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Anti-Diabetic and Anti-Oxidative Effect of *Aloe Vera (L.)* Gel: An In-Vitro Approach

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Abstract

Medicinal plants are utilized in various formulations to treat a wide range of illnesses, includig diabetes.The present study focused on anti-diabetic and anti-oxidative effect of *Aloe vera (L.)* in an *in-vitro* model. Wistar strain albino rats with less than 350 mg/dl of fasting blood glucose level on the 7th day of Streptozotocin (STZ) injection were considered for this study and left for obverting a chronic state of diabetes. On the 29th day of STZ injection, rats were sacrificed and targeted tissues were incubated for 2 hours in an *in-vitro* medium with 1, 2, and 4 mg of *Aloe vera (L.)* hydro-ethanol (40:60) extract (AVHE) separately and subjected to evaluation with adopted parameters. Results showed a significant ($p<0.05$) recovery in the glucose-6-phosphatase and oxidative stress markers along with GOT and GPT activities in extract-charged groups whereas no significant ($p>0.05$) changes was noted in hexokinase enzymes activity compared to the unexposed diabetic group. Statistical analysis showed no significant ($p>0.05$) difference in the recovery level among 1, 2, and 4 mg doses of AVHE. It can be concluded that AVHE has direct ameliorative effect on diabetes and its linked oxidative stress markers.

Keywords: Diabetes, Oxidative stress, *in-vitro* study, Glucose-6-phosphatase, Hexokinase

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Introduction

Diabetes and other non-communicable diseases have been linked to rapid changes in lifestyle and technological advancements **(Sun et al., 2023).** Diabetes mellitus is a metabolic cum life style disease marked by abnormalities in insulin secretion, action, or both, leading to disruptions in protein, lipid, and carbohydrate metabolism and impaired glucose homeostasis **(American Diabetes Association, 2009).** Globally, onset of diabetes is rising, but in developing nations like India, it poses a serious health risk and societal cost **(Pradeepa et al., 2021).**

For thousands of years, plants have been the foundation of traditional medical systems all across the world. Ethnobotanical remedies still have a significant impact on healthcare even in contemporary medical systems **(Yuan et al., 2016).** In developing nations, traditional medicine provides healthcare to about 80% of the population. Herbal medicines are gaining popularity due to their effectiveness, few adverse effects in clinical trials, and reasonable low cost. **(Oyebode et al., 2016).** The use of plant medicines for many illnesses, including diabetes mellitus, is even approved by the World Health Organization **(Patil et al., 2012).**

The succulent plant *Aloe vera (L.)* is utilized in traditional medicine. *Aloe vera (L.)* is widely recognized for its anti-inflammatory, anti-oxidant, immune-boosting, anti-aging, and anticancer properties. It also relieves sunburn. Owing to its distinct composition, *Aloe vera (L.)* has been used in a variety of industrial applications **(Surjushe et al., 2008).** In vitro tests can be employed as early screening methods for the evaluation of antidiabetic activity of different plants, allowing for the screening of a large number of possible therapeutic candidates. They could offer helpful details regarding the mode of action of medicinal substances **(Roy et al., 2013).** Considering this background, hydro-ethanol extract of *Aloe vera (L.)* was used for the assessment of in-vitro anti-diabetic and anti-oxidative activities.

Methods and materials

Hydro-ethanol extract preparation

Leafs of *Aloe vera (L.)* were collected from the local nursery in Midnapore and verified by the taxonomist of Botany and Forestry department, Vidyasagar University. Only the gel part was separated and pulverized by a grinder. The pulverized gel 500 ml mixed with hydro-ethanol (40:60) and concentrated in a rotary evaporator and then air drying for 48 hours in room temperature.

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Experimental design

Ethical concern was taken from the Institutional Animal Ethics Committee before execution of the experiment. Thirty matured, normoglycemic, male rats having bodyweight of 130 ± 10 g were purchased from an authorized vendor (Saha enterprise). Before the initiation of the experiment, animals were housed in ambient temperature (25 ± 2^0C), humidity, and 12-hour light/12-hour dark condition for 10 days with a sufficient amount of water and food supply. After the acclimatization period, rats were made diabetic by a single intramuscular injection of streptozotocin (STZ) at a dose of 4 mg/ 100 g of body weight and left in the favorable condition for 28 days to develop a chronic diabetic state. Fasting blood glucose level of more than equal to 350 mg/dl was considered as diabetes rats. Hydro-ethanol extract of 10, 20 and 40 mg was added to 10 ml of in-vitro media and so the concentrations of the extract were 1, 2 and 4 mg/ml.

