

BENEFICIAL EFFECT OF *ALOE VERA* GEL AND *MANGO* PULP ON STREPTOZOTOCIN-INDUCED DIABETIC RATS AND ITS REGULATION ON OXIDATIVE ENZYMES OF HEART AND KIDNEY

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Abstract:

Natural dietary supplements are essential for the treatment of Diabetes mellitus and its complications. So in our study we focused on combined effect of *Aloe vera* gel and Mango pulp on oxidative enzymes of heart and kidney of rat. In the present work eight groups of rats were taken Group – I, II and III were treated as control rats, Group – IV, V, VI, VII and VIII as experimental groups. As the oxidative enzymes play a key role in carbohydrate metabolism pathway and it is expected changes in citric acid cycle during diabetes. Hence it is essential to understand the function of some of the important marker enzymes and to find out their correlation with dietary supplements and diabetes. An attempt has been made in the present study to analyze the activity of dehydrogenase enzymes like MDH, SDH and LDH in both control and experimental rat kidney and heart tissues, as they exhibit important role in ATP production and in electron transport system also. The result reveals that MDH and SDH activities were decreased in Diabetes induced group and in contrast the LDH was increased. On treatment with *Aloe vera* gel and Mango pulps individually and in combination of both the alterations in the oxidative enzymes of heart and kidney were reestablished. Thus from these findings, it is possible to recommend natural dietary supplements such as mango pulp and *Aloe vera* gel for control of sugars in diabetic patients without any side effects.

Key words:

Diabetes mellitus, *Aloe vera*, Mango pulp, Dehydrogenase enzymes and Natural dietary supplements.

1. INTRODUCTION:

Diabetes mellitus is most common and epidemic in nature, the report of International Diabetes Federation (IDF) stated that in year 2019 around 463 million people were diagnosed with diabetes across the world and it is estimated that this number may rise to 700 million by 2045 (PouyaSaeedi et al., 2019, Eleftheria Papachristoforou et al., 2020). It is categorized in heterogeneous group of action or both (Zubin Punthakee et al., 2018). Alterations in the carbohydrate, protein and fat metabolisms are related to insulin secretions in general which play a vital role in energy production, (Radha Madhavi et al., 2012). All mitochondrial enzymes

are involved in energy generation, disturbance in these enzymes will cause change in the cycles of cell and lead to up and down of energy levels. Diabetes is associated with long term complications such as cardiovascular diseases, kidney syndromes, eye diseases and brain disorders (Radha Madhavi et al., 2012). Nature provide us many plants to treat many diseases among them we selected natural miracle medicinal plant *Aloe vera* and pride fruit of India the mango to treat Diabetes mellitus.

Aloe vera is a wonderful succulent plant widely used in traditional medicine. It contains 75 active compounds like anthraquinones, saponins, carbohydrates, chromones, hormones, minerals, vitamins, enzymes, lignin, salicylic acids, and Amino acids and it has many biological activities (SharifMoghaddasi and sandeep kumar varma, 2011). Many studies suggest that it has anti diabetic properties by controlling the blood glucose level, biochemical parameters and antioxidant stress enzymes in an alloxan or streptozotocin induced diabetic animal models (Radha Madhavi et al., 2012 , Abo-Youssef Messiha, 2013, Abd El-Kader et al., 2019)

Mangifera indica belongs to family Anacardiaceae and usually known as mango. It is used in traditional medicine for the cure of various ailments due to presence of more bioactive constituents like carotenoids, tocopherols, ascorbic acid, dietary fiber, and the phenolic compounds mangiferin, gallic acid and quercetin (USDA, 2005). The stem bark and leaves aqueous extract of mango was reported in lowering of blood glucose in streptozotocin-induced diabetic rats (Muruganandan et al., 2005) and glucose-induced hyperglycemia in rats and mice (Aderibigbe et al., 1999 & 2001). High-fat diet fed mice showed positive effect on body composition, blood glucose and lipid profile on supply of freeze dried mango pulp (Lucas et al., 2011). *Aloe vera* fresh juices and mango pulp showed a hypoglycemic effect by decreasing glucose levels and retaining serum enzymes (Dina Anwar et al., 2020). However, to our knowledge, there were no studies exploring the combined effect of Mango pulp and *Aloe vera* gel on oxidative enzymes and in the present study an attempt was made in this direction.

