ECRONICON OPEN ACCESS

EC DIABETES AND METABOLIC RESEARCH
Research Article

'Therapeutic Polymorphism' of Anti-Diabetic Herbal Drug

Amruthavalli GV*, Aruna V and Gayathri R

Dr. JRK's Research and Pharmaceuticals Pvt Ltd, Kunrathur, Chennai, India

***Corresponding Author:** Amruthavalli GV, Assistant Manager, Dr. JRK's Research and Pharmaceuticals Pvt Ltd, Kunrathur, Chennai, India.

Received: February 22, 2021; **Published:** February 25, 2021

Abstract

In the present paper, we have unveiled the science behind herbal medicine and have established the therapeutic polymorphism exerted by different phyto-active molecules in each herb in the formulation prepared with multiple herbs. The therapeutic benefits that we have established for a formulation were creatine degradation, degradation of creatinine and sarcosine. Further we have also studied the effect of the formulation on neuroblast cells and kidney cells. The findings clearly show that different phyto-active constituents in the herbs exhibit different therapeutic benefits which are in several occasions, bi, tri or quadri-polar in nature. Our study clearly suggests that the polyherbal formulations may be more advantageous for treating problems like diabetes mellitus and other auto immune disorders because all such disorders by itself and also by acting as the key predilecting factor for various complications can harm the host. Details are presented in the paper.

Keywords: *Diabetes Mellitus; Co-Morbidities of Diabetes; DCoD*

Introduction

Debate over the therapeutic value of the herbal drugs for the treatment of various diseases when exist strongly in the arena of medical science, the emergence and demand for new herbal drugs also grows unabated.

Herbal drugs have enormous scope and potential to alleviate man from several aliments if the herbal wealth is properly understood, studied and utilized.

The term 'therapeutic polymorphism' refers to the ability of the secondary metabolite(s) from a single plant species having repertoire of therapeutic benefits for managing various clinical symptoms or diseases which are unrelated.

The plant secondary metabolites are the conglomerations of several bioactive molecule(s) capable of exhibiting therapeutic effect individually, collectively and synergistically [1,2]. Unless we unravel the science that exist in the plant secondary metabolites, the therapeutic value that we expect and accordingly formulate the drug we may not achieve the desired therapeutic benefit and only such scientifically proven herbal drugs can put the debate over the efficacy of herbal drugs to rest.

More than the individual or collective benefits of various bioactive molecules in herbal drugs, the synergistic value will be relatively constant because the same species of plants that were grown in different agrochemical conditions although may show great variability in TLC profile but the basic characteristics of the plant shall remain constant, for example, bitterness in neem, astringent taste in amla etc.

The therapeutic polymorphism shown by the metabolites from a single plant itself is so complex and perplexing to understand means, the admixture of the metabolites of different plants will be far more difficult to understand and so shall the synergy between the metabolites of different plants in most of the polyherbal drug formulations.

Diabetes mellitus is an emerging health hazard and the disease (Diabetes mellitus) by itself and also as a single predisposing factor for various diseases, is causing great concern to the medical fraternity all over the world. Since the glucose being the key energy source for the cells of all organs, the cellular receptor for glucose, ability of glucose metabolism and glycogen conversion by the cells is considered to be universal code of cells in general.

The high glucose burden in the blood is likely to impair the functions of several organs and as a result, progressive multi-organ failure due to diabetes mellitus is quite common, such as diabetic cardiomyopathy, diabetic retinopathy, diabetic nephropathy and diabetic neuropathy etc. [3,4].

The polyherbal drugs will be useful in the initial stage of the disease and or post treatment management. Therefore, the role of herbal drugs must be placed appropriately in the treatment 'niche' and only then the real therapeutic polymorphic benefit of herbal drugs can be achieved.

