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ORIGINAL ARTICLE

EFFECT OF *CURCUMA LONGA* FREEZE DRIED RHIZOME POWDER WITH MILK IN STZ INDUCED DIABETIC RATS

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ABSTRACT

This study deals with the effects of freeze dried rhizome powder of Curcuma longa (C. longa) dissolved in milk on normal as well as diabetic models. Diabetes of type II and type I was within 3 days of a single administration of doses of 45 and 65 mg kg^{-1} of streptozotocin respectively. Various parameters such as blood glucose levels, triglycerides, total cholesterol, high density lipoprotein, very low density lipoprotein, low density lipoprotein, serum glutamic oxaloacetic transaminase, serum glutamic pyruvate transaminase, alkaline phosphatase, creatinine, hemoglobin, urine protein and urine sugar in addition to body weight were taken in to consideration and were analyzed after administration of variable doses of rhizome powder. The dose of 200 mg kg^{-1} was identified as the most effective dose as it increased HDL, Hb and bw ($P<0.05$) with significant decrease in the levels of blood glucose, lipid profile and hepatoprotective enzymes ($P<0.001$).

KEY WORDS

Curcuma longa rhizome, Freeze dried, Antidiabetic, Hypolipidemic, Hepatoprotective.

Food and medicine are, in fact, two sides of the same coin and man has been provided with both of these by plants. Herbal medicines have been used since ages for the treatment of diabetic patients and they are currently accepted as a complementary and alternative treatment for diabetes mellitus (1). More than 1200 plants have been screened till date scientifically as hypoglycemic agents (2, 3). Recently, awareness of synthetic drugs related health problems have increased tremendously resulting in an increasing demand of natural ingredients, as novel oral antidiabetic agents from these ethnobotanical plants (4).

From very early times spices have played an important role in Ayurvedic preparations. *Curcuma longa* (Zingiberaceae) commonly known as 'Haldi' in Hindi is also an important dietary spice. It is extensively used in Ayurveda, Unani and Siddha systems of medicine as home remedy for various diseases including bileary disorder, anorexia, diabetic wounds etc (5,6). Its aqueous extract has been screened for its protective effect against type 2 diabetes mellitus (7). Since, no work has been carried out so far on freeze dried powder of *C. longa* with milk and that too in type 1 diabetic models, therefore in the present study, an attempt has been made to evaluate the antidiabetic effect of freeze dried powder of *C. longa* rhizome dissolved in milk. The advantage of high solubility of *C. longa* in fat or other organic solvents has been taken in to consideration by using milk as a solvent for freeze dried *C. longa* powder (8). Freeze dried *C. longa* was preferred in order to preserve its active ingredients. The streptozotocin induced diabetic rats were considered as a valuable tool for the present pathophysiological and pharmacological studies. A number of biochemical parameters have been studied for validating its antidiabetic, hypolipidemic and hepatoprotective potential. Hence, dissolving the freeze-dried powder of *C. longa* rhizome in milk has facilitated the creative potential of the work. This report being an original research gives a vision of creativity

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by developing a novel oral herbal antidiabetic agent as a dietary supplement with triple action.

MATERIALS AND METHODS

Preparation of material and experimental animals: The rhizome of *C. longa* authenticated by Taxonomist, was freeze dried at – 40°C to get a powder. About hundred and twenty five male albino Wistar rats of body weight of 150-200 g, were housed under standard environmental conditions (25 ± 2°C temperature, 50 ± 5 % humidity with a 12 h each of dark and light cycle) and maintained with free access to water and a standard laboratory diet ad libitum. The Institutional Ethical Committee approved the study.

Induction of diabetes in rats: A single intraperitonial injection of two different doses of 45 and 65 $mgkg^{-1}$ of Streptozotocin (STZ) (Sigma Aldrich Chem. Co. USA.) were used for induction of diabetes of type II (9) and type I (10,11) respectively in over night fasted animals and were dived into sub, mild and severely diabetic models depending upon their FBG levels after 3 days of administration. Type II model: Sub diabetic: FBG 80-100 $mgdl^{-1}$; PPG 210-310 $mgdl^{-1}$; Mild diabetic: FBG 120-250 $mgdl^{-1}$; PPG 210-310 $mgdl^{-1}$. Type I models: Severely diabetic: FBG > 350 $mgdl^{-1}$; PPG >550 $mgdl^{-1}$.