2. MATERIALS AND METHODS

2.1 Plant Material Preparation:

2.1.1 *Aloe vera* extract:

Aloe vera plants were collected from Tirumala hills . The taxonomic identification of *Aloe vera* plant was confirmed by a senior Botanist, Dr. Madhava chetty, Department of Botany, S. V. University. *Aloe vera* leaves were taken from plant and washed under running tap water, then outer green color part was removed, later inner gel of leaf is separated, homogenized by using blender until formation of clear viscous solution then filtered with filter paper to avoid fibrous particles in gel solution. Daily this process is done throughout the experimental period and this gel (300mg/kg body weight) was given freshly to rats daily by oral gavage.

2.1.2 Mango pulp:

Mango pulp was purchased from the Srini food park Pvt. Ltd, Chittoor, Bangarupalem. Mango pulp was stored in refrigerator, 5 gms of mango pulp was mixed in 10ml of distilled water daily and administrated orally (300 mg/kg BW) to the rats.

2.2 Chemicals

Chemicals used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and plastics from Nunclon (Roskilde, Denmark).

2.3 Animals:

Male Wistar albino rats 48 in number weighing around 180 ± 20 gm of 3 months old obtained from animal house of Bangalore were used for this study. Each polycarbonate cage contains 6 rats, they were maintained at a room temperature of $24 \pm 2^\circ\text{C}$, humidity of 45-64% with 12h light/dark cycle and feed with standard rat pellet supplied by Hindustan Lever Ltd., Bangalore, India and water was supplied *ad libitum* through plastic bottle provided with nipples.

2.3.1 Experimental Design:

The animals were divided into eight groups (I, II, III, IV, V, VI, VII and VIII) containing 6 rats in each group.

2.3.2 Induction of Diabetes:

After fasting for 18 hours, Group IV, V, VI, VII, and VIII rats were injected intraperitoneally with a single dose of 40 mg Streptozotocin (STZ) freshly dissolved in 0.1 M cold sodium citrate buffer, (pH 4.5). After injection, they had a free access to food and water, later given 5% glucose solution to drink overnight to counter hypoglycemic shock.

Here Group I, II, III were selected as control group and group IV, V, VI, VII and VIII as experimental groups. Group I act as control which is fed with standard diet. Group II rats were nourished with standard diet and supplemented with 300 mg/kg BW of mango pulp. Group III rats were nourished with standard diet and supplemented with 300 mg/kg BW of *Aloe vera* gel. Group IV rats were nourished with standard diet and STZ induced diabetic group. Group V rats were STZ induced diabetic group and treated with 300 mg/kg BW of *Aloe vera* gel. Group VI rats were STZ induced diabetic group and treated with 300 mg/kg BW of mango pulp. Group VII rats were STZ induced diabetic group and treated with *Aloe vera* gel and mango pulp in 1:1 ratio. Group VIII rats were STZ induced diabetic group and treated with 600 µg/kg BW of Glibenclamide (Standard drug). After completion of experimental period of 21 days the rats were sacrificed, the tissues such as heart and kidney were isolated from rats and used for further biochemical analysis.

2.4 Oxidative enzymes assay:

10% (W/V) homogenates of the Heart and Kidney tissues were prepared in 0.25 M ice cold sucrose solution and centrifuged at 1000 g for 15 minutes at 4°C . The supernatant fraction was used for MDH, SDH and LDH enzyme assay.

2.4.1 Malate Dehydrogenase (MDH) (E.C: 1.1.1.37):

Malate dehydrogenase (L- Malate NAD^+ Oxidoreductase) activity was determined by the method of Lee and lardy (1965). The final volume of 2.0 ml in two separate test tubes were consists of 400 µ moles of sodium malate, 1.0 ml of phosphate buffer (pH 7.4), 100 µ moles of NAD, and 300 µ moles of INT. The

reaction was initiated by the addition of 0.2 ml of heart and kidney tissue enzyme sources were added individually to each tube and incubated for 30 minutes at 37°C. The reaction was stopped by the addition of 5 ml of glacial acetic acid. The formazan formed was extracted overnight by adding 5 ml of toluene to each tube and kept in refrigerator at 5°C, the final colour was read at 495 nm in a Spectrophotometer. The enzyme activity was expressed in μ moles of formazan formed / mg protein / minute.

