Synergistic potential of *Zingiber officinale* and *Curcuma longa* to ameliorate diabetic-dyslipidemia

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Abstract: To find the cure of world's one of the leading morbid and mortal disorders; diabetes mellitus and its most prevalent complication, 'diabetic-dyslipidemia', is one of the leading health challenges of 21st century. The use of phytomedicine is a glimmer of hope in this scenario. Studies of current decade have shown that methanolic extracts of *Zingiber officinale* and *Curcuma longa* have highly effective therapeutic potentials against the aforesaid disorders, however, which of the extracts has more potential is still unclear. Furthermore, synergistic effect of the extracts has never been studied. Forty-eight Albino adult rats of either sex were randomly divided into eight groups. A-D groups were containing healthy rats while E-H groups were of induced diabetic-dyslipidemic rats. For forty-two days, rats of each group were given either distilled water or *Zingiber officinale* methanolic extract (ZOME) or *Curcuma longa* methanolic extract (CLME) or ZOME+CLME therapies at dose rate of 300mg/100 mL dist. H_2O/kg body wt/day. FPG and lipid profiles were estimated before and after the trial, and were statistically analyzed by one-way ANOVA along with Post-hoc Tukey's multiple comparison tests. Although, ZOME and CLME significantly ($P<0.05$) lowered fasting plasma glucose (FPG) levels and controlled lipid profiles in diabetic-dyslipidemic rats; yet, synergistic therapy of both extracts (ZOME+CLME) most significantly ($P<0.05$) controlled all parameters of diabetic-dyslipidemia (78.00±1.06mg/dL FPG, 62.00±0.58mg/dL TG, 66.50±0.76mg/dL cholesterol, 32.00±0.36mg/dL HDL, 22.43±0.64 mg/dL LDL, and 12.40±0.12mg/dL VLDL). Our findings may be useful to formulate new medicines having multiple potentials to control diabetes mellitus, dyslipidemia, and diabetic-dyslipidemia.

Keywords: Diabetic-dyslipidemia, Phytomedicine, *Zingiber officinale*, *Curcuma longa*, 6-Gingerol, Curcumin, HPLC.

INTRODUCTION

Diabetes mellitus (DM) is one of the main causes of mortality and morbidity worldwide, which is characterized by chronic metabolic disorders of lipids and carbohydrates. Similarly, dyslipidemia is another life-threatening challenge of the time; and is also a major risk factor of DM. It may also inversely result from uncontrolled hyperglycemia and insulin resistance in both type 1 and type 2 diabetic patients and is called 'diabetic dyslipidemia' (Verges, 2011; Mooradian, 2009). DM and the associated dyslipidemia if left untreated or poorly controlled, may consequence to neuropathy, nephropathy, coronary artery disease (CAD) and non alcoholic fatty liver disease (NAFLD) (Targher *et al*,2010; Vincent *et al*, 2009). So, in order to prevent humans from both diabetes and dyslipidemia, and their mutually linked chronic life-threatening complications, there is a great demand of finding new drugs having no or least side effects with multiple therapeutic potentials to control hyperglycemia and dyslipidemia and their associated complications; as the currently available anti-hyperglycemic and anti-dyslipidemic drugs have lost their therapeutic potentials because of their crucial side effects and high cost rates (Fournier *et al,* 2014; Bhandari *et al,* 2002).

The detailed evaluation of traditional medicine especially phytomedicine or herbal medicine may be an excellent approach of the time in this regard (Nammi *et al,* 2009). Furthermore, the evaluation of phytomedicine has also been recommended by World Health Organization in circumstances where there is lack of potentially safe drugs (WHO, 2002; WHO, 2013).

Zingiber officinale (ZO) rhizome, Zingiberaceae family, is a famous spice commonly called ginger and has been widely used for the traditional treatment of many infirmities (Afzal *et al,* 2001). Many current studies (Rackova *et al,* 2013; Paul *et al,* 2012; Abdulrazaq *et al,* 2011) have shown the effectiveness of extract of ZO in lowering both plasma glucose levels and lipid profile in induced diabetic and fat-rich diet/cholesterol fed model of mice, rats and rabbits. However, there is quite discrepancy among the results of these studies. Such discrepancy may be associated to different protocols of extract preparation, storage, and different biochemical composition of ginger of different localities (Hussain *et al,* 2015). So, more studies are required to assess its optimum therapeutic potential.

