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EFFECT OF *ALOE VERA* GEL EXTRACT ON OXIDATIVE ENZYMES IN ALLOXAN–INDUCED DIABETIC ALBINO-RAT TESTIS

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ABSTRACT

The present study was aimed to evaluate the therapeutic potential of *Aloe vera* by assaying the activities of selective oxidative enzymes in Alloxan induced diabetic oxygen free radicals have been suggested to be a contributory factor in complications of Type-1 diabetes mellitus. *Aloe vera* leaf gel extract administered orally to different groups of rat at a dose of 300mg/kg body weight for three weeks, Albino-Rats were divided into four groups. Each group consists of six rats. Normal Rats, Normal Rats + *Aloe vera*, diabetic + *Aloe vera* (Alloxan 40mg/kg body weight). In this study, work was under taken to evaluate the oxidative enzymes activity of *Aloe vera* gel extract on testis tissue oxidative enzymes, Glucose-6-phosphate dehydrogenase(G-6-PDH), Lactate dehydrogenase(LDH), Succinate dehydrogenase(SDH), Glutamate dehydrogenase(GDH) in Alloxan induced diabetic rats. G-6-PDH, SDH, GDH, activities were decreased in diabetic albino-Rats. Where as LDH activity was increased. Oral administration of *Aloe vera* gel extract to diabetic Rats showed significantly reversal of disturbed oxidative enzyme system. The findings of this study indicate that the administration of *Aloe vera* resulted in a better oxidative profile levels in both normal and diabetic rats.

KEYWORDS: Diabetes, *Aloe vera*, Alloxan, oxidative enzymes, testis, male rats.

INTRODUCTION

Diabetes mellitus is a serious health problem being the third greatest cause of death all over the world. Diabetes mellitus results in hyperglycemia and is characterized as type-1 in absolute insulin deficiency or type-2 in insulin resistance due to receptor insensitivity to endogenous insulin.[1] Oxidative stress defined as an imbalance between oxidants and antioxidants plays an important role in the development of diabetic complications.[2]

Oxidative stress and oxidative damage to the tissue are common end points of chronic diseases, such as atherosclerosis, diabetes and rheumatoid arthritis.[3] Oxidative stress is currently suggested as mechanism underlying diabetes and diabetic complications.[4] During diabetes, persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS), for all tissues from glucose auto- oxidation and protein glycosylation. Some chemical drugs such biguanides and sulfonylureas are currently available to reduce hyperglycemia in diabetes mellitus.[5] These drugs have side effect and thus search for new drug compound is essential.[6, 7] Many herbs and plant products have been shown to have hypoglycemic action. This leads to increasing demand for herbal products plant with antidiabetic activity and lower side effects.[8,9] *Aloe vera* is one of these antidiabetic plants.[10] Clinical evaluations have revealed that the pharmacologically active in gradients are concentrated in both the gel and rind of *Aloe vera* leaves.[11] Polysaccharide containing plants which *Alo* barbadensis is also among are used in various diseases as anti inflammatory, antiulcer, antineoplastic and in wound healing and against hepatitis.[12] In some studies it is shown that *Aloe* has an antioxidative effect. Its antigenetoxic and chemopreventive effects also proven.[13, 14] Our previous experimental results were highly encouraging as they revealed that level of blood glucose was significantly lower after oral administration of ethanolic extract of *Aloe vera* gel in glucose load condition and in Alloxan induced diabetes.[11]

MATERIALS AND METHODS

Selection of Animals

Wistar strain albino rats (180±20g) were obtained from Indian Institute of science, Bangalore. The rats were housed in clean polypropylene cages having six rats cage and maintained under temperature controlled room (26±20C) with a photo period of 12 hours light and 12 hours dark cycle. The rats were fed with a standard rat pellet diet and water adlibitum. The study was carried out according to guidelines for the care and use of laboratory animals and approved by the Institutional Animal Ethical Committee at Sri Venkateswara University, Tirupati, India. (Regd. No.438/01a/CPCSEA, Dt: 17-07-2001, and its resolution no. 08/2012-2013/ (i)/a/ CPCSEA/IAEC/SVU/MBR-MRN/dt. 02-07-2012).