Group division

Rats were sacrificed on the 29th day of STZ injection by strictly maintaining CCSEA guidelines. Skeletal muscle, liver, cardiac muscle, and kidney were taken out for the *in-vitro* study.

Control: Targeted tissue from the non-diabetic rat + 10 ml Kreb's ringer solution (KRS, pH 7.4) + 0.5 ml distilled water (DW)

Diabetes: Targeted tissue from the diabetic rat + 10 ml KRS + 0.5 ml DW

1 mg *Aloe vera (L.)* hydro-ethanol extract (AVHE): Targeted tissue from the diabetic rat + 10 ml KRS + 0.5 ml DW + 0.5 ml of 10 mg AVHE

2 mg AVHE: Targeted tissue from the diabetic rat + 10 ml KRS+ 0.5 ml DW + 0.5 ml of 20 mg AVHE

4 mg AVHE: Targeted tissue from the diabetic rat + 10 ml KRS + 0.5 ml DW + 0.5 ml of 40 mg AVHE

The tissues were incubated for 2 hours with a constant oxygen supply at a rate of 30 bubbles/min.

Carbohydrate metabolic enzymes

Hexokinase and glucose-6-phosphatase enzyme activities were measured spectrophotometrically at 340 nm wavelength. The amount of NADPH reduction after initiation and end of the reaction in cuvette denoted the activity of hexokinase enzyme whereas liberation of inorganic PO_4^{3-}/g of tissue denoted the activity of glucose-6-phosphatase enzyme **(Chou et al., 1975; Swanson, 1955).**

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Oxidative stress markers

Catalase and superoxide dismutase (SOD) enzyme activities were measured and thiobarbituric reactive substances (TBARS) level was quantified by standard methods **(Beers et al., 1995; Marklund et al., 1974; Ohkawa et al., 1979).**

Toxicity markers

Glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities in liver were measured biochemically (**Jagadeesan et al., 2006**).

Statistical analysis

The collected data was evaluated statistically using Analysis of Variance (ANOVA) followed by multiple comparison Student's two-tail "t" tests. Differences were considered significant at the level of $p<0.05$. **(Sokal et al., 1997)**

Results

Carbohydrate metabolic enzymes

No significant ($p>0.05$) change was noted in hexokinase enzyme activity between extract charged groups (EEG) and unexposed diabetic group (UDG) (Fig 1). Inhibition in the glucose-6-phosphatase activity was noted significantly ($p<0.05$) in EEG after 2 hours of incubation compared to UDG. The percentage of inhibition in skeletal muscle was 18.10% by 1 mg, 21.12% by 2 mg and 23.06% by 4 mg. In liver, this inhibitory percentage was 13.91% by 1 mg, 16.14% by 2 mg, and 16.32% by 4 mg. In cardiac muscle, the said percentage was 16.27 % by 1 mg, 17.12 % by 2 mg, and 17.54 % by 4 mg. Statistical comparison showed no significant ($p>0.05$) differences in the level of inhibition among the said doses (Fig 2).

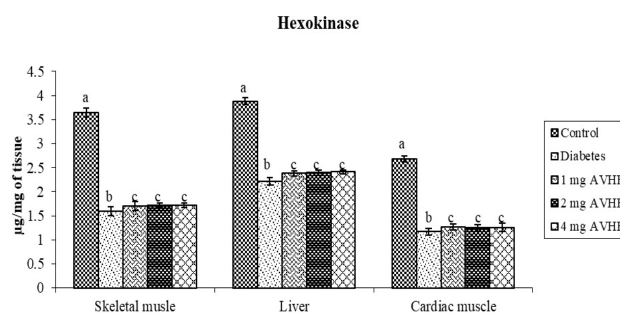

Fig 1 Direct effect of AVHE on hexokinase activities in targeted organs. Bars are expressed as mean ± SEM, n = 6. ANOVA followed by "Multiple comparisons Student's two-tail t-tests". Bars with different superscripts (a, b, c) differ from each other significantly, $p<0.05$.