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Another common use spice of Zingiberaceae; '*Curcuma longa'* (CL) rhizome or turmeric has shown its strong intrinsic activity as a therapeutic agent for various ailments. Although, some past studies had curbed its therapeutic potential due its poorer absorption and fast metabolism; however, recent studies have re-evaluated its anti-hyperglycemic and hypolipidemic potential and strongly advocate its medicinal importance (Abdelaziz *et al,* 2012; Ashour *et al,* 2011). Now, even though it is evident that the extracts of both spices have the same anti-diabetic and anti-dyslipidemic properties; yet, which one is more potent is still unclear. Moreover, synergistic effects of both extracts have never been clearly studied.

MATERIALS AND METHODS

Samples' collection of Z. officinale and C. longa rhizomes

Fresh ZO rhizome samples were collected from Trait (Murree Hills), Punjab, Pakistan while fresh CL rhizomes samples were collected from 25-km Wah Kharaan, Changa Manga, Kasur District, Punjab, Pakistan. The samples' species were verified, assigned voucher numbers (ZO: GC. Herb. Bot. 2406a; CL: GC. Herb. Bot. 2406b) by Dr. Muhammad Ajaib, [Department of Botany, Government College University (GCU), Lahore], and were submitted to Dr. Sultan Ahmad Herbarium, GCU, Lahore.

Extracts' preparations

After thoroughly washing both the samples with distilled water, they were slightly peeled off and then cut into smaller pieces. The pieces were dried at 25^{o}C for 15-20 days under the shade and were later ground into fine powders by using electric mill for 7-10 minutes. After bringing both the samples into uniform size, their methanolic extracts were prepared by using soxhlet apparatus. For ZOME preparation, 50g dried powder was evenly filled in a filter paper and put into a thimble to be extracted with 250 mL methanol taken in a receiver. Continuous extraction was performed for a duration of 24 hours until solvent in siphon tube became almost colorless. ZOME was then concentrated by using rotary evaporator (1200A EYELA, Japan) under reduced pressure of 23-26 mm Hg at 35^{o}C until a viscous golden brown substance was obtained. ZOME was further placed in hot air oven at 30^{o}C until all the remaining solvent was evaporated and finally, powdered extract was stored at -4^{o}C in a dark glass container (Hussain *et al,* 2015). For preparation of CLME, the same protocol was followed.

Extracts' standardizations

Both ZOME and CLME were standardized by HPLC analysis (Hussain *et al,* 2015) by using '6-Gingerol analytical standard' (Carbosynth Limited, UK) and 'Curcumin analytical standard (Sigma-Aldrich, USA) for the presence of main bioactive constituents: 6-gingerol in ZOME and curcumin in CLME respectively.

Experimental design

All experimental manipulations used in this study were approved by 'Ethical Review Committee for the Use of Laboratory Animals (ERCULA)', UVAS (DR/303/ORIC/14) in 2014. For the experimental design, forty-eight adult Wistar Albino rats of either sex, having 200-220g weight were selected from the Rat House, Institute of Biochemistry and Biotechnology, UVAS, Lahore, Pakistan. The selected rats were then categorized as follows:

Twenty-four healthy control rats

These rats were fed with normal diet (ND) that was a little modified AIN-93G rodent diet with 7.10% kcal from fats (table 1) as recommended by (Sasidharan *et al,* 2013).

These rats were randomly sub-divided into four equal groups and were given their respective therapies at the dose rate of 300 mg/100 mL dist. H_2O/kg body wt/day by using oral gavage for forty-two days. The rats' groups were as follows:

Group-A (healthy normal control group) on distilled water treatment.
Group-B on ZOME treatment.
Group-C on CLME treatment.
Group-D on ZOME+CLME (50% of each) treatment.

Twenty-four induced diabetic-dyslipidemic rats

To induce dyslipidemia in rats, a high fat diet (a modified AIN-93 diet with 45% kcal from fats, prepared as per formula described by Sasidharan *et al,* 2013) was used; and the induced dyslipidemia was confirmed by serum lipid profiling. After the induction of dyslipidemia, diabetes was induced by a single dose (150mg/kg body wt) of intraperitoneally injected alloxan monohydrate in a fasting condition of twelve hours. After that, rats had free access to water and food. Six hours after the alloxination, rats were given glucose solution (5%) to drink for twelve hours to evade the risk of hypoglycemic shock. Rats having random plasma glucose ≥200mg/dL, after seventy-two hours of alloxination, were confirmed as diabetic (Zhang *et al,* 2013).