Chemicals: The entire chemical used in the present study were Analar Grade (AR) and obtained from the following scientific companies: Sigma (ST. Louis, MO, USA), Fischer

(Pitrsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India).

Induction of Diabetes

The rats were injected intraperitonial with Alloxan monohydrate (Span chemical Co.Mimbai) dissolved in sterile normal saline at a dose of 40 mg/kg body weight. After injection, they had a free access to food and water was given 5% glucose solution to drink, overnight to counter hypoglycemic shock. The animals were considered as diabetic, if their blood glucose values were above 250 mg/dl on the third day After Alloxan injection the treatment was continued for three weeks.

Preparation of *Aloe vera* extract

The fresh *Aloe vera* was locally and authenticated by botanist in the department of Botany, S.V.University, and Tirupati. *Aloe vera* solid gel in the center of the leaf was collected and homogenized resulting, mucilaginous, thick and straw colored homogenate was obtained and lyophilized. Then the lyophilized sample was extracted using 95% ethanol. The filtrate was collected and evaporated to dryness under reduced pressure in a rotary evaporator at 60°C. The residue was stored in dry sterilized small containers at 4°C until further use. A Suspension which is the form customarily usual in folk medicine was prepared by dissolving suitable amount of ethanol free extra of *Aloe vera* leaf gel to get the desired concentration. The dosing schedule used was once per day. The extracts were administered orally, daily to different groups of rat at a dose of 300 mg/kg body weight.

Experimental design

Rats were randomly divided into four groups of six animals in each group.

Group-1: Control rats

Group-2: Control + *Aloe vera* (300mg/kg body weight of *Aloe vera*)

Group-3: Diabetic rats (40mg/kg body weight of Alloxan)

Group-4: Diabetic + *Aloe vera* extract (300mg/kg body weight in ethanol solution daily. Once in a day by an intragastic tube for three weeks)

After completion of three weeks treatment the animals were sacrificed by cervical dislocation and the testis tissue was excised at 4°C .The tissue was washed with ice-cold saline, and immediately stored in deep freeze at 80° C for further biochemical analysis.

Biochemical analysis and Enzymatic assays

Glucose-6-Phosphate dehydrogenase (G-6-PDH) (E.C: 1.1.1.49)

Glucose-6-posphate dehydrogenase activity was assayed by the method of,[15] as modified by.[16] 10% (W/V) testis tissue homogenate was prepared in ice cold sucrose 0.25 M solution and centrifuged at 1000 g for 15 min at 4^{Q}C. The reaction mixture in a total volume of 2 ml contained 100 µ moles of sodium phosphate buffer (pH 7.4), 20 µ moles of glucose-6-phosphate, 2 µ moles of INT and 0.3 µ moles of NADP. The reaction was initiated by adding 0.5ml containing 50 mg of tissue as an enzyme source. The reaction mixture was incubated at 37°C for 30 min and the reaction was stopped by the addition of 5 ml of glacial acetic acid. The formazan formed was extracted with 5 ml of toluene at 5°C. The optical density of the formazan was read at 495 nm against the toluene blank. The activity was expressed in µ moles of formazan formed/mg protein / hour.