2.4.2 Succinate dehydrogenase (SDH) (E.C: 1.3.99.1) :

The Spectrophotometeral analysis of SDH (Succinate acceptor oxidoreductase) activity was measured at 495 nm according to the method of Nachlas et. al., (1960) as modified by Prameelamma and Swami (1975) with little changes and expressed in μ moles of formazone formed/mg protein/min. The reaction mixture was prepared separately in two test tubes contained 2 ml possess 400 μ moles of sodium Succinate, 1.0 ml of phosphate buffer (pH 7.0), 100 μ moles of NAD and 300 μ moles of INT. The subsequent steps were followed same as described for MDH analysis.

2.4.3 Lactate dehydrogenase (LDH) (E.C: 1.1.1.27) :

According to Nachlas et. al., (1960) Lactate dehydrogenase activity (L-lactate: NAD⁺ Oxidoreductase) was measured with slight changes followed by Prameelamma and Swami (1975). The reaction mixture was prepared separately in two tubes contained 2 ml of 400 μ moles of sodium lactate, 100 μ moles of NAD and 300 μ moles of INT and 1.0 ml of phosphate buffer (pH 7.4). The subsequent steps were followed same as described for MDH. The enzyme utilizes NAD and INT was reduces to form formazan then it was extracted overnight into 5 ml of toluene. The activity noted as μ moles of formazan formed / mg protein / minute.

Statistical analysis

All the data are expressed as mean , \pm SD. One-way ANOVA.

3.RESULTS:

The experiments reported here were conducted to evaluate the impact of *Aloe vera* and mango pulp on diabetic rats. MDH, and LDH enzyme activity were high in kidney when compared to heart and low in SDH vice versa .

3.1 Malate Dehydrogenase (MDH) activity in Heart:

As shown in the table 1 the activity of MDH in control rats was found to be (5.03 mg protein / min) in heart. Group II and Group III the activity were shown no significant change over the control rats (5.23 mg protein / min) and (5.33 mg protein / min) respectively . When Group IV compared to the control rats found significantly decreased (4.31 mg protein/min). On treatment, Group V to Group VIII (4.88, 4.81, 4.99 and 4.84 mg protein/min) respectively showed significant increase over the Group IV i.e , diabetic rats and no significant change over the control rats.

3.2 Malate Dehydrogenase (MDH) activity in kidney:

As shown in the table 2 the activity of MDH in control rats was found to be (7.05 mg protein / min) in Kidney. In Group II and Group III the activity were shown no significant change over the control rats (7.14 mg protein / min) and (7.26 mg protein / min). When Group IV compared to the control rats found significantly decreased (5.06 mg protein/min). In treated Group V to VII (6.89, 6.82 and 7.01 mg protein/min) respectively showed significant increase over the Group IV and no significant change over the control rats. Group VIII (6.19 mg protein/min) showed significant decrease over the control rats.

3.3 Succinate dehydrogenase activity in Heart:

Our result showed that SDH activity in heart of control rats was (0.50 mg protein / min). Group II, III, V and VII (0.513, 0.508, 0.490 and 0.510 mg protein /min) respectively showed no significant change over the control rats. When Group IV, VI and VIII (0.230, 0.452 and 0.442 mg protein / min respectively) compared to the control rats found significantly decreased but Group VII and VIII showed significant difference when compared to Group IV.

3.4 Succinate dehydrogenase activity in Kidney:

In the kidney of control rats the SDH activity was found to be (2.01 mg protein / min). In Group II and Group III the activity were shown no significant change over the control rats (2.03 mg protein / min) and (2.12 mg protein / min). Group IV (1.13 mg protein / min) and Group VIII (1.63 mg protein / min) the activity was found to be significantly decreased over control rats. Group V to VII (1.98, 1.89 and 1.99 mg protein / min respectively) showed no significant change over the control rats but when compared to Group IV there was increase in the activity.