The induced diabetic-dyslipidemic rats were randomly sub-divided into four equal groups and were administrated their respective therapies at the dose rate of 300 mg/100 mL dist. H_2O/kg body wt/day by using oral gavage for forty-two days.

The diabetic-dyslipidemic rats' groups were as follows:
Group-E (*Diabetic-Dyslipidemic Control Group*) on distilled water treatment.
Group-F on ZOME treatment.
Group-G on CLME treatment.
Group-H on ZOME+CLME (50% of each) treatment.

Serum analyses

FPG levels on day 0 and day 42 were estimated with enzymatic kit (Human Diagnostics, Germany) by

Table 1: Composition of Diets

Macronutrients' Composition (%) with Respect to Calories (%)				
Nutrient	Normal Diet		High Fat Diet (45%)	
	g%	kcal%	g%	kcal%
Fats	3.0	7.1	24.0	45.0
Protein	20.3	21.3	25.4	21.3
Carbohydrates	68.1	71.6	40.0	33.7
Total kcal %		100		100
Quantitative Composition of Ingredients along Calories (kcal)				
Ingredients	Normal Diet		High Fat Diet (45%)	
	g	kcal	g	kcal
Sucrose	100	400	125	500
Cornstarch	438.59	1754	97.65	391
Maltodextrin	132	528	165.1	660
Casein	200	800	250.1	1000
L-Cystine	3	12	3.75	15
Tallow	0	0	200.3	1803
Soybean Oil	30	270	37.5	338
AIN-93 Vitamin Mix	10	40	12.5	50
AIN-93G Mineral Mix	35	0	43.8	0
Cellulose	50	0	62.5	0
Choline chloride	1.40	0	1.75	0
ENDOX	0.10	0	0.05	0
Total	1000	3804	1000	4757

following glucose peroxidase method (Trinder, 1969) on Merck Micro lab 300 (USA) at 500nm. For lipid profiling day 0 and 42nd day's serum levels of TG, cholesterol, HDL-cholesterol, and LDL-cholesterol were determined by GPO-PAP-enzymatic colorimetric method (Young *et al*, 1975), CHOD-PAP-enzymatic colorimetric method (Schetler *et al*, 1975), and phosphotungstic precipitation method (Lopezvirella, 1977) respectively. All of these parameters were estimated on Merck Micro lab 300 (USA) at 546 nm by using their respective kits (Human Diagnostics, Germany). Furthermore, levels of LDL-cholesterol and VLDL at the start and end of this experimental study were calculated by using the following formulas (Lopezvirella, 1977):

LDL-C = TC-HDL-TG/5

VLDL = TG/5

Lastly, TC/HDL, LDL/HDL and HDL/LDL ratios were also determined.

STATISTICAL ANALYSIS

One-way ANOVA along with Post-hoc Tukey's multiple comparison tests were used to compare means, standard errors, and differences of analyzed serum chemistry parameters by using SPSS-16 software (Chicago, IL, USA).

RESULTS

The comparisons of peaks in chromatograms of ZOME and CLME with their respective standards (fig. 1) showed the same retention times. 6.50% 6-gingerol in ZOME and 11.04% curcumin in CLME were found in this study.

FPG on Day 0 and Day 42

On day 0, FPG levels in all diabetic-dyslipidemic groups having homogeneity of means (b = 0.99, table 1) were significantly higher (P<0.05) vs. healthy control (Group-A). However, on 42nd day mean FPG level of only diabetic-dyslipidemic control group (357.67±3.32 mg/dL, table 2) was 339.77% higher (P<0.05) while no significant elevation of FPG values in diabetic-dyslipidemic groups on treatment of ZOME, CLME, and ZOME+CLME was noted.