Estimation: Blood glucose level (BGL) (12), total cholesterol (TC), high density lipoprotein (HDL) and triglyceride (TG) (13,14) asparate transferase (AST) and alanine transferase (ALT) (15), alkaline phosphatase (ALP) (16), total protein (TP) (17), creatinine (CRE) (18) and total haemoglobin (19) were measured using standard kits (Bayer Diagnostics India, Ltd.) by following known procedures. However, very low density lipoprotein (VLDL) and low density lipoprotein (LDL) was calculated by the formula. (20). Urine sugar (US) and urine protein (UP) were detected by reagent based Uristix of Bayer Diagnostics. All the parameters were measured initially before the treatment and then monitored regularly every week upto two weeks in case of severely diabetic animals.

Experimental Design: Hypoglycemic effect was studied in normal healthy rats by conducting fasting blood glucose (FBG) and glucose tolerance test (GTT) studies. Antidiabetic effect was assessed in sub diabetic as well as mild diabetic models of Type II by conducting similar set of GTT studies. The most effective dose identified in sub and mild diabetic cases was used for evaluating the antidiabetic, hypolipidemic and hepatoprotective potential in severely diabetic animals considered as type I models.

Assessment of hypoglycemic activity in normal healthy rats – FBG & GTT studies: Four groups of five rats each, fasted over night, were used in each of the experiments of FBG and GTT studies. Group I served as untreated control received vehicle (milk only). Groups II, III and IV received orally the doses of 150, 200 and 250 mg kg^{-1} respectively of the freeze dried rhizome powder of *C. longa* suspended in milk. Fasting blood samples were collected from tail vein initially before the treatment and then at 1.5, 3, 4.5 and 6 h after the treatment for FBG study. Whereas, for GTT study the effect of the above mentioned doses on FBG was studied initially at 2 h considered as '0' h value. The animals were then orally administrated with 2 g kg^{-1} of glucose and their glucose tolerance was studied at 1 h interval for the next 3 h.

Evaluation of antidiabetic activity in sub diabetic and mild diabetic rats – GTT studies: The antidiabetic effect of freeze dried rhizome powder of *C. longa* was also assessed by improvement of glucose tolerance in sub and mild diabetic rats. The overnight fasted rats were divided in to five groups of five rats each. Group I was control, received vehicle (milk only), whereas variable doses of 150, 200 and 250 mg kg^{-1} of freeze dried rhizome powder of *C. longa* dissolved in milk were given orally to group II, III and IV respectively after checking their FBG. Blood glucose levels were further checked firstly after 2 h of treatment considered as '0' h value and then 2 g/kg glucose was given orally to all the groups and their glucose tolerance was studied three hours at regular intervals of 1 h each, considered as 1 h, 2 h and 3 h values. The results were compared with group V treated with 0.5 mg kg^{-1} of Glipizide (hypoglycemic agent).

Determination of antidiabetic, antilipedemic & hepatoprotective attributes in severely diabetic rats: Three groups of five rats each were used in the experiment. Group I served as diabetic control received vehicle (milk only). Group II and III were treated with 3 units of insulin and 200 mg kg^{-1} of *C. longa* rhizome powder dissolved in milk respectively. A number of biochemical parameters such as FBG, PPG, lipid profiles, total protein, total haemoglobin, urine sugar, urine protein were studied. Enzymatic assays and body weight were also taken into consideration for defining a biomedical profile of *C. longa* rhizome powder with milk synergy.

