoology, Osmania University, Hyderabad, and our collaborators in Department of Zoology, Kakatiya University, Warangal and also thank the traditional healers of Adilabad district for their cooperation.

Competing interests

The authors have declared that no competing interests exist.

References

1. Anoop and M. Jegadeesan, “Biochemical studies on the anti-ulcerogenic potential of *Hemidesmus indicus* R. Br. var. indicus,” *Journal of Ethnopharmacology*, vol. 84, no. 2-3, pp.149–156, 2003.
2. Arky RA. Clinical correlates of metabolic derangements of diabetes mellitus in: Kozak GP.

(Ed), Complications of Diabetes mellitus, Saunders WB. Philadelphia, 1982;16-20.
3. Chakrabarti R, Rajagopalan R. Diabetes and insulin resistance associated disorders: Disease and the therapy. CurrSci 2002; 83: 1533-1538
4. E. Renold, Ching-Tseng Teng, F. B. Nesbett, and A. B. Hastings, J. Biol. Chem., 204, 533, 1953; J. Ashmore, A. E. Renold, F. B. Nesbett, and A. B. Hastings, Federation Proc., 13, No. 1, 176, 1954.
5. J. Anjaria, M. Parabia, G. Bhatt, and R. Khamar, *Nature Heals, a Glossary of Selected Indigenous Medicinal Plants of India*,SRISTI Innovations, Ahmedabad, India, 2002
6. K. N. Bopanna, N. Bhagyalakshmi, S. P. Rathod,R.Balaraman, and J. Kannan, "Cell culture derived *Hemidesmusindicus*in the prevention of hypercholesterolemia in normal and hyperlipidemic rats," *Indian Journal of Pharmacology*, vol. 29,no. 2, pp. 105–109, 1997.
7. Lampronti, M. T. H. Khan, M. Borgatti, N. Bianchi, and R. Gambari, "Inhibitory effects of Bangladeshi medicinal plantextracts on interactions between transcription factors and target DNA sequences," *Evidence-Based Complementary andAlternative Medicine*, vol. 5, no. 3, pp. 303–312, 2008.
8. M. I. Alam, B. Auddy, and A. Gomes, "Viper venom neutralization by Indian medicinal plant (*Hemidesmusindicus*and*Plucheaindica*) root extracts," *Phytotherapy Research*, vol. 10,no. 1, pp. 58–61, 1996.
9. M. Prabakan, R. Anandan, and T. Devaki, "Protective effect of *Hemidesmusindicus*against rifampicin and isoniazid-induced hepatotoxicity in rats," *Fitoterapia*, vol. 71, no. 1, pp. 55–59,2000.
10. M. S. Kotnis, P. Patel, S. N. Menon, and R. T. Sane, "Renoprotective effect of *Hemidesmusindicus*, a herbal drug used in gentamicin-induced renal toxicity," *Nephrology*, vol. 9, no. 3, pp. 142–152, 2004.
11. Mukherjee SK (1981). Indigenous drugs in diabetes mellitus. J. Diabetes Assoc. Ind., 21: 97-106.
12. N. K. Mary, C. R. Achuthan, B. H. Babu, and J. Padikkala, "In vitro antioxidant and antithrombotic activity of *Hemidesmusindicus*(L) R.Br," *Journal of Ethnopharmacology*, vol. 87, no.2-3, pp. 187–191, 2003.
13. Okokon JE, Udoh AE, Amazu L, Frank SG. Antiinflammatory and analgesic activities of Melantherascandens . Asian Pac J Trop Biomed 2012; 2 :144 - 148.
14. Oliver-BeverB (1986). Oral hypoglycemic action of medicinal plants in tropical West Africa. Cambridge University Press, 245-267.
15. Patil RN, Patil RY, Ahirwar B. DheerajAhirwarEvaluation of antidiabetic and related actions of some Indian medicinal plants in diabetic rats. Asian Pac J Trop Med 2011; 4(1): 20-23.
16. Pattanayak S, Nayak S, Panda D, Shende V. Hypoglycemic of *Cajanusscarabaeoides*in glucose overloaded and streptozotocin induced diabetic rats. Bangladesh J pharmacol 2009; 4: 131-135.
17. S. Murshed, B. Rokeya, N. Nahar, et al., "Hypoglycemic and hypolipidemic effect of *Hemidesmusindicus*root on diabetic model rats," *Diabetes Research*, vol. 39, pp. 15–23, 2005.
18. Seifter S, England S. Energy metabolism, In: Arias I, Popper H, Schacter D, et al(Eds.). The liver: Biology and Pathobiology, Rauen Press, New York, 1982; 219 – 49.
19. Swanston Flatt SK, Day C, Bailey CJ and Flatt RR. Traditional plant remedies for diabetes. Studies in the normal and Streptozotocin diabetic mice. Diabetologia 1990;33:462-4.
20. Swanston Flatt SK, Day C, Bailey CJ and Flatt RR. Traditional plant remedies for diabetes. Studies in the normal and Streptozotocin diabetic mice. Diabetologia 1990;33:462-4.
21. WHO Expert Committee on diabetes mellitus second report. Technical Report Series 646. World Health Organization. Geneva 1980; 61.

*****