3.5 Lactate dehydrogenase activity in Heart:

In this study LDH activity in control rats was found to be (0.305mg protein / min) in heart. Group II and Group III the activity were shown no significant change over the control rats (0.306 mg protein / min) and (0.310 mg protein / min). Group IV (0.390 mg protein / min) and Group VIII (0.343 mg protein / min) the activity was found to be significantly increased over control rats. Group V to VII (0.312, 0.314 and 0.302 mg protein / min respectively) showed no significant change over the control rats but when compared to Group IV there was decrease in the activity.

3.6 Lactate dehydrogenase activity in kidney:

LDH activity in kidney of control rats was found to be (3.37 mg protein / min). Group II and Group III the activity were shown no significant change over the control rats (3.48 mg protein / min) and (3.50 mg protein / min). Group IV (4.38 mg protein / min) and Group VIII (3.83 mg protein / min) the activity was found to be significantly increased over control rats. But when Group VIII compared to Group IV there was significant decrease in the activity. Group V to VII (3.41, 3.52 and 3.32 mg protein / min respectively) showed no

significant change over the control rats but when compared to Group IV there was decrease in the activity.

Table 1: Showing MDH, SDH and LDH enzyme activities in Heart of control and experimental rats.

HEART									
S.No	Parameter	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI	Group-VII	Group-VIII
1.	MDH μ moles of formazan formed / mg protein / minute	5.03 \pm 0.53	5.23 \pm 0.50 (+3.97) NS	5.33 \pm 0.52 (+5.96) NS	4.31 \pm 0.45 (-14.31) P<0.001	4.88 \pm 0.48 (-2.98) NS	4.81 \pm 0.49 (-4.37) NS	4.99 \pm 0.50 (-0.795) NS	4.84 \pm 0.42 (-3.77) NS
2.	SDH μ moles of formazan formed / mg protein / minute	0.50 \pm 0.05	0.513 \pm 0.051 (+2.60) NS	0.508 \pm 0.016 (+1.60) NS	0.230 \pm 0.02 (-54.00) P<0.001	0.49 \pm 0.048 (-2.00) NS	0.452 \pm 0.042 (-9.60) NS	0.510 \pm 0.052 (+2.00) NS	0.442 \pm 0.032 (+11.60) P<0.05
3.	LDH μ moles of formazan formed / mg protein / minute	0.305 \pm 0.06	0.306 \pm 0.03 (+0.32) NS	0.310 \pm 0.031 (+1.63) NS	0.390 \pm 0.036 (+27.86) P<0.001	0.312 \pm 0.032 (+2.29) NS	0.314 \pm 0.027 (+2.95) NS	0.302 \pm 0.022 (-0.98) NS	0.343 \pm 0.012 (+12.45) P<0.05

Values are mean , \pm S.D. of 6 individual rats

Values in the parenthesis are % change from that of control

NS means No significant change. P<0.05,P<0.001 statistically significant

Table 2: Showing MDH, SDH and LDH enzyme activities in kidney of control and experimental rats.

KIDNEY									
S.No	Parameter	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI	Group-VII	Group-VIII
1	MDH μ moles of formazan formed / mg protein / minute	7.05 \pm 0.61	7.14 \pm 0.59 (+1.27) NS	7.26 \pm 0.60 (+2.97) NS	5.06 \pm 0.59 (-28.22) P<0.001	6.89 \pm 0.70 (-2.26) NS	6.82 \pm 0.66 (-3.26) NS	7.01 \pm 0.69 (-0.56) NS	6.19 \pm 0.51 (+12.19) P<0.05
2	SDH μ moles of formazan formed / mg protein / minute	2.01 \pm 0.23	2.03 \pm 0.24 (+0.99) NS	2.02 \pm 0.21 (+0.49) NS	1.13 \pm 0.15 (-43.78) P<0.001	1.98 \pm 0.19 (-1.49) NS	1.89 \pm 0.14 (-5.97) NS	1.99 \pm 0.19 (-0.99) NS	1.63 \pm 0.17 (+18.90) P<0.05
3	LDH μ moles of formazan formed / mg protein / minute	3.37 \pm 0.21	3.48 \pm 0.23 (+0.29) NS	3.50 \pm 0.23 (+0.89) NS	4.38 \pm 0.34 (+29.97) P<0.001	3.41 \pm 0.32 (+1.18) NS	3.52 \pm 0.39 (+4.45) NS	3.32 \pm 0.32 (-1.48) NS	3.83 \pm 0.31 (+13.64) P<0.05