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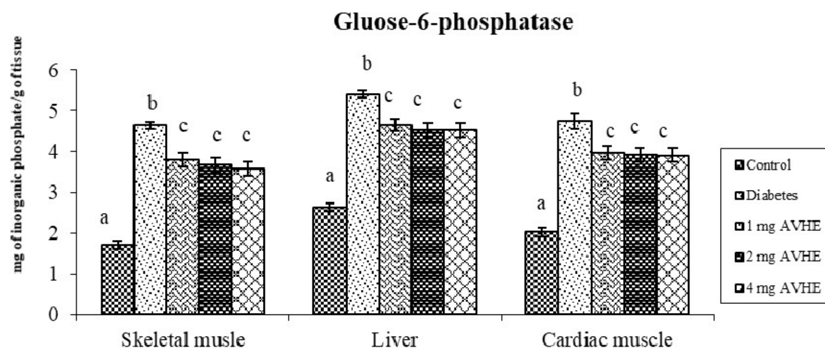

Fig 2 Inhibitory activity of AVHE on glucose-6-phosphatase enzyme after 2 hours of incubation in an in-vitro medium. Bars are expressed as mean ± SEM, n = 6. ANOVA followed by "Multiple comparison Student's two-tail t-tests". Bars with different superscripts (a, b, c) differ from each other significantly, p<0.05.

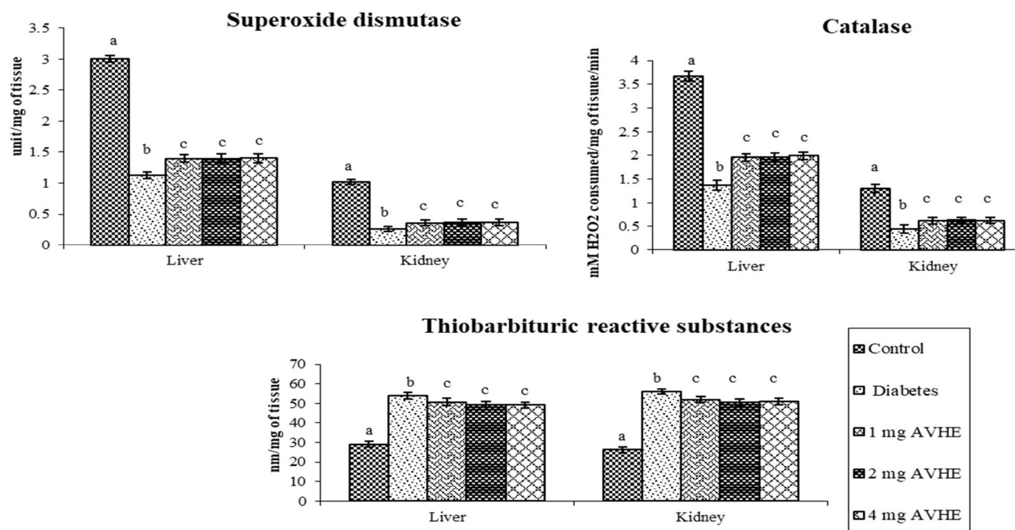

Fig 3 In-vitro effect of AVHE on antioxidant enzyme kinetics and TBARS level. Bars are expressed as mean ± SEM, n = 6. ANOVA followed by "Multiple comparison Student's two-tail t-tests". Bars with different superscripts (a, b, c) differ from each other significantly, p<0.05.

Oxidative stress marker:

Catalase and SOD activities were reduced significantly (p<0.05) in UDG than the control group (CG). A significant (p<0.05) level of recovery was noted in EEG than UDG but not in a dose-dependent manner. The recovery percentage of SOD activity in kidney and liver was 36.12 %

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and 24.64 % by 1 mg, 39.16 % and 23.82 % by 2 mg, 39.34 % and 24.00 % by 4 mg respectively. For catalase activity the percentage of recovery in kidney and liver was 36.48 % and 43.38 % by 1 mg, 38.68 % and 44.11 % by 2 mg, and 38.46 % and 46.32 % by 4 mg respectively. The level of TBARS was increased significantly (p<0.05) in UDG than CG. Two hours of AVHE exposure decreased TBARS level at a significant level (p<0.05) against UDG. The percentage of improvement of TBARS in kidney and liver were 7.06 % and 6.10 % by 1 mg, 9.44 % and 8.58 % by 2 mg, and 8.83 % and 9.01 % by 4 mg respectively (Fig 3).

Toxicity markers

Glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities were higher in UDG than CG. After incubation for 2 hours with 1, 2, and 4 mg AVHE, significant (p<0.05) diminution was noted in the said parameter than UDG. The recovery percentage of hepatic GOT activity was 13.43 % in 1 mg, 15.23 % in 2 mg and 17.52 % in 4 mg of EEG. Similarly, in GPT activity the percentage of recovery was 9.72 % in 1 mg, 10.70 % in 2 mg, and 11.49 % in 4 mg of EEG. No significant (p>0.05) difference in the recovery of GOT and GPT activities were noted among 1, 2, and 4 mg doses of AVHE (Fig 4).