In the present paper we have done an elaborate study on the synergy linked activity mapping of 8 herbs such as *Andrographis paniculata, Syzygium cumini, Tinospora cordifolia, Momordica charantia, Cyperus rotundus, Zingiber officinale, Piper nigrum, Adhatoda vasica* and a final formulation has been thus arrived. [5-12] And the formulation was studied in detail for its effect on creatine, creatinine and sarcosine at *in vitro* level. Subsequently, the formulation was also studied on renal cell line after inducing oxidative damage. Similarly, the effect of formulation was also studied on neuro blastoma cell line because AKD (Acute Kidney Diseases) often cause neuritis and therefore the diabetic people need polymorphic treatment such as regulation of blood glucose, glucose impedance in the blood and finally the reduce glucose induced organ impairments which is collectively called as diabetic co-morbidities.

The polyherbal drugs due to inter and intra-herb linked synergy may offer better therapeutic polymorphism for managing Diabetes mellitus, the epicenter of various disease radiation and several pathogen invasions. Our present study highlights the therapeutic polymorphism of the 'synergy linked activity mapping of a polyherbal formulation' for managing Diabetes mellitus. Details are presented in the paper.

Materials and Methods

The formulation studied is coded as D-Co-D and details are given below:

Each tablet contains

- Nilavembu (*Andrographis paniculata*): 100 mg
- Naval (*Syzygium cumini*) : 50 mg
- Seenthil (*Tinospora cordifolia*): 50 mg
- Pagal (*Momordica charantia*): 50 mg
- Koraikizhangu (*Cyperus rotundus*): 50 mg

- Sukku (*Zingiber officinale*): 50 mg
- Milaghu (*Piper nigrum*): 50 mg
- Adathodai (*Adhatoda vasica*): 50 mg
- Excipients: Q.S

Enzymatic assay

Creatinine amidohydrolase assay

The enzyme activity was assayed by estimating the decrease in creatinine level as a result of enzyme reaction. 0.5 ml of 50 mm potassium-sodium phosphate buffer with pH of 7.5 was prepared and then one µmole of creatinine was added to the above and then 0.5 ml of enzyme solution was incorporated to the above reaction mixture and was incubated at 37°C for 10 to 30 min. After incubation, 2 ml of 1% picric acid, 0.5 ml of 7.5% NaOH and 10 ml of deionized water were added and was called as Jaffe reaction.

To the Jaffe reaction mixture, before and after enzyme addition, the aqueous extract of DCOD (at varying concentrations such as 1, 2, 3 mg/ml) was added separately and studied the role of DCOD in enhancing either the enzyme activity or degradation of creatinine. The Jaffe reaction mixture was read at 520 nm and the difference in the OD values before and after reaction with the enzyme and the unit activity per ml of the enzyme solution was calculated. Thus, the role of DCOD in enhancing any of the above activity was arrived:

Creatininase units/ml = (O.D.b-O.D.t)/O.D.b x l/ x 2 x dilution factor

Where T was the reaction time in min.

Creatine amidinohydrolase

We have employed the method wherein the decrease in creatine by the enzyme reaction and followed by the addition of diacetyl alpha -naphthol.

In brief, to 0.5 ml of 0.1M phosphate buffer (pH 8.0) containing 0.5 µmole of creatine was added and 0.5 ml of the enzyme solution. And then the mixture was incubated at 37°C for 10 to 30 min. To the above reaction mixture 1.0 ml of diacetyl (1% stock diluted 20 fold with water) and 2.0 ml of alpha-naphthol (freshly prepared, 1% in 1.5 N NaOH) and 6.0ml of deionized water were added. After 30 min, the color developed was measured at 530 nm. The activity unit(s) per ml of the original enzyme solution was calculated by using the following formula:

Creatinase units/m1 = (O.D.b-O.D.t)/O.D.b x 1/T x dilution factor

Where the value of O.D.b was that of color development due to 0.5 µmole Creatine under the conditions and was usually between 0.7 and 0.72.

Sarcosine dehydrogenase

Diformazane formed by the reduction of nitrotetrazolium blue (NTB) which was coupled with phenazine metho sulfate (PMS) and the dehydrogenase reaction was then measured.

In brief, to 0.5 ml of 0.5 M sarcosine solution was prepared in 50 mm Tris-HCl buffer (pH 7.5) containing 0.1 Triton X-100. To the above mixture, 0.5 ml of the enzyme and 0.1 ml of PMS-NTB were added and incubated at 37°C for 15 min. After incubation, 3 ml of 0.3 N HCl was added to the above reaction mixture and the absorbance of the same were read at 570 nm for diformazane. The activity in units per ml of the original enzyme solution was also calculated.

