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REGULATION OF DIABETES AND OXIDATIVE ENZYMES ON SUPPLEMENTATION OF *ALOE VERA* EXTRACT IN LIVER ALLOXAN INDUCED DIABETIC MALE RATS.

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ABSTRACT

The present work was under taken to evaluate the oxidative Enzymes activity of *Aloe vera* plant extract on liver tissue oxidative Enzymes, Glucose-6-Phosphate dehydrogenase (G-6-PDH), Lactate dehydrogenage (LDH), Succinate dehydrogenase (SDH), Glutamate dehydrogenase (GDH) in Alloxan induced diabetic rats. *Aloe vera* leaf get extract administered orally to different groups of rat at a dose of 300 mg/kg body weight for 21 days. Three months old male wistar rats were divided into 4 groups (n=6) namely: Control, control + Aloe vera, diabetic (Alloxan 40mg/kg body weight), diabetic + Aloe vera (300 mg/kg body weight). G-6-PDH, SDH, GDH, activities were decreased in diabetic rats. Whereas LDH activity was increased. Oral administration of *Aloe vera* leaf extract to diabetic rats showed significant reversal of disturbed oxidative enzyme system. The findings of the present study suggest that oxidative stress starts at Type-I diabetes mellitus and increases progressively. In conclusion, the *Aloe vera* extract played a key role in reduction of diabetes for diabetic patients.

KEY WORDS: Diabetes, *Aloe vera*, Alloxan, Oxidative stress, Rats.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both,[1] besides hyperglycemia, several other factors like hyperlipidemia and enhanced oxidative stress play a major role in diabetic pathogenesis. The disease is progressive and is associated with high risk of complications.[2] Oxidative stress and oxidative damage to the tissue are 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 liver tissues from glucose auto-oxidation and protein glycosylation.

Oxidative stress can occur as results of either excess production, or impaired antioxidant system. The primary ROS produced in the course of oxygen metabolism is superoxide, which is highly reactive, cytotoxic ROS. Superoxide is dismulated to a far less reactive product, hydrogen peroxide (H_2O_2) by a family of metalloenzymes knows as Superoxide dismulate (SOD).[5] The increased production of free radicals in mitochondria may damage Beta cells which known to be very sensitive to free radicals.[6] Also decrease in oxygen consumption and respiratory ratio were observed by.[7] Furthermore, lowering in the activities of pyruvate dehydrogenase and increase in NAD+/NADH ratio were reported in Alloxan induced diabetic rats.[8] Recent decades have shown a resurgent interest in traditional plant treatments for diabetes. Plants often contain substantial amounts of antioxidants including α-tocopherol (Vitamin E), carotenoids, ascorbic acid (Vitamin C) Flavonoids and tannis.[9] *Aloe vera* is a perennial plant belonging to the family of Liciaceae, which includes about 360 species.[10] Toxonomists now refer to Aloe barbadensis as *Aloe vera*.[11] *Aloe vera* is the one of the few medical plants that has maintained its popularity for a long period of time. The plant has stiff gray green lance-shaped leaves containing clear gel in a central mucilaginous pulp. Clinical evaluations have revealed that the pharmacologically active ingredients are concentrated in both the gel and rind of *Aloe vera* leaves. The present study attempted to clarify the changes in oxidative status in liver tissue with development of diseases. We therefore investigated the effects of diabetes on levels of reactive oxygen species (ROS) and the activity of oxidative Enzymes, namely Glucose-6-phosphate dehydrogenase (E.C:1.1.1.49), Succinate dehydrogenase (E.C:1.3.99.1) Glutamate dehydrogenase (E.C: P 1.4.1.3) and Lactate dehydrogenase (E.C: 1.1.1.27) in liver of control and Alloxan induced diabetic rats at various stages of development of diabetes (21 days).

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 21 days.

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 21 days)

After completion of 21 days treatment the animals were sacrificed by cervical dislocation and the liver 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,[12] as modified by.[13] 10% (W/V) Liver 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[14] as suggested by[15] with slight modifications. 10% (W/V) homogenates of the Liver 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.