Lactate dehydrogenase (LDH) (L-lactate: NAD^{+} Oxidoreductase -E.C: 1.1.1.27)

Lactate Dehydrogenase activity was determined by the method described by (17) as suggested by (18) with slight modifications. 10% (W/V) homogenates of the testis tissue was prepared in ice cold 0.25 M sucrose solution and centrifuged at l000g for 15 minutes at 4°C. The supernatant fraction was used for enzyme assay. The reaction mixture in a final volume of 2 ml contained 40 (µ moles of sodium lactate, 100 µ moles of phosphate buffer (pH 7.4), 0.1 µ mole of NAD and 4 µ moles of INT. The reaction was initiated by the addition of 0.2 ml of homogenate containing 20 mg of tissue as an enzyme source and incubated for 30 minutes at 37°C and the reaction was stopped by the addition of 5 ml of glacial acetic acid. Zero time controls (ZTC) were maintained by addition of 5 ml of glacial acetic acid prior to the addition of the enzyme source to the incubation mixture. The formazan formed was extracted over night into 5 ml of toluene at 5^{0}C. The color developed was measured at 495 nm in a Spectrophotometer against the toluene blank. The enzyme activity was expressed in moles of formazan formed / mg protein / hour.

Succinatedehydrogenase (SDH) (Succinate acceptor oxidoreductase-E.C: 1.3.99.1)

The specific activity of SDH was assayed by the method of,[17] as suggested by[18] with slight modifications. 10% (W/V) homogenates of the testis tissue was prepared in ice cold 0.25 M sucrose solution and centrifuged at l000g for 15 minutes at 4°C. The supernatant fraction was used for enzyme assay. The reaction mixture in a final volume of 2 ml contained 40 (µ moles of sodium Succinate and 100 µ moles of phosphate buffer (pH 7.0) and 4 µ moles of INT. The reaction was initiated by adding 0.2 ml of homogenate containing 20 mg of tissue as an

enzyme source. The incubation was carried out for 15 minutes at 37°C and the reaction was stopped by the addition of 5 ml of glacial acetic acid. The subsequent steps were followed same as described for LDH. The activity was expressed in µ moles of formazan formed / mg protein / hour.

Glutamate dehydrogenase (GDH-L-Glutamate; NAD oxidoreductase -EC:1.4.1.3)

Glutamate dehydrogenase (GDH) activity was assayed by the method of.[19] 5% (W/V) of testis tissue homogenates were prepared in ice cold sucrose (0.25M) solution and the contents were centrifuged at l000g for 15 minutes at 4°C. The supernatant part was used as an enzyme source. The reaction mixture in a total volume of 2 ml contained 100 µ moles of phosphate buffer (pH 7.4), 40 µ moles of sodium glutamate, 0.1 µ mole of NAD, 2 µ moles of INT and 0.2 ml containing 10 mg of tissue as an enzyme source. The reaction mixture was incubated at 37°C for 30 minutes. The reaction was arrested by the addition of 5 ml glacial acetic acid and the formazan formed was extracted into 5 ml of toluene. The intensity of the color was read at 495 nm against the toluene blank. The enzyme activity was expressed as µ moles of formazan formed/mg protein/hour.

Statistical analysis

The data has been analyzed by using one-way Analysis of Variance (ANOVA) followed by Dunnet's-test and 'P' value < 0.001 was considered significant. The data were presented as Mean ± S.D. And analysis was carried out by using SPSS 16.0.1 program.

RESULTS

GLUCOSE-6-POSPHATE DEHYDROGENASE (G-6-PDH).

The activity of G-6-PDH in Control rats testis 0.499 mg protein/minute. In the group-II, the activity was slightly increased. In group-III it was found significantly decreased, as increased trend was observed in group-IV rats.

LACTATE DEHYDROGENASE (LDH)

The activity of LDH in Control rats testis 0.309 mg protein/minute. In the group-II, the activity was slightly increased. Increased trend was observed in group-III. But when compared to the group-IV they had shown decreased activity. Observe that the alterations in activity of LDH in different groups when compared to the control rats had showed a different trend.

SUCCINATE DEHYDROGENASE (SDH)

The activity of SDH in control rats testis was found to be 0.619 mg protein/minute. In the groups-II, where rats were non diabetic but treated with *Aloe vera* extract the activity was very slightly increased. In group-III, the activity testis was found to be 0.424 mg protein/minute. Group-IV had also showed decreased levels of activity when compared to control rats. But, the activity in them was increased when compared to Group-III rats.