Cell culture assay

Neuroblastoma cells

Analogue of Human neuroblastoma SK N SH cells were cultured in minimum essential medium (MEM) supplemented with 1 mM non-essential amino acids, 0.5 mM L-glutamine, 0.1 mM sodium pyruvate and 10% FBS and maintained at 37°C in a humidified atmosphere of 5% CO_2. When SK N SH cells reached 80% confluence, following tests were conducted.

Cytotoxicity assay

Cell viability of SK N SH cells was measured by MTT assay. Cultured SK N SH cells (0.2×10^6 cells per well) were seeded into 96-well-plate containing in 200 mL of medium supplemented with 10% FBS. Extract of D-Co-D at various concentrations in DMSO were added to the well and plate was incubated in humidified CO_2 incubator with 5% CO_2 at 37°C for 24h. 20 µL of MTT reagent at a final concentration of 5 mg/mL were added to each well and incubated for an additional 4h period in humidified atmosphere. The medium was then removed; the insoluble formazan crystals were dissolved in 200 mL of 0.1N acidic isopropyl alcohol. Calorimetric measurement of MTT reduction was recorded at 570 nm.

Neuroprotection assay

Neuroprotection effect of D-Co-D extract was assayed by measuring induced neuronal cell death of SK N SH cells. Control and treated SK N SH cells (10, 20, 30 µg/ml of D-Co-D) were incubated with MTT for 3h in a humidified CO_2 incubator with 5% CO_2.

To induce oxidative stress (neural death), 1.0 mM H_2O_2 was added and incubated for 24 hours. After incubation cell viability was tested by MTT. Cells pre-treated with D-Co-D were used for neuro-protection assay.

Kidney HK-2 cells

Analogue of Human kidney HK-2 cells were grown in keratinocyte-SFM media supplemented with 5% FBS, 0.005 g/ml, rhEGF and 0.05 mg/ml bovine pituitary extract and then incubated at 37°C in a 5% CO_2 in a humidified atmosphere. Cells were enumerated by Trypan blue dye exclusion and seeded at a density of 2×10^4 cells in 96-well plates, the perimeter wells were filled with 100 µl of sterile water to avoid evaporation effects in the inner wells.

Stock of D-Co-D extract (10, 20 and 30 µg/ml was used for treatment and nephron protectant (negative control) valproic acid was prepared using DMSO and diluting this mixture further with media (for a final DMSO concentration of ≤ 0.4% in media). After overnight incubation, cells were treated D-Co-D in triplicate for 24h at the concentration described above.

Result

Enzymatic assay

Creatinine amidohydrolase assay

The addition of DCOD to the Jaffe mixture at 1 mg/ml and incubated for 10 minutes prior to the addition of enzyme has resulted in 52% reduction in creatinine formation and the percentage reduction of creatinine increased to 56 and 64 respectively when the concentration of DCOD was increased to 2 and 3 mg/ml respectively.

The addition of DCOD to the Jaffe mixture along with the enzyme showed a marginal reduction in creatinine. For the three concentrations of DCOD such as 1, 2, 3 mg/ml respectively, the percentage reduction of creatinine was 44, 52 and 60 respectively. The above findings suggest that DCOD may possess bidirectional action viz., directly degrading creatinine and also promoting the enzymatic activity (Table 1).

S.no	Test details	Enzyme activity (Units/ml) vs concentration of DCOD	% reduction of creatinine
1	Jaffe mixture (with creatinine)- devoid of enzyme (A)	2.5	-
2	Jaffe reaction mixture (Creatinine + enzyme) (B)	1.8	28
3	To A, addition of 1 µg/ml DCOD 10' before enzyme treatment	1.2	52
4	To A, addition of 2 µg/ml DCOD 10' before enzyme treatment	1.1	56
5	To A, addition of 3 µg/ml DCOD 10' before enzyme treatment	0.9	64
6	To B, 1 µg/ml DCOD added along with enzyme	1.4	44
7	To B, 2 µg/ml DCOD added along with enzyme	1.2	52
8	To B, 3 µg/ml DCOD added along with enzyme	1.0	60

Table 1: *Bidirectional effect of D-Co-D on creatinine and Creatinine amidohydrolase.*

Creatine amidinohydrolase

DCOD marginally reduced the secondary by product of creatinine i.e. creatine.