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

The specific activity of SDH was assayed by the method of,[14] as suggested by[15] with slight modifications. 10% (W/V) homogenates of the Liver 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.[16] 5% (W/V) of Liver 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-Phosphate Dehydrogenase (G-6-PDH)

The activity of G-6-PDH in Control rats liver was found to be 0.630 mg protein/minute. In group-II, the activity was slightly increased. In group-III it was found significantly decreased, as increased trend was observed in group-IV rats. (Table)

Lactate dehydrogenase (LDH)

Control rat in liver the Lactate Dehydrogenase activity was found to be 0.510 mg protein/minute. In 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. (Table)

Succinate Dehydrogenase (SDH)

The activity of SDH in control liver was found to be 0.690 mg protein/minute. In groups-II, where rats were non diabetic but treated with *Aloe vera* extract the activity was very slightly increased. In group-III, the activity was found significantly decreased to 0.462 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. (Table)

Glutamate Dehydrogenase (GDH)

The activity of GDH in control liver rats was found to be 0.650 mg protein/minute. In 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 liver was found significantly decreased to 0.360 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)

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

Parameter	Group I (non diabetic rats)	Group II (non diabetic rats + *Aloe Vera*)	Group III (diabetic rats)	Group IV (diabetic rats + *Aloe Vera*)
G-6-PDH (µ moles /mg of protein/hr)	0.630±0.051	0.648±0.054 (+3.39)	0.428±0.031 (-38.11)	0.590±0.049 (-7.34)
LDH (µ moles /mg of protein/hr)	0.510±0.04	0.519±0.039 (+2.19)	0.642±0.042 (+32.19)	0.489±0.032 (-5.12)
SDH (µ moles /mg of protein/hr)	0.690±0.056	0.709±0.061 (+3.22)	0.462±0.031 (-38.64)	0.559±0.028 (-22.20)
GDH (µ moles /mg of protein/hr)	0.650±0.063	0.749±0.061 (+3.04)	0.460±0.032 (-42.85)	0.602±0.05 (-20.31)