Both ZOME and CLME treatments' individual FPG lowering effects (74.09% and 74.14%) were although significant (P<0.05) but their effects were lower (4.10% and 4.06% respectively) than ZOME+CLME treatment's synergistic effect (78.19%). Moreover, FPG level (78.00±1.06 mg/dL) lowered by ZOME+CLME therapy had homogeneity of means with healthy control group (a=0.068). On the other hand, no significant change in FPG values in healthy groups treated by ZOME, CLME, and ZOME+CLME doses vs. healthy control was noted on day 42 (a=0.068, table 2).

Lipid profiles on day 0 and day 42

Triglycerides (TG) levels in all diabetic-dyslipidemic groups (a=0.997, P<0.05) were significantly raised on day 0 vs. healthy control group (P<0.05, table 2). Yet, on day 42 only mean TG level in diabetic-dyslipidemic control

Table 2: One Way ANOVA along with Post-hoc Tukey's Multiple Comparison Test and Range Test Analysis of Day 0 Fasting Plasma Glucose Levels and Lipid Profiles of Healthy and Induced Diabetic-Dyslipidemic Groups on Treatment of Distilled Water, ZOME, CLME, and CLME+ZOME (Mean±SEM)

Group	FPG (mg/dL)	Chol (mg/dL)	TG (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	VLDL (mg/dL)	LDL /HDL	HDL /LDL	TC /HDL
Group A	80.33 ±1.28^{a}	71.00 ±1.32^{a}	69.17 ±1.14^{a}	27.18 ±1.44^{a}	30.00 ±0.73^{a}	13.82 ±0.23^{a}	0.91 ±0.06^{a}	1.13 ±0.90^{a}	2.38 ±0.07^{a}
Group B	78.00 ±0.82^{a}	69.83 ±1.01^{a}	70.67 ±1.12^{a}	25.53 ±1.28	29.83 ±0.48^{a}	14.13 ±0.22^{a}	0.86±0.05^{a}	1.18± 0.99^{a}	2.34 ±0.06^{a}
Group C	79.83 ±0.94^{a}	71.67 ±0.88^{a}	69.17 ±0.94^{a}	28.28 ±0.89^{a}	29.50 ±0.76^{a}	13.83 ±0.19^{a}	0.96 ±0.04^{a}	1.08 ±0.97^{a}	2.44 ±0.05^{a}
Group D	78.00 ±0.82^{a}	70.83 ±0.83^{a}	69.50 ±0.7^{a}	27.30 ±1.17^{a}	29.67 ±0.56^{a}	13.87 ±0.17^{a}	0.93 ±0.05^{a}	1.10 ±0.92^{a}	2.40 ±.06^{a}
Group E	262.50 ±1.94^{b*}	156.00 ±3.30^{b*}	139.67 ±2.85^{b*}	107.38 ±4.73^{b*}	17.50 ±0.76^{b*}	27.93 ±0.57^{b*}	6.23 ±0.50^{b*}	0.16 ±0.13^{b*}	8.04 ±0.80^{b*}
Group F	262.33 ±1.98^{b*}	156.33 ±2.69^{b*}	138.17 ±1.35^{b*}	111.23 ±3.51^{b*}	17.50 ±0.88^{b*}	27.60 ±0.29^{b}	6.46 ±0.51^{b*}	0.15 ±0.11^{b*}	9.08 ±.59^{b*}
Group G	260.83 ±1.58^{b*}	154.50 ±4.04^{b*}	139.00 ±1.75^{b*}	110.20 ±4.66^{b*}	16.50 ±0.88^{b*}	27.80 ±0.35^{b}	6.85 ±0.05^{b*}	0.15 ±0.12^{b*}	9.57 ±.79^{b*}
Group H	261.00 ±2.29^{b*}	156.00 ±3.49^{b*}	138.67 ±1.43^{b*}	111.27 ±4.19^{b*}	17.00 ±0.93^{b*}	27.73 ±0.29^{b}	6.68 ±0.56^{b*}	0.17 ±.13^{b*}	9.34 ±.64^{b*}
F Value	394.5	323.824	575.055	197.057	78.178	563.239	56.197	100.981	49.854
Tukey Range Test P Values Subset	a =0.96^{ns} b =0.994^{ns}	a =1.000^{ns} b =1.000^{ns}	a =0.997^{ns} b =0.997^{ns}	a =0.998^{ns} b =0.987^{ns}	a =0.982^{ns} b =1.000^{ns}	a =0.996^{ns} b =0.994^{ns}	a =1.000^{ns} b =0.958^{ns}	a =1.000^{ns} b =0.854^{ns}	a =1.000^{ns} b = 0.410^{ns}