GLUTAMATE DEHYDROGENASE (GDH)

The activity of GDH in control testis rats was found to be 0.550 mg protein/minute. In the groups-II, where rats were non diabetic but treated with *Aloe vera* extract respectively, the activity was very slightly increased. In group-III, the activity was found significantly decreased to 0.501 mg protein/minute. Group-IV had also showed decreased levels of activity when compared to control rats. But, the activity in them was increased when compared to group-III rats. The increase was more pronounced in group-IV where the rats were subjected to *Aloe vera* extract.

Table: Showing Oxidative Enzymes activities in testis tissue of Control and Experimental rats.

Parameter	**GroupI (normal rats)**	**GroupII (normal rats + *Aloe Vera*)**	**GroupIII (diabetic rats)**	**Group IV (diabetic rats + *Aloe Vera*)**
G-6-PDH µ moles /mg of protein/hr	0.499±0.05	0.517±0.051 (+3.6)	0.374±0.032 (-25.01)	0.449±0.041 (-10.0)
LDH µ moles /mg of protein/hr	0.309±0.03	0.324±0.032 (+4.84)	0.389±0.036 (+25.81)	0.283±0.027 (-8.38)
SDH m moles /mg of protein/hr	0.619±0.061	0.631±0.064 (+1.94)	0.424±0.041 (-31.45)	0.567±0.052 (-8.39)
GDH µ moles /mg of protein/hr	0.550±0.062	0.580±0.067 (+4.62)	0.360±0.031 (-44.61)	0.501±0.050 (-22.92)

Values are mean ± S.D. of 6 individual rats

Values in the parenthesis are % change from that of control

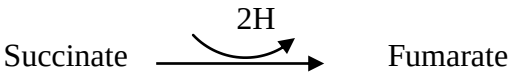
Values are significantly different from control at $P < 0.001$

DISCUSSION

In the present study, the observed decreases in the activities of mitochondrial enzymes in the testis of the diabetic rats were significantly enhanced upon *Aloe vera* Treatment (Table). The normal rats treated with *Aloe vera* gel extract showed increased activity of G-6-PHD. This could be due to elevation of mitochondrial enzymes by plant extract. The activity of glucose was found to be lowered in diabetic tissues.[20] G-6-PDH is the rate-limiting enzyme in the pentose phosphate pathway. G-6-PHD activity is reported to increase in the testis of Alloxan induced diabetic rats.[21] These data may indicate that G-6-PDH regulation varies indifferent tissues. With the treatment of *Aloe vera* plant extract to diabetic rats, which could have resulted in an increase in the reducing agent NADPH and a decrease in oxidative stress.

In the present study normal rats showed low levels of LDH. The normal rats treated with *Aloe vera* extracts showed still more decreased activity of LDH. Normal LDH activity is indicate of improved channeling of (Pyravate) glucose for mitochondrial oxidation. The protective effects due to treatment with plant extracts strongly indicate the possibility of the extract being able to prevent any leakages of marker enzymes. The significant increase in the activity of LDH in Alloxan induced diabetic rats could be due to excessive accumulation of pyruvate. The excessive pyruvate is conveted to lactate for which LDH is needed and therefore the activity of LDH may be increased due to less insulin availability in diabetes.[22,23]

SDH is a key enzyme in krebs cycle, that catalyses the reversible oxidation of succinate to fumarate. It is the only enzyme in TCA cycle which is an inner membrane bound and involves the direct transfer of hydrogen atoms from the substrate, succinate to flavor protein FAD.



In the present study the normal rats treated with *Aloe vera* extracts showed increased activity of SDH. This is due to the antioxidants present in the plant extracts that which influence the citric acid cycle including SDH activity. In case of Alloxan treated normal rats the activity was found decreased significantly. It has been suggested that the diabetogenicity of Alloxan is dependent on the inhibition of citric acid cycle enzymes like SDH.[24] When Alloxan subjected rats were treated with *Aloe vera* extract as in the case of group-IV, the activity was

increased. Same results were seen in UDCA (Ursodeoxycholic acid) treated diabetic and ethanol treated rats.