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

Values in the parenthesis are % change from that of control

Values are significance different from control at P < 0.00

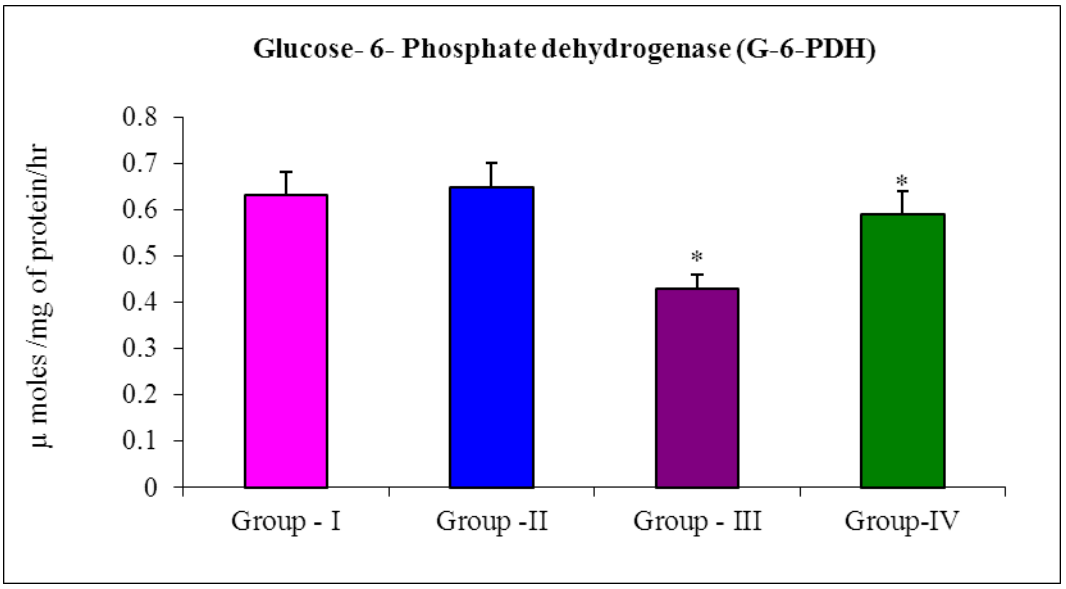
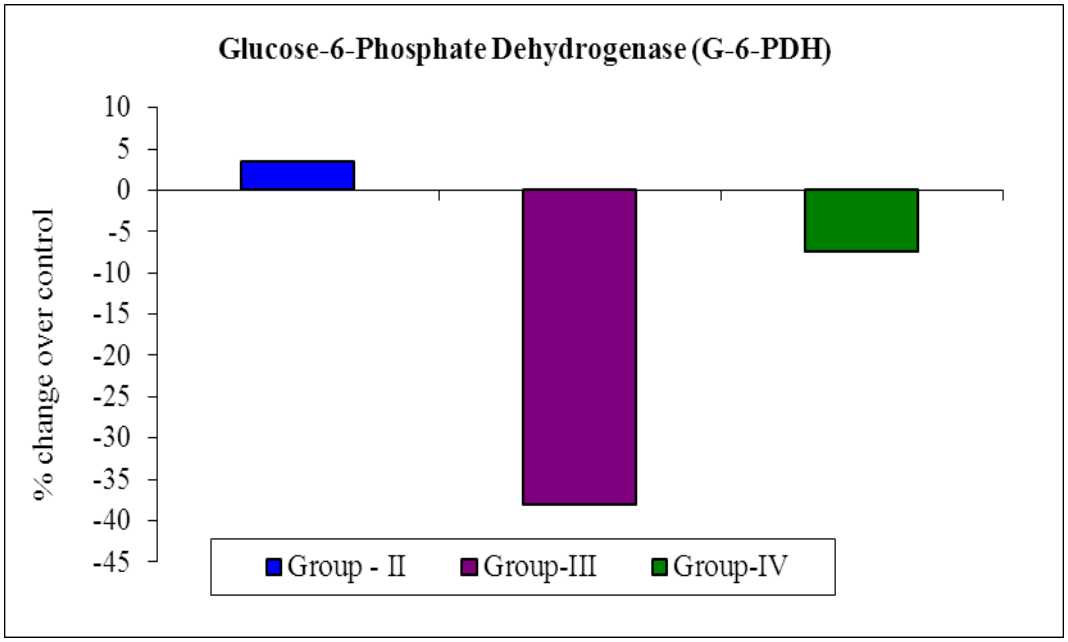


Fig: 1.1 Showing G-6-PDH levels in Liver tissue of control and experimental animals

* Significant difference from that of Diabetic Control animals P < 0.001.
Values are mean, SD: n=6



Values in the parentheses are % change from Control

Fig: 1.2 Showing % change of G-6-PDH levels in Liver tissue of control and experimental animals

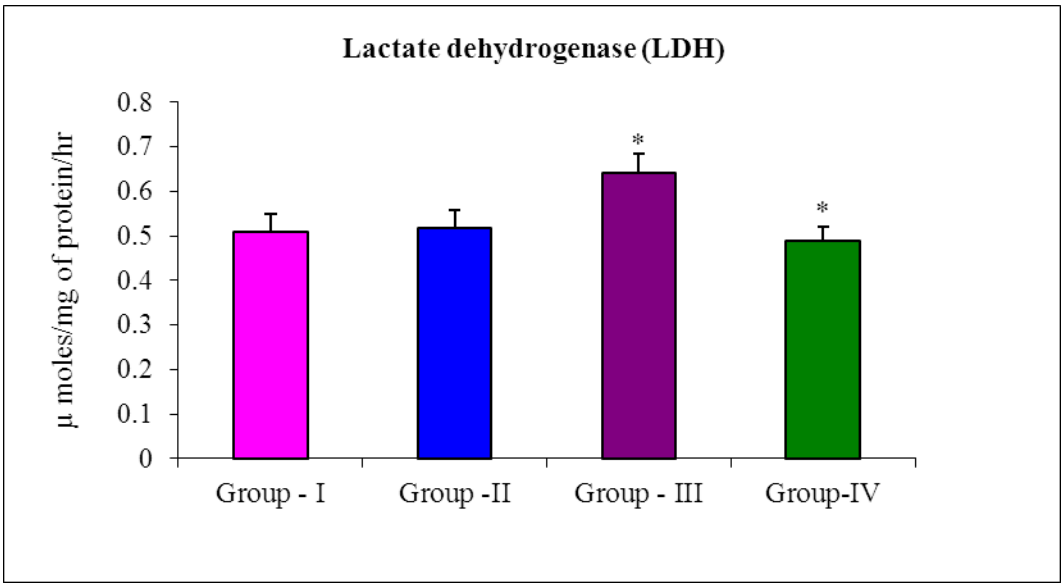


Fig: 2.1 Showing LDH levels in Liver tissue of control and experimental animals

* Significant difference from that of Diabetic Control animals $P < 0.001$.