*Mean values were significantly different from the healthy control group (P<0·05).
ZOME: *Zingiber officinale* Methanolic Extract, CLME: *Curcuma longa* Methanolic Extract, FPG: Fasting Plasma Glucose, Chol: Cholesterol, TG: Triglycerides, LDL: Low Density Lipoproteins, HDL: High Density Lipoproteins, VLDL: Very Low Density Lipoproteins, TC: Total Cholesterol, ns: non-significant, a and b: Tukey's range test analysis divides p values into two subsets (a and b) to assess homogeneity of means.

group was significantly raised (68.92%, P<0.05, table 2); and no rising was noted in rats' groups treated by ZOME, CLME and ZOME+CLME. Both ZOME and CLME treatments for 42 days were equally effective in their TG lowering potentials (66.59%); however, their synergistic effect (ZOME+CLME) was comparatively 5.48% more potent. Moreover, mean TG level (62.00±0.58 mg/dL) lowered by ZOME+CLME treatment was homogenous with healthy control group (a = 0.929). Lastly, no significant effects of ZOME, CLME and ZOME+CLME doses on TG levels were observed in healthy groups vs. healthy control group.

Similarly, no significant effects in serum cholesterol levels of healthy groups treated by of ZOME, CLME, and ZOME+CLME for 42 days were noted; and all healthy groups had homogeneity of means on day 0 (a=1.000) and day 42 (a=0.485). Significantly high cholesterol levels (P<0.05) of diabetic-dyslipidemic groups (table 2) on day 0 were greatly decreased by 69.44%, 70.42% and 74.00% with 42 days' treatments of ZOME, CLME and ZOME+CLME in comparison with diabetic-dyslipidemic control group (267.20% high cholesterol level vs. healthy control group). Although, both ZOME and CLME treatments had almost same significant cholesterol lowering potentials, but mean cholesterol level (66.50±0.76 mg/dL) lowered by ZOME+CLME was closer to that of the healthy control rats (a=0.485); and it had 4.6% and 3.58% more cholesterol lowering effect than ZOME and CLME respectively.

Induced diabetic-dyslipidemic rats' low serum high density lipoprotein (HDL) levels on day 0 (table 2) were significantly improved by 42 days' ZOME, CLME and ZOME+CLME therapeutic treatments i.e. 182.58%, 169.09%, and 269.09% respectively vs. control group: in which significantly low HDL levels of day 0 (table 2) were highly decreased (71.10%, P<0.05) on 42nd day (table 3). ZOME+CLME medicinal effect to improve HDL was 86.51% and 100% more than that of ZOME and CLME. On the other hand, no significant effect of either treatment was noted in healthy rats.

The low density lipoprotein (LDL) levels in healthy rats were remained unaffected by all aforesaid treatments; while, 80.36%, 81.61%, and 88.67% reductions in LDL level was recorded in diabetic-dyslipidemic groups treated by ZOME, CLME, and ZOME+CLME respectively for 42 days. Although, CLME had 1.25% more LDL lowering potential than ZOME; yet ZOME+CLME therapy was more effective than both of the individual therapies (8.31% and 7.07% more than ZOME and CLME respectively). Mean LDL levels lowered by ZOME+CLME therapy (22.43±0.64 mg/dL) were homogenous with that of the healthy control rats (a=0.422). Furthermore, in diabetic-dyslipidemic control group, LDL levels were increased by 685.44% (P<0.05) on 42nd day (table 3).

In investigation of said therapies' effects on serum very low density lipoprotein levels (VLDL), it was noted that healthy groups (B-D) on their respective treatments remained unaffected at the last day of the trial (table 3).