Glutamate dehydrogenare (GDH) is a homohedameric mitochondrial matrix enzyme that catalyses the reversible oxidative deamination of glutamate to α-ketoglutarate plus free ammonia using either NAD or NADP as a co-factor. In the *Aloe vera* gel extract treated rats GDH activity was increased. The elevated levels of GDH activity may be due to the antioxidants present in the extracts. These antioxidants had the capacity to utilize energy yielding metabolites and hence on *Aloe vera* extract treatment GDH activity were increased. In case of Alloxan induced diabetic rats the significant decrease in its activity was due to increased LDH that which leads to progressive alteration and degeneration of mitochondira. An investigation reveals that the activity of GDH was significantly decreased with the ethanol stressed young rats. The decrease in GDH activity is attributed to its inhibition by elevated ammonia levels (product-inhibitor) which diminish the catalytic efficiency of the enzyme molecule.[25] The increased LDH also reported in the present study, consonance with that lactate inhibits the GDH activity. A high NADDH/NAD was envisaged in the animals exposed to ethanol treatment, which might play a prominent role in the inactivation of GDH.[26]

CONCLUSION

The effect of the ethanolic of *Aloe vera* on testis tissue in oxidative enzyme profiles due to reduction enzymes in activities of diabetic rats. Oxidative stress is associated with the development and progression of diabetes mellitus.

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REFERENCE

1. EI-Hilaly J, Adil T, Zafar H I and Badiaa L.A. Hyperglycemic, hypocholesterolemic and hypotriglyceridemic effects of continuous intravenous infusion of a lyophilized aqueous extract of ajuga iva 1. Schreber whole plant in streptozotocin – induced diabetic rats. *Pak J Pharm sci*, 2007; 20(4): 261-268.
2. B. Halliwell and J.M.Gutteridge, “Role of free Radicals and Catalytic Metal Ions in Human Disease: An overview”, *Methods* in enzymology, 1990; 186: 1-85.

3. John W Baynes and Suzanne R Thorpe. Role of oxidative stress in Diabetic complications. Diabetes., 1999; 48: 1-9.
4. SA Moussa. Oxidative stress in diabetes mellitus. Romanian J Biophys., 2008; 18(3): 225-236.
5. Mutalik. S, B. Sulochana, M. Chetana, N. Udupa and V.P. Devi, 2003. Preliminary studies on acute and subacute toxicity of an antidiabetic herbal preparation. Indian J. Experimental Biol, 41: 316-320.
6. Noor, A.S. Gunasekaran, A. Soosai Manickam and M.A. Vijay Lakshmi. Antidiabetic activity of *Aloe vera* and histology of organs in streptozotocin induced diabetic rats. Current Sci., 2008; 94: 8-25.
7. Nirmala, A., S. Saroja, H.R. Vasanthi and G. Lalitha,. Hypoglycemic effect of Basella rubra in streptozotocin induced diabetic albino rats. Journal of pharmacognosy and phyotherapy, 2009; 1: 025-030.
8. Shane – Whorter, L. Biological complementary therapies: a foucs on botanical products in diabetes. Diabetes spectrum, 2001; 14: 199-208.
9. Bolkent, S. N. Akev, A. Can, S. Bolkeat, R. Yanardag and A. Okzar, 2005. Immunohistochemcial studies on the effect of *Aloe vera* on the pancreatic – cells in neonatal streptozotocin – induced type –II diabetic rats. Egyptian J. Boil., 2005; 7: 14-19.
10. Can A. and A. Nurize, 2004. Effect of *Aloe vera* leaf gel and pulp extracts on the testis in type – II diabetic rat models. Biological and pharmaceutical Bulletin, 2004; 27: 694-698.
11. Rajasekaran, S.K. Sivagnanam and S.Subramanian. Antioxidant effect of *Aloe vera* gel extract in streptozotocin – induced diabetes in rats. Pharmalogical Report, 2005; 57: 90-96.
12. Kim, H.S., S. Kacew and B.M. Lee. In vitro chemopreventive effects of plant polysaccharides (*Aloe* barbadensis Miller, Lentinus cdodes, Ganoderm lucidum and coriolus versicolor). Carcinogen, 1999; 20: 1637-40.
13. Hu, Y., J. Xu and Q. Hu. Evaluation of antioxidant potential of *Aloe vera* (*Aloe* barbadesis Miller) extracts. J. Agriculture and Food Chemistry, 2003; 51: 77888-91.
14. Kosif, R., G. Akta and A. Zeekin, Microscopic examination of placenta of rats prenatally exposed to *Aloe* barbadensis: A preliminary study, International J. Morphol., 2008; 26: 275-81.
15. Lohr GD, Waller HD. In: Bergmeyer HV (ed). Method of Enzymatic Analysis. 1965. Academic Press, New York, London.