Values are mean, SD: n=6

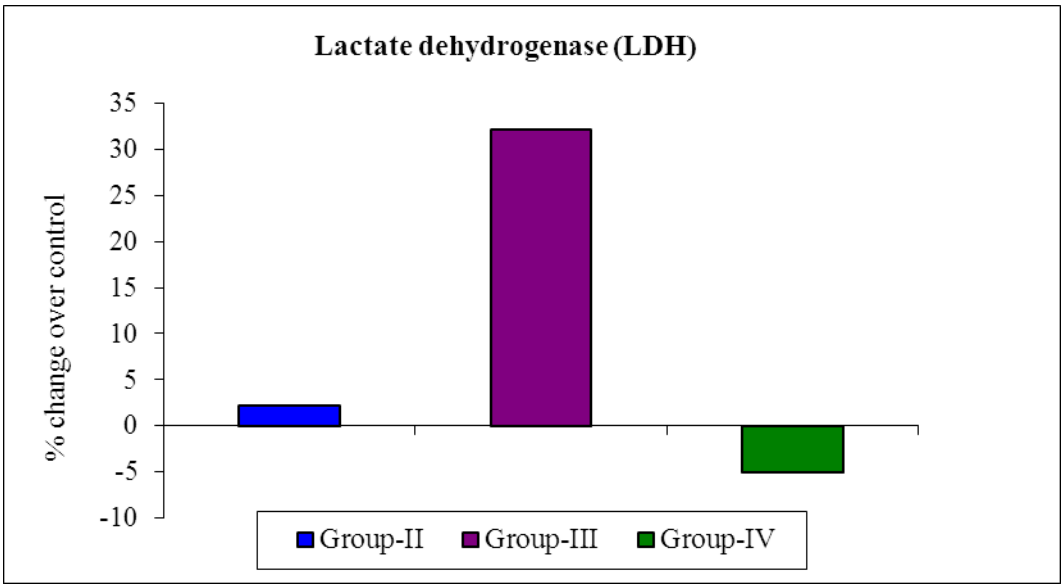


Fig: 2.2 Showing % change of LDH levels in Liver tissue of control and experimental animals