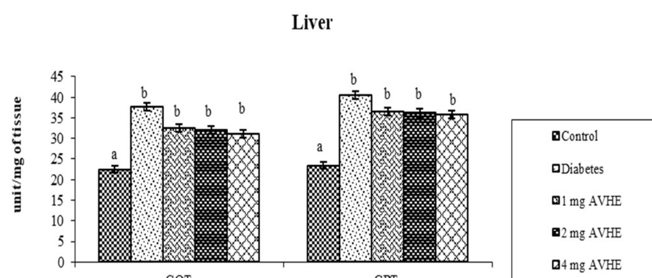

Fig 4 Changes in the activities of GOT and GPT after 2 hours of incubation with AVHE in an in-vitro medium. Bars are expressed as mean ± SEM, n = 6. ANOVA followed by "Multiple comparison Student's two-tail t-tests". Bars with different superscripts (a, b) differ from each other significantly, p<0.05.

Discussion

The purpose of the in vitro-experiment was to investigate whether *Aloe vera (L.)* has any direct effects on oxidative stress management and carbohydrate metabolic enzymes in diabetes condition for its management. The results showed significant inhibition in the activity of glucose-6-phosphatase in EEG which catalyzes the terminal step of gluconeogenesis and glycogenolysis is compatible with other reports **(Mitra et al., 2020; Rosas-Ramírez et al., 2020)** in this domain and can be an important therapeutic tool for the *in-vivo* regularization of blood glucose level. The possible mechanism for such inhibitory activity may be due to the binding of the phyto-consitituents of the said extract in competitive aspect or it can bind with the concerned enzyme and inhibit it by allosteric modulation **(Mata-Torres et al., 2020).** The unchanged activity of hexokinase in EEG compared to UDG may be either the said

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extract has no direct effect on the activity of the hexokinase or it may be caused by inadequate processing time for the execution of any potential non-genomic effect.

Diabetes is associated with low genomic expression of anti-oxidant enzymes as well as hyper-activation of lipid peroxidation pathways **(Ara et al., 2020)** and this is substantiate with our results. After direct exposure to *Aloe vera (L.)* antioxidant enzyme activities like catalase and SOD were recovered towards the control may be due to the activation of the antioxidant enzymes after they bound to the phytomolecule (s) present in the said extract (**Tripathy et al., 2016**). Another possible way may be due to the potential non-genomic stimulatory effect of AVHE on oxidative stress averting enzymes through providing necessary co-factors needed for their catalytic proceeding as diabetes is linked with deficiency of certain minerals **(Joe, 2009)** and *Aloe vera (L.)* is enriched with micro-nutrients **(Asadi et al., 2017).** The metabolomics study showed a decreased level of TBARS in EEG than UDG. This may be due to the free radical quenching or scavenging activity of the secondary metabolites present in the said extract or may be due to the inhibition of mitochondrial uncoupling reaction followed by attenuation of reactive oxygen species linked lipid peroxidation **(Ademowo et al., 2017).** Tentative mechanism of improvement in GOT and GPT activity in EEG than UDG could be either phytomolecule (s) of the said extract directly binds to GOT and GPT to reduce the activity of these enzymes, or it could stabilize lysosomes to reduce the amount of GOT or GPT released into the cytosolic compartment of the concerned cell **(McCarty, 2013).**

The results of the study suggested that the active phytocontituents present in the AVHE has anti-diabetic and anti-oxidative effect. The way in which the said extract executes the anti-oxidative and anti-diabetic activities in animal models and the selection of the potent dose for this purpose may be the subject of future research.

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References

Ademowo, O.S., Dias, H.K.I., Burton, D.G.A and Griffiths, H.R. (2017): Lipid (per) oxidation in mitochondria: An emerging target in the ageing process?, Biogerontology. 18: 859-879.

American Diabetes Association. (2009): Diagnosis and classification of diabetes mellitus. Diabetes. Care, Erratum, pp S62-S67.

Ara, F., Tripathy, A., Ghosh, P and Ghosh D. (2020): Correction of glycemic and oxidative stress sensors in streptozotocin induced diabetic rat model: Impact of dose dependent study of n-butanol solvent fraction of hydro-methanol extract of banana flower (*Musa balbisiana*), J. Food. Biochem. 44: 1-13.