16. Mastanaiah SD, Chengalraju, Swamy KS. Circadian rhythmic activity of lipase in the scorpion, Heterometrus fulvipes. Current Sci., 1978; 20(47): 130-131.
17. Nachlas, M.M., Marguil, S.I. and Seligman, A.M. A colorimetric method for determination of succinate dehydrogenase activity. *J. Biol. Chem.*, 1960; 235: 499-505.
18. Prameelamma, Y., and Swami, K.S. Glutamate dehydrogenase activity in the normal and denervated gastrocnemius muscle of frog Rana hexadactyla. Curr. Sci., 1975; 44: 739- 740.
19. Lee, Y.L and A.A. Lardy., Influence of thyroid hormones on L-glycerophosphate dehydrogenases in various organs of the rat. Journal of Biological Chemistry, 1965; 240: 1427- 1430.
20. Saraswathi Panneerselvam and Swaminathan Govindaswamy. Effect of sodium molybdate on carbohydrate metabolizing enzymes in alloxan-induced diabetic rats. *The Journal of Nnutritional Biochemistry.,* 2002; 13(1): 21-26.
21. Sochor, M., Kunjara, S., Greenbaum, L., and P. Testis hypertrophy in experimental diabetes: effects of Diabetes on the pathwaysof glucose metabolism:differential response in adult and immature rats. Biochem, J, 1986; 234: 573-577.
22. Jones, A. L., ruderman, N. B., Herrera, M, G. Electron microscopic and biochemical study of lipoprotein synthesis in the isolated perfused rat testis. *Journal of lipid Research.,* 1967; 8: 429-446.
23. Awaji, Y., Hastimoto, H., Matsui, Y., Kawaguchi, K., Okumura, K., Lto, T., and Stake, T. Isoenzyme profiles of creatine kinase, lactate dehydrogenase and aspartate aminotransferase in diabetic heart: Comparison with hereditary and catecholamine cardio myopathies. *Cardivasic Res.*, 1990; 24(7): 547-54.
24. Boquist, L., Ericsson, I., Lorentzon, R., and Nelson, L. Alterations in mitochondrial aconitase activity and respiration and in concentration of citrate in some organs of mice with experimental or genetic diabetes. *FEES Lett.*, 1985; 183: 173 - 176.
25. Dudley, G. A., Staron, R. S., Murray, T. F., Hagerman, F. C. and Luginbuhal. A. Muscle fiber composition and blood ammonia levels after intense exercise in humans. *J. Apl. Physiol.*, 1983; 54: 582-586.
26. Sugano, T., Hasndler, J. A., Youshiva, H., Kizaki, Z and Thurman. R. G. Acute and chronic ethanol treatment *in vivo* increases malate-aspartate shuttle capacity in perfused rat liver. *J. Biol Chem.*, 1990; 265: 21549-21553.