Values in the parentheses are % change from Control

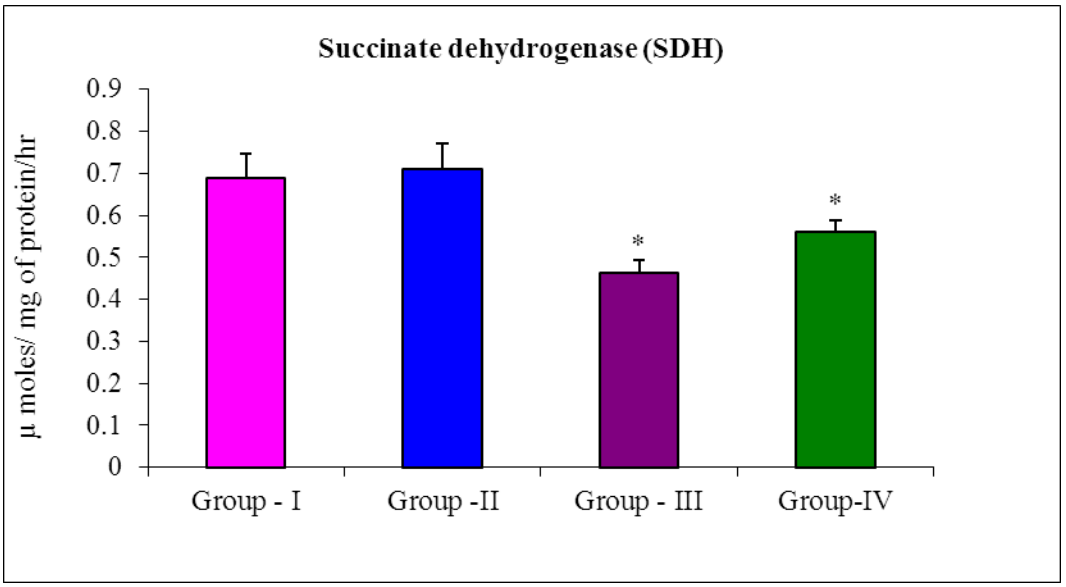


Fig: 3.1Showing SDH levels in Liver tissue of control and experimental animals

* Significant different from that of Diabetic Control animals P < 0.001.

Values are mean SD: n=6

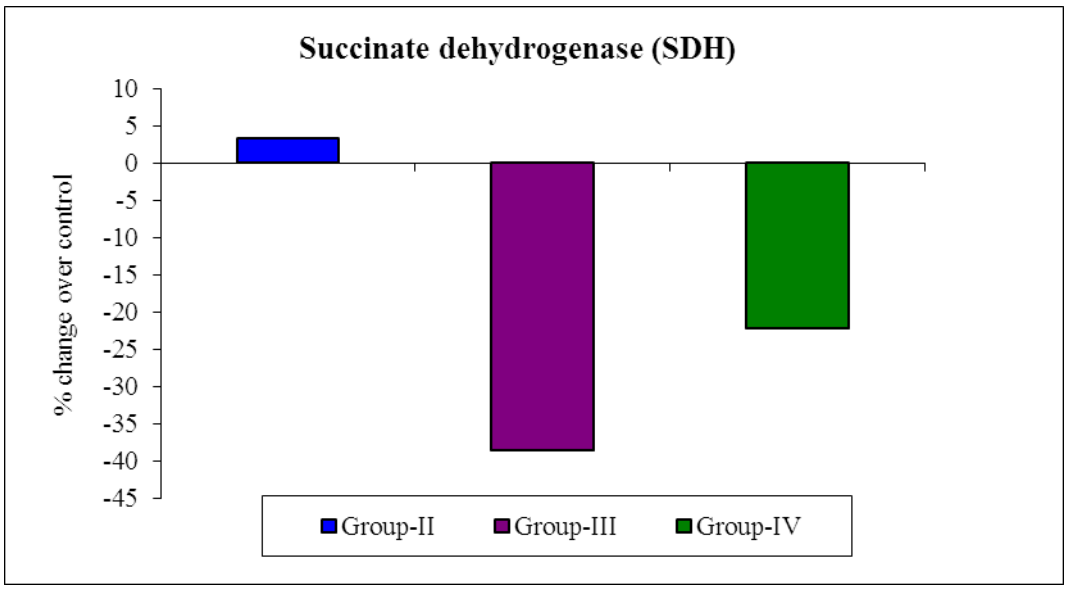


Fig: 3.2 Showing % change of SDH levels in Liver tissue of control and experimental animals