The % reduction of creatine was 10.5, 21.0 and 31.5 respectively for 1, 2 and 3mg/ml of DCOD (Table 2).

S.no	Test details	Enzyme activity (Units/ml) vs concentration of DCOD	% reduction of creatine
1	Reaction mixture (with creatine) and Creatinase (A)	1.9	-
2	A with 1 µg/ml DCOD	1.7	10.5
3	A with 2 µg/ml DCOD	1.5	21.0
4	A with 3 µg/ml DCOD	1.3	31.5

Table 2: *Creatine amidinohydrolase.*

Sarcosine dehydrogenase

DCOD reduced the enzyme demand of sarcosine and which was directly relatable to the concentration of D-Co-D (Table 3).

S.no	Test details	Enzyme activity (Units/ml) required for activity vs concentration of DCOD
1	Reaction mixture (A)	30
2	A with 1 µg/ml DCOD	25
3	A with 2 µg/ml DCOD	19
4	A with 3 µg/ml DCOD	16

Table 3: *Sarcosine dehydrogenase.*

Cell culture assay

Cytotoxicity assay

D-Co-D upto a concentration of 30 µg/ml did not exhibit cytotoxic effect to SK N SH cells (Analogue of Human neuroblastoma) (Table 4).

Concentration of D-Co-D in µg/ml	% of viable cells
Control	100
10	60
20	50
30	40

Table 4: *Effect of D-Co-D on neuroblastoma.*

Neuroprotection assay

D-Co-D showed neuro protection effect to neuroblastoma cells from oxidative damage and the effect was directly proportional to the concentration of D-Co-D (Table 5).

Concentration of D-Co-D in µg/ml	% of viable cells
Treated control	-
Untreated control	100
10	40
20	50
30	60

Table 5: *Effect of D-Co-D on neuro-protection.*

Kidney HK-2 cells

D-Co-D did not induce any cytotoxic effect on Kidney HK-2 cells (Table 6).

Concentration (µg/ml)	% Death
10	10
20	20
30	40

Table 6: *Cytotoxicity assay on Kidney HK-2 cells.*

Discussion

The poly herbal formulation that we made after ascertaining the synergy linked activity of the secondary metabolites of 8 plants at inter and intra level showed significant effect in decreasing creatine. Interestingly the formulation induced both the enzyme-creatine amidinohydrolase and degradation of the substrate - creatine.

The binary activity of the formulation can be assumed due to the synergy linkage that exists between different herbs in the formulation. The secondary metabolites of all the eight herbs used in the formulation such as *Andrographis paniculata, Syzygium cumini, Tinospora cordifolia, Momordica charantia, Cyperus rotundus, Zingiber officinale, Piper nigrum, Adhatoda vasica* when tested individually both on creatine and in the reaction mixture with the substrate and the enzyme-creatine amidinohydrolase showed that the formulation does not possess any substrate degradation effect. However, the metabolites of some of the herbs showed strong enzyme up regulation. Interestingly, the formulation showed substrate degradation effect and it might be due to the synergy between different herbs. Further the formulation also up-regulated the enzyme activity.

We could not explain why the individual herbs did not show any effect on substrate-creatine degradation but the polyherbal mixture showed such activity except the reason that we could attribute to the above is synergy. The synergy that we have established is although difficult to explain or demonstrate experimentally at the micro level but such limitation should not be allowed to deny the existence of such scientific miasma.

In our earlier study we have established different fractions of the herbal extracts when tested individually showed wide spectrum of activity such as anti-bacterial, anti-viral, anti-fungal and anti-larvicidal. Similarly, different fractions also showed wide range of activities at various biochemical processes. When we re-agglomerated all the different fractions and then pooled and tested, to our surprise we observed a new bio-chemical activity which was never seen in any of the individual fractions.