Table 3: One Way ANOVA along with Post-hoc Tukey's Multiple Comparison Test and Range Test Analysis of Day 42 Fasting Plasma Glucose Levels and Lipid Profiles of Healthy and Induced Diabetic-Dyslipidemic Groups on Treatment of Distilled Water, ZOME, CLME, and CLME+ZOME (Mean±SEM)

Group	FPG (mg/dL)	Chol (mg/dL)	TG (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	VLDL (mg/dL)	LDL/HDL	HDL/LDL	TC/HDL
Group A	81.33 ±0.80^{a}	69.67 ±0.80$^{a, b}$	69.00 ±0.36$^{a, b}$	25.20 ±0.57^{a}	30.00 ±0.73^{c}	13.80 ±0.073^{a}	0.86 ±0.04^{a}	1.20± 0.053$^{c, d}$	2.32 ±0.06^{a}
Group B	79.67 ±0.42^{a}	69.17 ±0.48$^{a, b}$	68.83 ±0.40$^{a, b}$	24.57 ±0.63^{a}	30.83 ±0.31$^{c, d}$	13.77 ±0.08$^{a, b}$	0.80 ±0.03^{a}	1.30± 0.047$^{d, e}$	2.24 ±0.03^{a}
Group C	80.67 ±0.67^{a}	70.67 ±0.95$^{a, b}$	70.83 ±0.79^{b}	27.83 ±0.59^{a}	28.67 ±0.67^{c}	14.17 ±0.16^{b}	0.97 ±0.02^{a}	1.03± 0.024^{c}	2.47 ±0.03^{a}
Group D	79.67 ±0.42^{a}	70.67 ±0.42$^{a, b}$	69.33 ±0.42^{a,b}	26.53 ±0.91^{a}	29.83 ±0.60^{c}	13.97 ±0.11^{b}	0.90 ±0.04^{a}	1.22± 0.087$^{c, d, e}$	2.38 ±0.05^{a}
Group E	357.67 ±3.32^{c***}	255.83 ±3.56^{d***}	222.00 ±4.46^{c***}	197.93 ±4.51^{c***}	8.67 ±0.67^{a***}	44.40 ±0.89^{c***}	23.67 ±2.22^{b***}	0.04± 0.006^{a***}	30.44 ±2.45^{b***}
Group F	92.67 ±1.31^{b†††}	78.17 ±1.19^{c†††}	74.17 ±0.90^{b†††}	38.87 ±1.14^{b†††}	24.50 ±0.43^{b†††}	14.80 ±0.17^{b†††}	1.59± 0.06^{a†††}	0.64± 0.026^{b†††}	3.19± 0.07^{a†††}
Group G	92.50 ±1.18^{b†††}	75.67 ±0.67$^{b, c†††}$	74.17 ±0.83^{b†††}	36.40 ±1.44^{b†††}	23.33 ±1.02^{b†††}	14.83 ±0.17^{b†††}	1.51± 0.13^{a†††}	0.69± 0.067^{b†††}	3.20± 0.25^{a†††}
Group H	78.00 ±1.06^{a†††}	66.50 ±0.76^{a†††}	62.00 ±0.58^{a†††}	22.43 ±0.64^{a†††}	32.00 ±0.36^{d†††}	12.40 ±0.12^{a†††}	0.70± 0.023^{a†††}	1.43± 0.049^{e†††}	2.08± 0.03^{a†††}
F Value	451.3	199.2	101.8	110.9	143.601	101.6	103.436	81.444	128.278
Tukey Range Test P Values Subset	a = 0.068^{ns} b = 1.000^{ns} c =1.000^{ns}	a = 0.485^{ns} b = 0.057^{ns} c = 0.925^{ns} d = 1.000^{ns}	a = 0.929^{ns} b = 0.357^{ns} c =1.000^{ns}	a = 0.422^{ns} b = 0.977^{ns} c =1.000^{ns}	a = 1.000^{ns} b = 0.895^{ns} c =0.265^{ns} d = 0.265^{ns}	a = 0.094^{ns} b = 0.357^{ns} c =1.000^{ns}	a = 0.992^{ns} b = 1.000^{ns}	a = 1.000^{ns} b = 0.994^{ns} c =0.190^{ns} d = 0.836^{ns} e = 0.083^{ns}	a = 0.983^{ns} b = 1.000^{ns}

***Mean values were highly significantly different from the healthy control group (P<0·05). †††Mean values were highly significantly different from the diabetic control group (P<0·05).
ZOME: *Zingiber officinale* Methanolic Extract, CLME: *Curcuma longa* Methanolic Extract, FPG: Fasting Plasma Glucose, Chol: Cholesterol, TG: Triglycerides, LDL: Low Density Lipoproteins, HDL: High Density Lipoproteins, VLDL: Very Low Density Lipoproteins, TC: Total Cholesterol, ns: non-significant, a, b, c, d and e: Tukey's range test analysis divides p values into subsets of a, b, c, d, and e to assess homogeneity of means.

