DcoD ameliorate diabetic retinopathy through aldose-sorbitol cleavage

Comment [s1]: The type of study should be mentioned at the end of the title.

Abstract

Comment [s2]: Write again based on the scientific abstract

A new effect of D-Co-D, polyherbal Siddha drug for the management of diabetes mellitus has been discovered in the present study relating to its effect in reducing the episode of sugar cataract a common ocular problem encountered among diabetic people. D-Co-D found to exert biphasic action on both aldose reductase and sorbitol dehydrogenase. Aldose reductase expression increase over 30% in diabetic condition to convert excess glucose to sorbitol which is further cleaved into fructose by another enzyme sorbitol dehydrogenase; D-co-D inhibited the activity of both enzymes comparable to the known positive control. The herbs in the formulation suh as Tinospora cordifolia was effective on aldose reductase while Momordica charantia and Zingiber officinale where effective on sorbitol dehydrogenase. Result of the findings and the treatment advancement of D-Co-D is discussed in the article.

Keyword

Comment [s3]: Write based on the MeSH system

D-Co-D, Aldose reductase, Sorbitol dehydrogenase, diabetic retinpathy

Introduction

Comment [s4]: Use newer sources
Explain the scientific gap better

Excessive blood glucose burden in the blood stream resulting in both high HbA1c and advanced glycation end product accumulation occur during diabetic condition; does not speak only about aberration of glucose metabolism but also about lipid and protein as well along with deteriorating organ (vital organs) health. [1, 2, 3]

The high blood glucose would naturally increase the biological systemto respond and react in an anomalous manner through several mechanisms (enzymatic and hormonal) to utilize the excess sugar and the same situation also may compel various vital organs like kidney, liver, nerve cells etc., to re-adjust to the 'high sugar' environment by adopting partial damage either at structural or functional level. The most consequent effect of hyperglycemic condition is, increased aldose reductase activity. This enzyme is abundantly present in all mammalian cells and more so in cornea, lens epithelial cells, kidney, retina and sciatic nerves.[4, 5, 6]

The major role aldose reductase enzyme is to breakdown excess sugar and convert thesame into sorbitol and then sorbitol to fructose through another enzyme, sorbitol dehydrogenase. In normal condition, the above process would account for about 3% consumption of glucose which increases by 30% during diabetic condition. The end product accumulation would increase the osmotic pressure and severely damage the tissues, resulting in 'sugar eye', followed by microvascular damage and nerve damage resulting in diabetic retinopathy. [7, 8, 9]

Sugar cataract and diabetic retinopathy are the second most common co-morbidity conditions associated with and due to diabetes mellitus. Therefore, early intervention to scissor out aldose reductase enzyme and sorbitol dehydrogenase enzyme are necessary to prevent the damages of lens, cornea, retina and optic nerve, the imminent medical complications waiting to maul.

For the proactive treatment measure of various diabetic co-morbidities, especially to prevent/delay/reduce the organ damage due to hyperglycemia, instead of relying on 'synthetic' drugs which have unitary therapeutic effect, although with high target specificity, herbal products and preparations are highly desired purely due to pluripotent pharmacological action.

Herbal preparations have multi-various pharmacological values due to the multitude of phytoactives present in every individual herb. This make the polyherbal preparations the

most effective for the management of not just the given medical condition alone, but also for managing various secondary, tertiary and even the contralateral complications due to the main medical condition. Further, the pro-drug nature of the herbal drugs will not cause 'drug fatigue' and also the possible toxic effect due to prolonged usage. Further, marginal efficacy is sufficient to delay the complication if the herbal preparation is relayed upon with onset of diagnosis, however proper scientific validation for such herbal preparation is necessary.[10]

DcoD is a polyherbal Siddha product studied extensively for its effect in inhibiting comorbidities and improving organ health both at laboratory level and through clinical trial.

The present research work reports the inhibitory effect of DcoD on aldose reductase and sorbitol dehydrogenase enzymes and the implications of the above to the overall medical benefit in the context of increasing incidences of diabetes mellitus is discussed in detail.

Materials and Methods

Comment [s5]: The following items should be mentioned in the method
Type of study, study population and number of samples
Sampling method
Citing the source for the drug preparation method
Time to intervene
Where is the study permit obtained?
What were the methods of statistical analysis?

Description of the Siddha drug – D-Co-D

The Siddha drug DcoD is formulated the following medicinal herbs such as *Andrographis paniculata, Syzygium cumini, Tinospora cordifolia, Momordica charantia, Cyperus rotundus, Zingiber officinale, Piper nigrum and Adhatoda vasica.*

Preparation of extract for study

All the nine herbs (shade dried and pulverized) were weighed individually to 1 gm and 1 gam of each powder was dispensed into 100 ml of distilled water and then heated to 80ºC for 15 minutes. Then the mixture was filtered and the filtrate was cooled then stored at 4ºC until use.