Although the scientific intrigue behind the synergy needs to be experimentally explained but in any case the synergy holds a positive value for therapy and also the synergy emphasize that the native healing recipes must be formulated the way they are enshrined in the ancient texts.

Interestingly we have also observed a stable synergistic benefit in several herbal metabolites of the same species sourced from different agro-climatic regions (data submitted elsewhere for publication) however the thin layer chromatographic pattern of the herbs showed great variability. Therefore, we hypothesize that the synergistic benefit may be linked at species or genus level, whereas the activity of different bio-active molecules in the extract conglomerate may not be stable because the formation of several secondary metabolites in the plants is stimuli specific and therefore the plants grown in different agro climatic zones may not possess all the secondary metabolites. If we identify the constitutional synergistic marker benefit of the herbal metabolites of the plant chosen and accordingly the formulation is made, a credible science over herbal drugs can be easily achieved.

Creatine is the source material for creatinine. Creatine is released from the muscles where it is stored, subsequent to its synthesis by kidney and liver. During diabetes mellitus, the creatine breakdown occurs and as a result, creatinine is released. The creatine impairs renal function due to its abundance in the blood stream and therefore the degradation of creatine is necessary to bring down the burden of the kidney.

The polyherbal formulation that we have tested showed both creatine degradation as well as the upregulation of creatine amidinohydrolase. Subsequently we have focused on the effect of polyherbal drug on sarcosine and its accumulation in human body, as it can lead to prostate cancer. Sarcosine is also known to get accumulated during Diabetes mellitus. Therefore, cleansing the body of sarcosine is necessary. The polyherbal formulation showed great effect in upregulating the activity of sarcosine dehydrogenase. However, we could not establish any sarcosine decomposing effect of the formulation.

We do not know whether the formulation actually increases the enzyme synthesis or increases the activity. In *in vitro* system the increased enzyme synthesis cannot be established therefore the finding has to be squired up to the enzyme activity enhancement alone. When none of the individual herbs have shown any effect on the substrate, how the formulation made with the above herbs has effect on enzyme and substrate remains a mystery to us. The above finding once again compelled us to believe that a synergy may exist between the herbs.

The underlying mechanism of action around the herbal drugs is quite puzzling. Whether the formulation directly exhibits certain pharmacological action or the broken down end product(s) of the formulation in mammalian system is responsible for the above is difficult to ascertain. If the end product of the metabolism of the herbal formulation is responsible means the end product level synergy between different herbs also cannot be ruled out. From the scientific prospective although knowing 'what, why, how, where and when' needs to be established but from the medical requirements point of view, mere benefit alone is sufficient.

Patients need relief and the disease needs treatment. If the treatment and relief can be achieved but at the same time the underlying cause and effect relationship (structure and activity) of the drug cannot be established or difficult to establish should not limit or dismiss the value of herbal drugs for treating various diseases.

Sometime in medical science especially in treating patients from various medical agonies, the Machiavelli principle may be more appropriate than theorizing and questioning several aspects of the drug in practical sense such information has no bearing on relief and treatment.

We have studied the polyherbal formulation on kidney cell line SK N SH cells. The cellular protection in response to treatment with the polyherbal drug after intentional induction of oxidative damage upon the cells showed that the formulation could protect the cells greatly from the oxidative damage. However, in our earlier study on the reaction between the formulation and DPPH showed that the formulation does not have any significant effect in preventing DPPH. This once again reiterates the possibility of a synergy between cells and the formulation; the formulation may be eliciting cellular response and thus the biochemical reaction has occurred. The findings also suggest that the formulation may directly establish synergy with the cells and the metabolic end product of the formulation may not be responsible for the pharmacological effect. Strikingly our above observation also indicates the misnomer status of the universal notion about the herbal drugs that the herbal drugs are slow acting and therefore prolonged therapy may be required to experience the result.

Neural blastoma cells are quite vulnerable and susceptible to high blood glucose and renal impairment. Therefore, we were a bit curious to study whether the formulation would offer protection to neuro blastoma cells. The neuro blastoma cells did not show any cytotoxic susceptibility to the formulation and no significant decline in the population of cells were observed. Since the renal impairment and nerve damage are interlinked, the present therapeutic polymorphism shown by the formulation assumes great significance in the treatment of diabetes and co-morbidities associated with Diabetes mellitus.