LD_{50} experiment: Two groups of rats of both the sexes (6 animals per group, 3 females and 3 males), weighing 180-200 grams were orally administered by a single dose of 2.0 g and 3.0 g of the *C. longa* freeze dried powder with milk. The rats were then observed for gross behavioural neurologic,

autonomic and toxic effects continuously. Food consumption, faeces and urine were also examined at 2 h and then at 6 h intervals for 24 h.

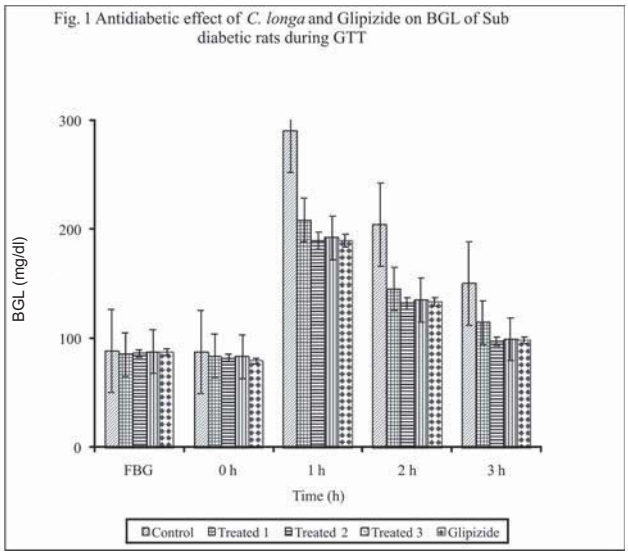
Statistical analysis: Data were statistically evaluated using one-way ANOVA, followed by a post hoc Newman-Keuls Multiple Comparison Test. The values were expressed as mean ± SD and considered significant at (P<0.05).

RESULTS

Hypoglycemic effect on FBG of normal healthy rats: Table 1 depicts the blood glucose lowering effect of a single oral administration of variable doses of 150, 200 and 250 mg kg^{-1} of freeze dried *C. longa* rhizome powder dissolved in milk in normal healthy rats on their FBG levels. The maximum fall observed was 13.1, 17.7 and 17.6 % (P <0.001) at 6 h respectively.

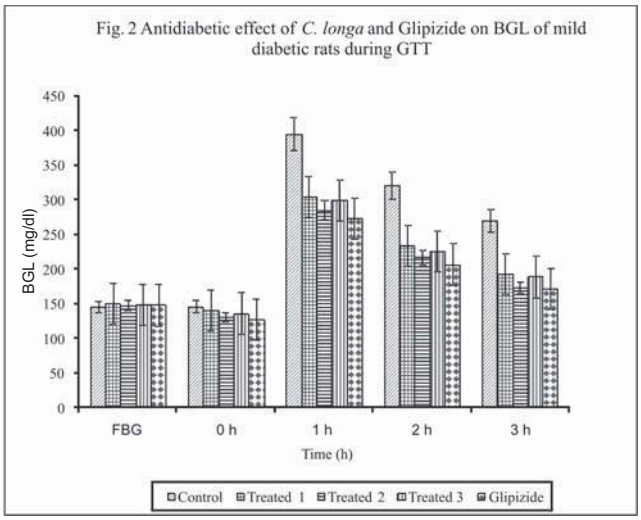
Hypoglycemic effect on GTT of normal healthy rats: Table 2 deals with the hypoglycemic effect of *C. longa* rhizome powder on BGLs during GTT of normal healthy rats. Different doses of 150, 200 and 250 mg kg^{-1} of C. longa were given orally to overnight fasted normal healthy rats and the fall observed after 3 h of glucose administration was 22.1, 27.9 and 26.0 % (P<0.01) respectively. The dose of 200 $mgkg^{-1}$, associated with the maximum fall of 27.9%, was identified as the most effective dose for further studies.

Effect on diabetic rats during GTT: Fig 1 and Fig 2 demonstrate the antidiabetic effect of freeze dried *C. longa* with milk on BGLs of sub and mild diabetic animal respectively during GTT studies. Different doses as mentioned above along with the standard drug Glipizide 0.5 mg kg^{-1} were given orally to the groups as defined in the experimental design. The fall of 23.4, 34.9 and 33.7 % (P<0.01) was observed with the doses of 150, 200 and 250 $mgkg^{-1}$ of *C. longa* respectively.