In the case of DcoD, 1 gm of the finished product was weighed into 100 ml of distilled water, boiled for 15 minutes at 80ºC and then filtered and filtrate was used.

Determination of aldose reductase inhibitory activity

The extract combination of DcoD and individual herbal extracts of DcoD were tested for inhibitory activity of aldose reductase. In brief, the test materials such as 0.7 mL of phosphate buffer (0.067 M), 0.1 mL of NADPH ($25X10^{5}$ M), 0.1 mL of aldose source material (lens supernatant), 0.1mL of DL-glyceraldehyde (substrate) ($5X10^{-4}$ M) were taken and the final volume was made up to 1 mL and was taken in a cuvette.

Absorbance value was read at 340 nm by using a reference cuvette containing all components but not DL-glyceraldehyde.

The final pH of the reaction mixture was adjusted to the pH 6.2. Once the substrate was added to the solution, enzymatic reaction would start, and the absorbance (OD) was recorded for 3 min at 30 sec intervals continuously. Aldose reductase activity was calculated and expressed as ΔOD/min/mg protein. IC50 value was calculated.

Determination of sorbitol dehydrogenase inhibitory activity

The assay was performed spectrophotometrically using 0.050M of glycine/NaOH (pH 10.0) by using sorbitor 10mM and NAD^{+}(470mM) as substrate. The activity with and without the test compounds was assayed at 340nm. IC50 value was calculated.

Results

Appreciable aldose reductase inhibition effect could be observed only for Tinospora cordifolia extract where the IC50 value was 24 micrograms per millilitre whereas other individual herbal extracts showed IC50 value at much higher concentration. Total extract of DcoD showed activity at very low concentration, i.e., 11 micrograms per millilitre, Table 1

Table 1 Inhibitory effect of DCoD extracts on aldose reductase enzyme

Sl. No.	**Sample details**	**Aldose reductase inhibition –IC_{50} (µg/ml)**
1	*Andrographis paniculata*	66±0.2
2	*Syzygium cumini*	89±0.7
3	*Tinospora cordifolia*	24±0.12
4	*Momordica charantia*	99±0.5
5	*Cyperus rotundus*	112±0.12
6	*Zingiber officinale*	109±0.23
7	*Piper nigrum*	52±0.5
8	*Adhatoda vasica*	128±0.7
9	*Extract combination*	11±0.2
10	*Quercetin*	*1.4*±0.1

Momordica charantia and Zingeber officinale showed appreciable sorbitol dehydrogenase inhibition effect at very low concentration whereas other herbal extracts although exhibited a level of activity but was not as good as the above two herbs. The DcoD total extract also exhibited activity at very low concentration, Table 2

Table 2 Inhibitory effect of DcoD extract on sorbitol dehydrogenase enzyme

Sl.No.	**Sample details**	**Sorbitol dehydrogenase inhibition –IC_{50} (µg/ml)**
1	*Andrographis paniculata*	26±0.1
2	*Syzygium cumini*	22±0.4
3	*Tinospora cordifolia*	44±0.2
4	*Momordica charantia*	9±0.4
5	*Cyperus rotundus*	70±0.1
6	*Zingiber officinale*	6±0.4
7	*Piper nigrum*	22±0.3
8	*Adhatoda vasica*	39±0.5
9	*Extract combination*	7±0.1
10	*Quercetin*	9±0.2

Discussion

Comment [s6]: The discussion needs to be rewritten
Compare the results of the study with other related studies and cite the source.
At the end, suggestions for future studies should be mentioned.
What was the result of your study?

Comment [s7]: Do not speak with certainty about the results of the study

Our present investigation has indeed brought out the most defining, silent science of polyherbal Siddha products for the management of various ailments. It is already known that several phytoactives are hidden in every herb and each of the phytoactive alone or synergistically can exhibit innumerable pharmacological delights and can bewilder the entire medical fraternity around the world. Ancient scholars practiced the above in healing ailments and imparting the essential of health and wellness presumably knew the above silent science and that is how India is gifted with Ayurveda and Siddha wellness practices.

Our present investigation has revealed that DcoD can effectively manage the second most common co-morbidity associated with diabetes mellitus, that is sugar cataract and diabetic retinopathy. High glucose burden is known to trigger a cascade of bio-chemical/hormonal changes in the system for homeostasis and re-adjustment. Some of such re-adjustments would more often take their best price from our health and quality of life. The high glucose burden in the ocular region is dealt initially by an enzyme called aldose reductase which is vastly distributed in all most all mammalian cells. Aldose reductase would reduce glucose into sorbitol and then sorbitol dehydrogenase would reduce sorbitol into fructose through polyol pathway. The resultant product would accumulate and cause osmotic pressure resulting in retinal and lens related complications. The pressure difference also would result in nerve damage and microvascular damage, terminating in retinopathy. The above process is accounted for the consumption of about 3% of glucose which, during hyperglycemic condition is reported to increase by 30%. Therefore, early intervention to impair the above two enzymes with the onset of diagnosis of the problem is the best strategy to delay/prevent/reduce the diabetic co-morbidity occurrence.