Values in the parentheses are % change from Control

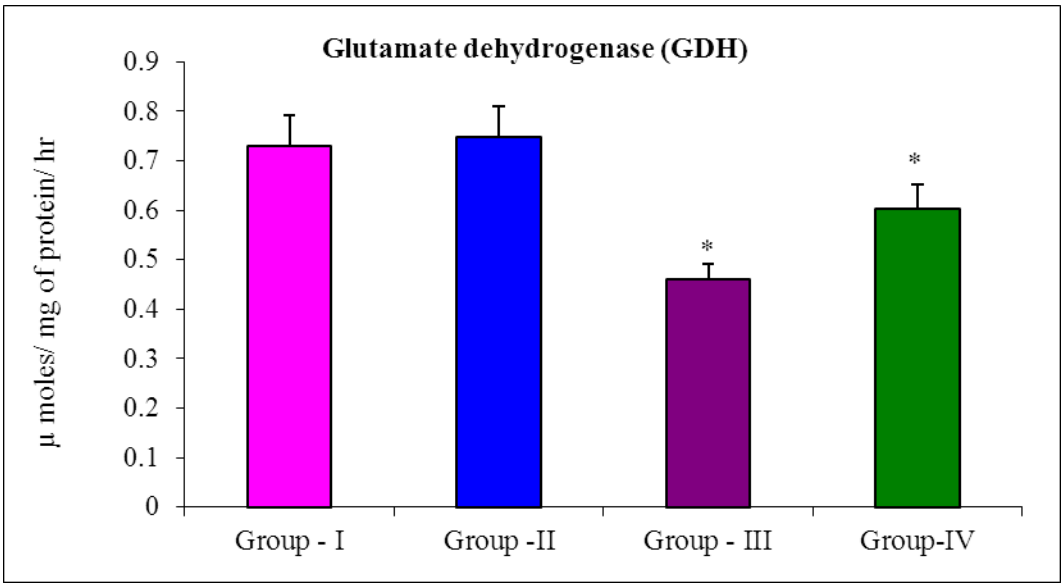


Fig: 4.1 Showing GDH levels in Liver tissue of control and experimental animals

* Significant difference from that of Diabetic Control animals $P < 0.001$.

Values are mean, SD: n=6

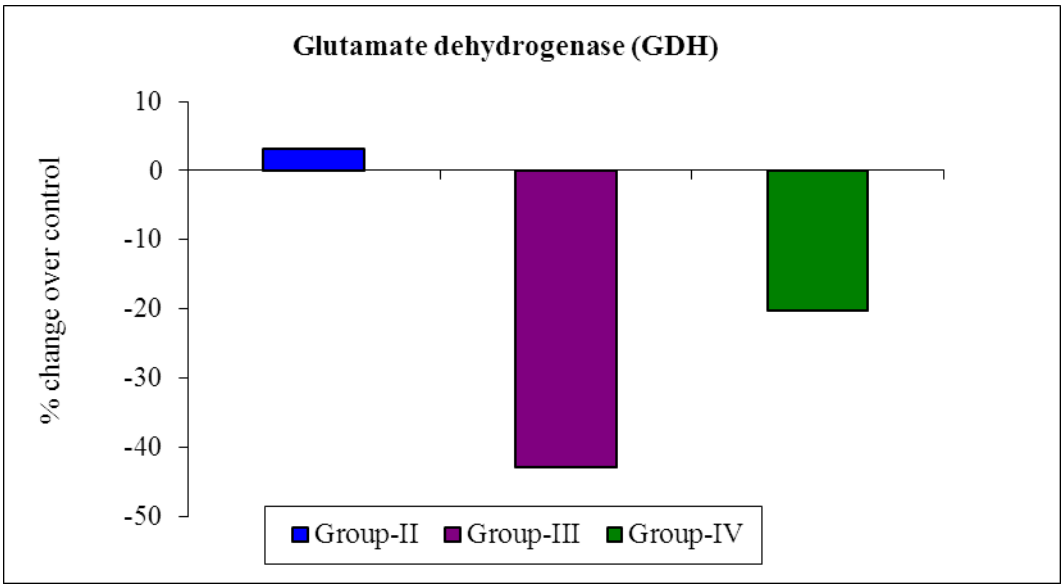


Fig: 4.2 Showing % change of GDH in Liver tissue of control and experimental animals

Values in the parentheses are % change from Control

DISCUSSION

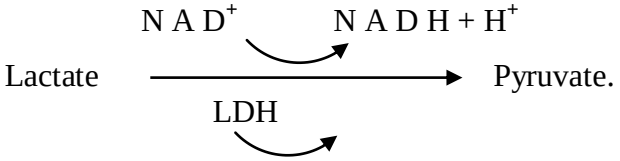
The normal rats treated with *Aloe Vera* plant extract showed increased activity of G-6-PDH. This could be due to elevation of mitochondrial enzymes by plant extract. The activity of G-6-PDH was found to be lowered in diabetic tissue.[17] Glucose-6-phosphate dehydrogenase (G-6-

PDH) is the rate-limiting enzyme in the pentose phosphate pathway. The pentose phosphate pathway is responsible for ribose synthesis and is the main source of NADPH, for GSH reductase and aldose reductase. The decrease in the activity of this enzymes in diabetic condition may result in the diminished functioning of hexose monophosphate shunt and there by the production of reducing equivalents such as NADP and NADPH.