** P < 0.01, * P <0.05 as compared with control.

Control: Milk, Treated 1: 150 mg kg^{-1} of *C. longa* Powder, Treated 2: 200 mg kg^{-1} of *C. longa* Powder, Treated 3: 250 mg kg^{-1} of *C. longa* Powder, Glipizide: 0.5 mg kg^{-1}.



** P < 0.01, * P <0.05 as compared with control.

Control: Milk, Treated 1: 150 mg kg^{-1} of *C. longa* Powder, Treated 2: 200 mg kg^{-1} of *C. longa* Powder, Treated 3: 250 mg kg^{-1} of *C. longa* Powder, Glipizide: 0.5 mg kg^{-1}

Table 1: Hypoglycemic effects of freeze dried *C. longa* with milk on FBG of normoglycemic rats

		Blood glucose levels (mg/dl)				
		Pretreatment	Post treatment (hours)			
Groups Treatment	Doses (mg kg^{-1})	FBG	1.5	3.0	4.5	6.0
I (milk)	Milk	70.2 ± 3.9	70.5 ± 3.2	70.8 ± 4.6	69.4 ± 3.8	69.1 ± 4.2
II (*C. longa* + milk)	150	71.5 ± 3.2	68.2 ± 4.4	65.6 ± 4.6**	62.1 ± 5.1	62.3 ± 3.8*
III (*C. longa* + milk)	200	70.3 ± 3.2	66.9 ± 4.4	63.4 ± 4.6	57.8 ± 5.1**	58.1 ± 3.8*
IV (*C. longa* + milk)	250	72.1 ± 3.2	67.4 ± 4.4	65.1 ± 4.6	59.3 ± 5.1	60.1 ± 3.8*

Values are in mean±SD; **P<0.01, *P<0.05 as compared with initial

Table 2: Hypoglycemic effects of freeze dried *C. longa* with milk on BGL during GTT of normoglycemic rats

Groups Treatment	Doses (mg kg^{-1})	Blood glucose levels (mg/dl) Pretreatment FBG	Post treatment (hours) 0	1	2	3
I (milk)	Milk	72.3 ± 4.7	72.5 ± 4.1	106.9 ± 4.6	102.5 ± 3.2	94.8 ± 4.3
II (*C. longa* + milk)	150	71.8 ± 42	69.6 ± 4.6	88.9 ± 4.9	80.6 ± 4.4**	73.8 ± 3.8
III (*C. longa* + milk)	200	71.4 ± 3.5	69.1 ± 3.6	83.2 ± 4.9	74.3 ± 4.6**	68.3 ± 3.7
IV (*C. longa* + milk)	250	72.8 ± 4.2	70.5 ± 5.6	85.4 ± 4.9	76.1 ± 4.8*	70.0 ± 4.4*

Values are in mean±SD; **P<0.01, *P< 0.05 as compared with control

Whereas, the dose of 0.5 mgkg^{-1} of Glipizide reduced BGL by 34.5 % (P<0.01) at 3 h of glucose administration during GTT in sub diabetic rats. However, in case of mild diabetic rats the fall was 28.5, 35.8 and 29.9 % (P<0.001) with the doses of 150, 200 and 250 mg kg^{-1} of *C. longa* respectively at 3 h of glucose administration. The fall observed with the dose of 0.5 mg kg^{-1} of Glipizide was of 36.2 % (P<0.001) at 3 h during GTT in mild diabetic rats. Moreover, the maximum fall was associated with the dose of 200 mg kg^{-1} and was comparable too with the dose of 0.5 mg kg^{-1} of Glipizide in both sub and mild diabetic animal.