Asadi, N., Bahmani, M., Kheradmand, A and Rafieian-Kopaei, M. (2017): The impact of oxidative stress on testicular function and the role of antioxidants in improving it: A review, JCDR. 11: IE01–IE05.

Beers, R.F and Sizer, I.W. (1952): A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase, J. Biol. Chem.195: 133–140.

Chou, A.C and Wilson, J.E. (1975): Carbohydrate metabolism. In: Methods in Enzymology (W.A. Wood, ed.), Academic Press. New York, 20-21.

Joe, M., Mae, C., Ali, S and Arshag, D. (2009): Mooradian; The role of micronutrients in managing diabetes, Diabetes. Spectr. 22: 214–218.

Maan, A.A., Nazir, A., Khan, M.K., Ahmad, T., Zia, R., Murid, M and Abrar, M. (2018): The therapeutic properties and applications of *Aloe vera:* A review, J. Herb. Med. 12: 1-10.

Marklund, S and Marklund, G. (1974): Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase, Eur. J. Biochem. 47: 469–474.

Mata-Torres, G., Andrade-Cetto, A., Espinoza-Hernández, F.A and Cárdenas-Vázquez, R. (2020): Hepatic glucose output inhibition by Mexican plants used in the treatment of type 2 diabetes, Front. Pharmacol. 11: 215-224.

McCarty, M. F. (2013): Nutraceutical strategies for ameliorating the toxic effects of alcohol, Med. Hypotheses. 80: 456–462.

Mitra, D., Tripathy, A and Ghosh, D. (2020): Antidiabetic and antioxidative efficacy of the constituents present in the fraction solvents of *Curcuma amada* rhizomes: An experimental study on the diabetic rat model, J. Food. Biochem. 44: 1-11.

Ohkawa, H., Ohishi, N and Yagi, K. (1979): Assay for lipid peroxides in animal tissues by thiobarbituric

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acid reaction, Anal. Biochem. 95: 351–358.

Oyebode, O., Kandala, N.B., Chilton, P.J and Lilford, R.J. (2016): Use of traditional medicine in middle-income countries: a WHO-SAGE study, Health. Policy. Plann. 31: 984-991.

Patil, A., Nirmal, S., Pattan, S., Tambe, V and Tare, M. (2012): Antidiabetic effect of polyherbal combinations in STZ induced diabetes involve inhibition of α-amylase and α-glucosidase with amelioration of lipid profile, Phytopharm. 2: 46-57

Pradeepa, R and Mohan, V. (2021): Epidemiology of type 2 diabetes in India, Indian. J. Ophthalmol. 69: 2932-2938.

Rosas-Ramírez, D., Escandón-Rivera, S., Andrade-Cetto, A and Arreguín-Espinosa, R. (2020): Glucose-6-phosphatase and α-glucosidase inhibitors from *Smilax moranensis* roots identified by affinity-directed fractionation, Rev. Bras. Farmacogn. 30: 832-837.

Roy, A and Geetha, R.V. (2013): In vitro α-amylase and α-glucosidase inhibitory activities of the ethanolic extract of *Dioscorea villosa* tubers, Int. J. Pharm. Bio. Sci. 4: 49-54.

Sokal, R.R and Rohle, F.J. (1997): Introduction to Analysis of Variance. In: Biometry (R.R. Sokal and F.J. Rohle, eds.), WH Freeman and Company. New York, 179–206.

Sun, X., Yon, D.K., Nguyen, T.T., Tanisawa, K., Son, K., Zhang, L., Shu, J., Peng, W., Yang, Y., Branca, F and Wahlqvist, M.L. (2023): Dietary and other lifestyle factors and their influence on non-communicable diseases in the Western Pacific region, Lancet Reg. Health West. Pac. 2023: 1-23.

Surjushe, A., Vasani, R and Saple, D.G. (2008): *Aloe vera:* a short review, Indian. J. Dermatol. 53: 163-166.

Swanson, M.A. (1955): Glucose-6-phosphatase from liver. In: Methods in enzymes (S. P. Colowick and N. O. Kaplan, eds.), Academic Press. New York. 154.

Tripathy, A., Ara, F., Ghosh, P and Ghosh, D. (2020): Effect of lycopene on testicular androgenic and anti-oxidative enzymes in cyproterone acetate-induced male infertile Wistar strain albino rats: An in vitro study, Andrologia. 52: 1-6.

Yuan, H., Ma, Q., Ye, L and Piao, G. (2016): The traditional medicine and modern medicine from natural products, Molecules. 21: 559-577.

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