With the treatment of *Aloe vera* plant extract to diabetic rats, the G-6-PDH activity was increased. This increase in G-6-PDH activity may be due to antioxidants which are present in plant extract that elevate the G-6-PDH in induced diabetic rats. Some other studies had showed that the treatment of diabetic rats with curcumin drastically elevated G-6-PDH. Similarly ginger was also found as more effective herb in attenuating diabetes.[18] The extracts of *Coccinia indica* stimulated the shunt enzyme G-6-PDH.[19] The ethanol extract activated G-6-PDH in treated diabetic rats, which could have resulted in an increase in the reducing agent NADPH and a decrease in oxidative stress.

Lactate dehydrogenase (LDH) is a key enzyme of anaerobic glycolysis and catalyses the reversible oxidation of lactate to pyruvate in the terminal step of glycolysis. The reaction catalyzed by LDH interlinks anaerobic and aerobic oxidation of glucose. In view of its role in glucose oxidation the NAD dependent LDH activity was assayed to asses the metabolic significance of this enzyme in compensatory mechanism operating in the tissues of diabetic (Alloxan) and *Aloe vera* plant extract treated diabetic rats.

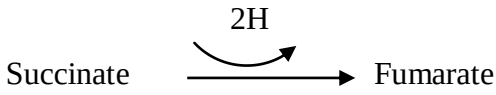
In the present study control rats showed low levels of LDH. The normal rats treated with *Aloe vera* extracts showed still more decreased activity of LDH. This is due to the regulation of NAD+/NADH ratio following stimulation of oxidation of NADH. Normal LDH activity is indicative of improved channeling of (pyruvate) 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. This excessive pyruvate is converted to lactate for

which LDH is needed and therefore the activity of LDH may be increased due to less insulin availability in diabetes.[20, 21] It is clear from the results that oxidative stress caused by Alloxan/STZ increased LDH levels.

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 flavoprotein 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 activities of citric acid cycle enzymes like SDH and ICD.[22] Hyperglycemia results in decreased activities of citric acid cycle and pentose phosphate pathway enzymes as the phosphorylated glucose enters into the pathways like glucogenesis and glycopreotein synthesis.[23, 24, 25] Alloxan exerts secondary toxic effects on the hepatocyte. Deleterious effects of oxidative stress on mitochondrial respiration, ATP synthesis and membrane properties are mainly connected with extensive peroxidation of membrane properties and mainly connected with the extensive peroxidation of membranous polyunsaturated phospholipids, the integrity of which is important for the functioning of mitochondrial respiratory chain. When Alloxan subjected rats was treated with *Aloe vera* extract as in the case of groups-IV, the activity was increased. This elevation is due to the antioxidant compounds present in these plant extracts which have the capacity of normalizing the levels of SDH. Increase in SDH activity in plant extract treated rats indicates better utilization of energy yielding intermediates by TCA cycle. Same results were seen in UDCA (Ursodeoxycholic acid) treated diabetic and ethanol treated rats. This acid ameliorates the oxidative phosphorylation and normalizing mitochondrial enzymes.[26]

Glutamate dehydrogenase (GDH) is a homohexameric 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. The enzyme that is linked to NAD is involved primarily in oxidation of glutamate, whereas the one linked to NADP is associated

with biosynthetic process. This enzyme is also important because of its pivotal position in metabolism occupied by the glutamate and α-ketoglutarate and the ability of these compounds to enter into various metabolic pathways. GDH is a key pyridine nucleotide enzyme which is involved in the oxidative deamination of nitrogen into organic compounds and forms link between carbohydrate and amino acid metabolism. The changes in GDH activity indicate alterations in the production of ammonia and oxidative deamination of glutamate. Hence, its activity was assayed to asses the metabolic significance of this enzyme in Alloxan treated rats with plant *Aloe vera* extract treatment groups.

In plant 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 plant 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 mitochondria. 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.[27] The increased LDH also reported in the present study, consonance with that lactate inhibits the GDH activity. A high NADH/NAD was envisaged in the animals exposed to ethanol treatment, which might play a prominent role in the inactivation of GDH.[28]

CONCLUSION

Oxidative stress is associated with the development and progression of diabetes mellitus.

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