Impact on FBG, PPG and Lipid profile of severely diabetic rats: Table 3 shows the impact of the most effective dose identified i.e. 200 mg kg^{-1} of *C. longa* with milk and 3 units of insulin on FBG, PPG and lipid profile of severely diabetic rats on two weeks treatment. At the end of the *C. longa* treatment, the animals were compared with their own

Table 3: Effect of most effective dose of *C. longa* on BGL and lipid profile of severely diabetic rats

Groups Treatment	Pre treatment level	Post-treatment levels 7 days	14 days
FBG (mg/dl)			
Milk	342 ± 4.5	354.1 ± 5.8	361.8 ± 6.1
Insulin	372.6 ± 5.7	290.7 ± 5.4***	234.8 ± 5.1***
C. longa + milk	377.8 ± 7.5	316.2 ± 8.6**	233.4 ± 6.8**
PPG (mg/dl)			
Milk	549.9 ± 10.2	550.1 ± 8.7	554.4 ± 7.8
Insulin	563.4 ± 9.8	419 ± 8.1*	302.5 ± 7.8
C. longa + milk	553.7 ± 9.5	414.9 ± 9.0**	307.2 ± 8.7**
Triglycerides (mg/dl)			
Milk	178.7 ± 6.4	179.2 ± 6.9	176.9 ± 7.4
Insulin	172.6 ± 8.4	132.6 ± 5.1*	94.5 ± 3.5*
C. longa + milk	180.2 ± 5.2	142.8 ± 4.6***	89.5 ± 4.8**
Total cholesterol (mg/dl)			
Milk	119.9 ± 4.2	118.5 ± 6.5	117.4 ± 5.8
Insulin	123.7 ± 7.8	109.2 ± 5.2*	91.6 ± 6.8
C. longa + milk	122.5 ± 6.3	111.2 ± 5.2***	93.8 ± 5.9***
HDL cholesterol (mg/dl)			
Milk	19.2 ± 4.8	19.0 ± 5.2	17.6 ± 2.8
Insulin	20.9 ± 5.2	23.6 ± 4.8*	26.8 ± 4.8
C. longa + milk	20.7 ± 3.2	24.3 ± 4.4**	26.5 ± 3.8**

Values are in mean±SD; ***P<.001 as compared to pretreatment levels; **P<0.01 as compared to pretreatment levels; *P<0.05 as compared to pretreatment levels.

Table 4: Impact of the most effective dose of freeze dried *C. longa* with milk on serum enzymes of severely diabetic rats on two weeks treatment (mean ± SD)

Groups Treatment	Pre treatment level	Post-treatment levels	
		7 days	14 days
SGOT (U/L)			
Milk	28.7 ± 4.5	29.4 ± 3.8	30.2 ± 5.5
Insulin	29.9 ± 4.1	24.1 ± 3.9**	21.5 ± 3.2**
C. longa + milk	30.5 ± 4.5	27.9 ± 3.8*	23.6 ± 4.6**
SGPT (U/L)			
Milk	31.6 ± 3.5	32.1 ± 2.8	32.9 ± 2.1
Insulin	30.8 ± 4.5	24.5 ± 5.1*	19.6 ± 3.4*
C. longa + milk	30.1 ± 4.7	26.8 ± 3.9**	20.7 ± 4.1**
ALP (U/L)			
Milk	144.5 ± 6.4	144.8 ± 6.8	145.3 ± 2.8
Insulin	158.5 ± 4.4	131.2 ± 5.6**	103.6 ± 5.1**
C. longa + milk	155.8 ± 5.8	135.2 ± 4.6**	105.6 ± 4.8***
CRE (U/L)			
Milk	2.2 ± 0.2	2.2 ± 0.1	2.3 ± 0.2
Insulin	1.2 ± 0.6	1.1 ± 0.3#	1.1 ± 0.8#
C. longa + milk	2.1 ± 0.5	1.9 ± 0.3*	1.5 ± 0.4**

Values are in mean±SD; **P<0.01 as compared to pretreatment levels. ***P<0.001 as compared to pretreatment levels. *P<0.05 as compared to pretreatment levels. # Not Significant as compared to pretreatment levels.