We are the first to report the possible synergy in plant secondary metabolites and study them in detail for validating the synergic hypothesis and therapeutic polymorphism of a poly herbal formulation. Our findings clearly show that the polyherbal formulation that we have studied may be useful for the treatment of the comorbidities associated with Diabetes mellitus.

Conclusion

DCOD, a polyherbal formulation was found to degrade creatine and creatinine *in vitro* as well as offers neuroprotection to SK NSH cells and kidney cells HK-2. Further the formulation also inhibited sugar assimilation by yeast cells as well as exhibited glucose absorption in situ indicating the possible anti-diabetic effect of the formulation. The formulation is comprised of 8 medicinal herbs and each herb in its native form is a combination of several phyto-active molecules. The spectrum of therapeutic benefit we observed for the formulation may be due to the polymorphic phytoactives of different herbs and associated polymorphic pharmacologic benefit. The formulation clearly gives a scope for the effective treatment and management of diabetes mellitus in future especially for preventing organ level damages.

Bibliography

1. Kishan Gopal Ramawat., *et al.* "The Chemical Diversity of Bioactive Molecules and Therapeutic Potential of Medicinal Plants". Chapter 2 (2008): 15.
2. Ramawat KG. "Secondary metabolites in nature". In: Ramawat KG, Merillon JM (eds) Biotechnology: Secondary Metabolites". Science Publishers, Enfield, CT (2007): 21.
3. Kawahito S., *et al.* "Problems associated with glucose toxicity: role of hyperglycemia-induced oxidative stress". *World Journal of Gastroenterology* 15.33 (2009): 4137-4142.
4. M Loredana Marcovecchio. "Complications of Acute and Chronic Hyperglycemia" (2017): 1-5.
5. Nugroho AE., *et al.* "Pancreatic effect of andrographolide isolated from *Andrographis paniculata* (Burm. f.) Nees". *Pakistan Journal of Biological Sciences* 1 (2014): 22-31.
6. Claudio Coimbra Teixeira., *et al.* "*Syzygium cumini* (L.) Skeels in the Treatment of Type 2 Diabetes". *Diabetes Care* 27.12 (2004): 3019-3020.
7. Rohit Sharma., *et al.* "Antidiabetic claims of *Tinospora cordifolia* (Willd.) Miers: critical appraisal and role in therapy". *Asian Pacific Journal of Tropical Biomedicine* 5.1 (2015): 68-78.
8. Rufine Fachinan., *et al.* "Effectiveness of Antihyperglycemic Effect of *Momordica charantia*: Implication of T-Cell Cytokines". *Evidence-Based Complementary and Alternative Medicine* (2017): 8.
9. Studies on the antidiabetic activities of *Momordica charantia* fruit juice in streptozotocin-induced diabetic rats". *Pharmaceutical Biology* 55.1 (2017): 758-765.
10. Hong Hanh Thi Tran., *et al.* "Inhibitors of αglucosidase and α-amylase from *Cyperus rotundus*". *Pharmaceutical Biology* 52.1 (2014): 74-77.
11. Araujo AJS., *et al.* "Effect of Ginger (*Zingiber officinale*) Supplementation on Diabetes: An Update". *American Journal of Phytomedicine and Clinical Therapeutics* 6.3 (2018): 13.

12. Uddandrao VVS., *et al.* "Evaluation of the Antioxidant and Antidiabetic Potential of the Poly Herbal Formulation: Identification of Bioactive Factors". *Cardiovascular and Hematological Agents in Medicinal Chemistry* 18.2 (2020): 111-123.

13. Mohan Patil., *et al.* "Anti-oxidant, anti-inflammatory and anti-cholinergic action of *Adhatoda vasica* Nees contributes to amelioration of diabetic encephalopathy in rats: Behavioral and biochemical evidences". *International Journal of Diabetes in Developing Countries* 34.1 (2013).

Volume 5 Issue 3 March 2021