Values are mean , \pm S.D. of 6 individual rats

Values in the parenthesis are % change from that of control

NS means No significant change. P<0.05, P <0.001 statistically significant

4. DISCUSSION:

In our study *Aloe vera* gel and Mango pulp combination had restored the alterations in the mitochondrial enzymes that were affected by streptozotocin induced diabetes. In our study MDH was decreased in streptozotocin induced diabetic rat heart and kidney tissues which indicate the decrease in usage of malate. The reason behind it may be due to changes in TCA cycle, as MDH a key enzyme in catalyzing the interconversion of malate and oxaloacetate using NADP⁺ or NAD⁺ as cofactor which in turn leads to variations in oxygen level which affect the known pathways such as glycolysis, citric acid cycle and oxidative phosphorylation (Takahashi-Iniguez et al., 2016). As on treatment with *Aloe vera* gel and mango pulp and in combination showed positive sign in diabetic rats, probably this is due to presence of beneficial phytochemicals with good antioxidative properties in both the plants, which have showed similar pattern of impact compared to Glibenclamide, a synthetic drug which is used for diabetes. In streptozotocin induced diabetic rats showed reduced oxidative enzymes and the some were regulated by the ethanolic extract of *Aloe vera* (Radha Madhavi et al., 2012). These findings support our results as *Aloe vera* gel has accelerated oxidative properties which is evidenced by restoration of MDH activity to the normal level in streptozotocin induced diabetic rats.

SDH play an important role in the citric acid cycle and respiratory electron transfer chain. SDH enzyme catalysis the succinate to fumarate. On oxidation of succinate, SDH reduces the FAD^+ cofactor and followed by the electron transfer, (Jared Rutter et al., 2010; Payani et.al, 2019). In our study SDH activity was decreased in streptozotocin induced diabetic rat heart and kidney tissues may be due to development of oxidative stress. Both the oxidant and anti oxidative enzymes regulate the imbalance raised due to oxidative stress which was the main cause of a diabetes. In diabetic rats the antioxidative enzymes are regulated by the *Curcuma longa* and *Trigonella foenumgraecum* (Guru sekhar et al., 2010). This supports our results, that the oxidative stress was suppressed by elevation of SDH activity due to presence of antioxidant properties in *Aloe vera* gel and mango pulp.

Lactate dehydrogenase (LDH) is a terminal product of glycolytic pathway. It plays major role in the development of pyruvate to lactate and vice-versa to produce energy under anaerobic conditions (Pradeepkiran and Bhaskar, 2016). Free radicals cause tissue damage that leads to the increased levels of the LDH activity. In support to this (Sadak Basha et al., 2010) studies also reported tissue damage in the diabetic rats similarly. In our study LDH activity was increased in streptozotocin induced diabetic rat heart and kidney tissues, this may be due to low availability of insulin. The decreased insulin levels might be anticipated due to the damaged pancreas. As streptozotocin mainly damage the β cells of pancreas, its chief function is to produce insulin. However, treatment of diabetic rats with *Aloe vera* gel and mango pulp and in combination of both lowered the activity of LDH where its level is almost similar to glibenclamide treated rats. This may be due to regulation of production of pyruvate and NADH^+ , which leads to the oxidation of glucose. Earlier studies also reported elevated glucose levels in blood of Alloxan and STZ induced diabetic rats and the same levels were controlled by the *Aloe vera* (Mude Ravi Naik et al., 2012). Our results coincides with other research findings like (Mishra et al., 2019) they stated that *Cymbopogon citratus* herb helps in lowering of LDH activity in STZ/HFD induced diabetic dyslipidemia rats. *Aloe vera* extract treated rats maintained low LDH activity in streptozotocin-induced diabetic rats was also reported by (Rajasekaran et al., 2004). To our knowledge literature on the mango pulp effect on oxidative enzymes were not found earlier.

5.CONCLUSION

Now days more concern in usage of Natural dietary supplements so, from this study we recommend the combination of *Aloe vera* gel and mango pulp to reduce the oxidative stress. Good and great useful for diabetic patients and need little more work on industrial standards for commercialization.

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