However, considerably high VLDL levels on day 0 (table 2) were 221.74% increased (P<0.05) in diabetic-dyslipidemic control group; while, these were 66.67% and 66.60% reduced in diabetic-dyslipidemic groups treated by ZOME and CLME respectively for 42 days. Although, both therapies were equally effective (b=0.357); yet their synergistic therapy was comparatively more effective (72.07%) than their individual effects and mean VLDL level (12.40±0.12mg/dL) was homogenous with healthy control group (a=0.094).

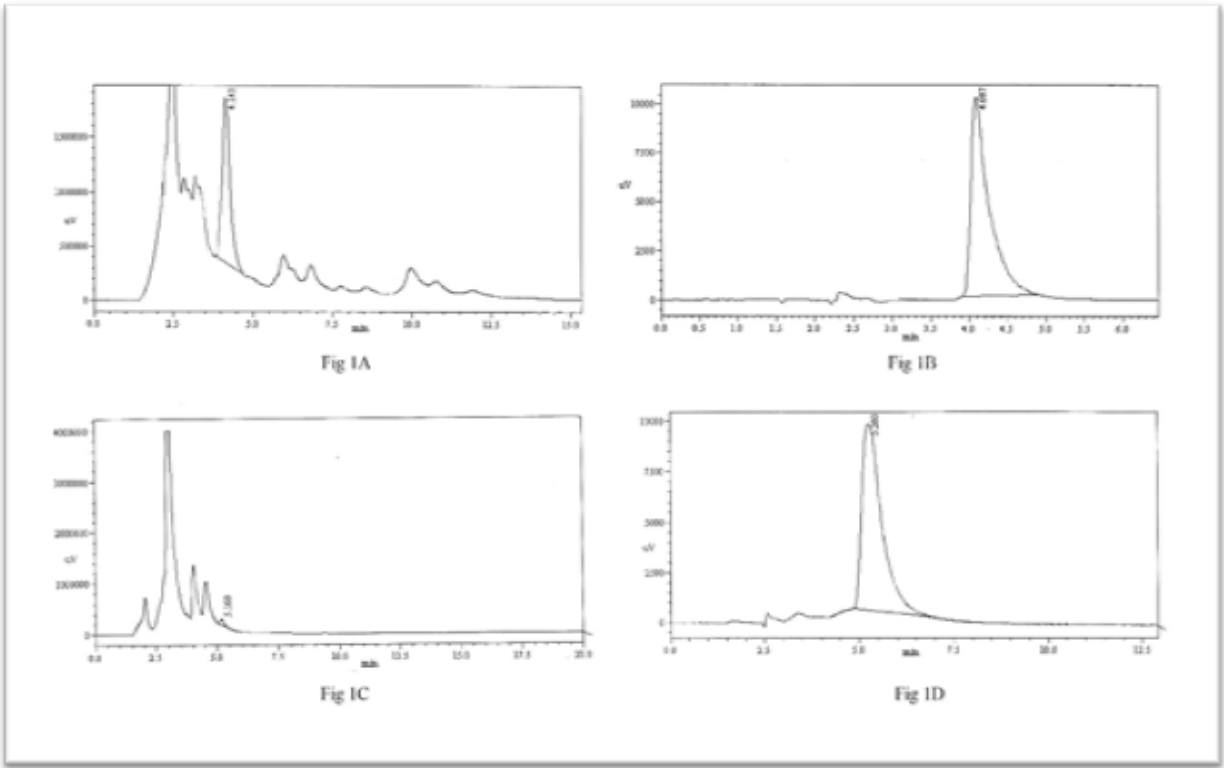
Lastly, TC/HDL, LDL/HDL ratios were also significantly (P<0.05) decreased while HDL/LDL ratio was significantly (P<0.05) increased in diabetic-dyslipidemic groups treated by ZOME and CLME respectively for 42 days. In each case, both therapies ZOME and CLME were although almost equally effective (table 3); but their synergistic therapy was comparatively more effective. Moreover, no significant change in any ratio was noted in healthy groups.