But the question is, should the diabetic patients require target specific, synthetic drug of modern system of medicine for the management of above problem with the onset of diagnosis of diabetes mellitus. Scientifically, not medically, the allopathic drug at this stage may not be required provided the patients relay of drug to control hyperglycemia along with life style and diet change. Further, the herbal preparations like DcoD can be taken for the management of several of the associated complications of diabetes mellitus because the polyherbal preparations have versatile pharmacological activity which may appear like a pyramid where broad effect ending up in offering the overall wellness and health. At this stage, broad management approach is sufficient than targeted treatment with synthetic drug.

Two enzymes studied are also involved in other pathological events in diabetes mellitus. Therefore, inactivation of the above enzymes would increase insulin sensitivity of cells by decreasing accumulation of sorbitol and fructose would cause nerve damage. The oxidative damage is also triggered by the above enzymes which can be reduced greatly by inhibiting the enzymes. Further, the inhibition the above enzymes also would help to reduce the release of TNF-α and IL6, the pro-inflammatory molecules that cause inflammatory changes. The synergistic value of DcoD also we could establish in the present study and that may be reason why the efficacy of DcoD at1/10 level of each herb could exhibit better activity than the activity of some of the individual herbal extracts.

Conventionally Epalrestat and Tolrestat are used for inhibiting aldose reductase enzyme and Ranirstat and Statins are used for sorbitol dehydrogenase inhibition. Due to severe hepatotoxicity, Tolrestat has be banned from human use. To address the above two enzymes, two separate medications are required in allopathic stream of medicine whereas in Siddha system, a single polyherbal preparation called DcoD is sufficient. Besides all the above, DcoD is known to provide wide spectrum of therapeutic benefit for preventing other co-morbidity conditions associated with diabetes mellitus. The present study clearly indicatesthe significant role of DcoD in the management of various diabetic complications and more so in dealing diabetic retinopathy.

References

Comment [s8]: References are few and old.

1. Sherwani SI, Khan HA, Ekhzaimy A, Masood A, Sakharkar MK. Significance of HbA1c Test in Diagnosis and Prognosis of Diabetic Patients. Biomark Insights. 2016 Jul 3;11:95-104. doi: 10.4137/BMI.S38440.
2. Deshpande AD, Harris-Hayes M, Schootman M. Epidemiology of diabetes and diabetes-related complications. Phys Ther. 2008 Nov;88(11):1254-64. doi: 10.2522/ptj.20080020.
3. Kirkness CS, Marcus RL, LaStayo PC, et al. Diabetes and associated risk factors in patients referred for physical therapy in a national primary care electronic medical record database. Phys Ther. 2008;88:1408–1416
4. Tang WH, Martin KA, Hwa J. Aldose reductase, oxidative stress, and diabetic mellitus. Front Pharmacol. 2012 May 9;3:87. doi: 10.3389/fphar.2012.00087.
5. Cheng H. M., Gonzalez R. G. (1986). The effect of high glucose and oxidative stress on lens metabolism, aldose reductase, and senile cataractogenesis. Metab. Clin. Exp. 35, 10–14 10.1016/0026-0495(86)90180-0
6. Drel V. R., Pacher P., Ali T. K., Shin J., Julius U., El-Remessy A. B., Obrosova I. G. (2008). Aldose reductase inhibitor fidarestat counteracts diabetes-associated cataract formation, retinal oxidative-nitrosative stress, glial activation, and apoptosis. Int. J. Mol. Med. 21, 667–676
7. Gonzalez R. G., Barnett P., Aguayo J., Cheng H. M., Chylack L. T., Jr. (1984). Direct measurement of polyol pathway activity in the ocular lens. Diabetes 33, 196–199 10.2337/diabetes.33.2.196
8. Nentwich MM, Ulbig MW. Diabetic retinopathy - ocular complications of diabetes mellitus. World J Diabetes. 2015 Apr 15;6(3):489-99. doi: 10.4239/wjd.v6.i3.489.
9. Kramer CK, Retnakaran R. Concordance of retinopathy and nephropathy over time in Type 1 diabetes: an analysis of data from the Diabetes Control and Complications Trial. Diabet Med. 2013;30:1333–1341.
10. Kumar S, Mittal A, Babu D, Mittal A. Herbal Medicines for Diabetes Management and its Secondary Complications. Curr Diabetes Rev. 2021;17(4):437-456.