DISCUSSION

To our knowledge, this is the first study wherein the comparative and synergistic therapeutic potentials of methanolic extracts of both phytomedicines have been investigated. Even though, Madkor *et al* (2011) has previously studied the combined effect of both ZO and CL crude drugs, yet their study lacked the information about alcoholic extracts of ZO and CL; which contain mainly important bioactive compounds. Among these bioactive compounds, 6-gingerol and curcumin are the most potent constituents of ZO and CL; which have the great therapeutic potential to cure diabetes and dyslipidemia (Abdelaziz *et al,* 2012; Li *et al,* 2013; Singh *et al,* 2009; Shao *et al,* 2012; Elmoselhy *et al,* 2011).

It has been found in a study (Li*et al,* 2013) that 6-gingerol increases antioxidant enzyme activities and act as free radicals scavenging agent because of its protective role against reactive oxygen species (ROS): are mostly increased in both type of diabetes by glucose auto-oxidation, non-enzymatic glycation of proteins, and polyol pathway (Obrosova *et al,* 2002). Moreover, it also lowers increased lipid peroxidation induced by diabetic-dyslipidemia (Singh *et al,* 2009; Aldebasi *et al,* 2013; Kumari *et al,* 2008).

The insulin restoring and β-cells protective properties of 6-gigerol (Chakraborty *et al,* 2012) may be due to its possible interaction with 5-HT3 receptors (Heimes *et al,* 2009); as it has been studied that it may act on 5-HT3 receptor ion channel complex by binding to modulatory



*ZOME: *Zingiber officinale* Methanolic Extract, **CLME: *Curcuma longa* Methanolic Extract.

Fig. 1: HPLC chromatograms of ZOME* (A), 6-Gingerol analytical standard (B), CLME** (C), and Curcumin analytical standard (D)

sites clearly different from 5-hydroxytryptamine (serotonin) binding sites (Abdel-Aziz *et al,* 2006).

Likewise 6-gingerol, curcumin also protects cells from oxidative damage caused by chronic diabetic-dyslipidemia as it has strong intrinsic anti-lipoperoxidase and free radical scavenging activities. It increases superoxide dismutase, catalase, and glutathione peroxidase levels along with glutathione level. It has been studied that curcumin performs its anti-oxidative role via generating hydroxyl radicals by reduction of Fe^{3+} to Fe^{2+} through a reaction called 'Fenton reaction' (Kohli *et al,* 2005; Rajesh *et al,* 2013).

Recently, the anti-lipogenic molecular mechanism of curcumin in high-fat fed induced obese and type 2 diabetic rats was investigated; and it was noted that it inhibits lipogenesis by decreasing mRNA contents of SREBP1-c and ChREBP: are two main transcription factors of lipogenesis in liver (Shao *et al,* 2012).

The anti-diabetic mechanism of curcumin was best studied by Best *et al,* (2007). They found that it boosts up the insulin release from rats' pancreatic β-cells, and thus controls hyperglycemia because of its induced generation of electrical activity resulted from the activation of volume-regulated anion channel and cell membrane potential's depolarization. Furthermore, curcumin's anti-hyperglycemic potential was also supported by another study (Kanitkar *et al,* 2008). They found that it induces Hsp70 and improves pancreaticβ-cells recovery.

Besides the presence of potent bioactive compounds in ZOME and CLME, both extracts also have alkaloids, terpenoids, sterols, saponins, and flavonoids (Hussain *et al,* 2015; Best *et al,* 2007; Bhargava *et al,* 2012). All these phytochemicals also have strong intrinsic antidiabetic and anti dyslipidemic therapeutic potentials (Gulfraz *et al,* 2011; Asai *et al,* 2007; Eliza *et al,* 2009a; Eliza *et al,* 2009bLeite *et al,* 2007; Sharma *et al,* 2008; Meliani *et al,* 2011).

CONCLUSION

Keeping in view, almost same medicinal potential of ZOME and CLME, it is obvious that if both extracts are used in combination, then their potent synergistic effect may control diabetic-dyslipidemia more effectively. This probable synergism was confirmed in our study; however, the underlying molecular mechanism of the synergistic effect is still not fully explained and needs future studies. Lastly, synergistic therapeutic potential of both phytomedicines may be used in the composition of new safer and more effective drugs having dual properties to control diabetes and dyslipidemia or diabetic-dyslipidemia.

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