initial values and showed a significant reduction of 38.2 % (P<0.001) in FBG and 44.5 % (P<0.001) in PPG levels. Almost similar reduction of 36.9 and 46.3% (P<0.001) in FBG and PPG levels was observed with insulin treatment. The enhanced levels of TG, TC, LDL and VLDL cholesterol were brought down significantly by 50.3, 23.4, 24.9, and 50.2 % (P<0.001) after the *C. longa* treatment. The group treated with 3 unit of insulin showed practically the same fall in TG, TC, LDL, and VLDL 45.2, 25.9, 17.6, and 45.2 % (P<0.01) respectively. However, *C. longa* as well as insulin treatment both improved HDL cholesterol levels by 28.4 and 28.2 % (P<0.001) respectively.

Impact on SGOT, SGPT, ALP, and CRE levels of severely diabetic rats: Table 4 demonstrates the hepatoprotective effect of two weeks long-term treatment on SGOT, SGPT, ALP and CRE levels in blood serum of severely diabetic rats. In diabetic control rats there was a gradual increase in all these parameters. Whereas, the most effective dose of 200 mg kg^{-1} of the *C. longa* produced a fall of 22.6, 31.2, 32.2 % (P<0.01) and 28.5 % (P<0.05) in SGOT, SGPT, ALP and CRE levels respectively and the insulin treatment showed reduction of 28.0 % (P<0.01) in SGOT, 34.6 % (P<0.001) in SGPT, 34.6 % (P<0.01) inALP, and 8.3 % (P<0.001) in CRE levels.

Impact on Hb, TP, UP, US and bw of severely diabetic rats : Table 5 reveals the impact of two weeks treatment with the most effective dose of 200 mg kg^{-1} of *C. longa* with milk and 3 unit of insulin on Hb, TP, UP, US, and bw of severely diabetic rats. It has been observed that on *C. longa* treatment UP and US, levels were decreased by 50, and 66.6% (P<0.01). Whereas, TP, Hb and bw increased by 6.2, 7.2 and 3.4 % (P<0.05).

LD_{50} : LD_{50} Experiment was carried out on normal healthy rats. The behavior of the treated rats appeared normal. No toxic effect was reported up to 10 and 15 times of the effective dose as no mortality was observed in any of the groups.

DISCUSSION

The present study was under taken to explore the vision of creativity scientifically by assessing the antidiabetic, hypolipidemic and hepatoprotective potential, of freeze dried *C. longa* rhizome powder dissolved in milk, in both type II as well as type I diabetic models. Glipizide a potent inhibitor of tyrosine phasphatase (21) and insulin were used as reference drugs for type II and type I models respectively. It is known that Glipizide mimics several insulin actions *in vivo* such as

Table 5: Impact of the most effective dose of *C. longa* on Hb, TP, UP, US and bw of severely diabetic rats on two weeks treatment

Groups Treatment	Pre treatment level	Post-treatment levels	
		7 days	14 days
***Haemoglobin* (mg/dl)**			
Milk	9.6 ± .7	8.7 ± .5	8.0 ± .8
Insulin	7.8 ± .5	8.0 ± .6**	8.2 ± .7**
C. longa + milk	9.7 ± .5	9.9 ± .5***	10.4 ± .4***
Total protein (mg/dl)			
Milk	6.1 ± .5	5.9 ± .9	5.4 ± .8
Insulin	6.4 ± .6	6.9 ±.3**	7.3 ± .5**
C. longa + milk	6.5 ± .6	6.7 ± .8*	6.9 ± .4*
Urine protein			
Milk	+++	+++	+++
Insulin	+++	++	+**
C. longa + milk	+++	++**	++**
Urine Sugar			
Milk	++++	++++	++++
Insulin	++++	+++	++**
C. longa + milk	++++	++**	++**
Body weight (g)			
Milk	150 ± 8.0	150 ± 7.5	145± 5.5
Insulin	150 ± 5.0	150 ± 6.5*	150± 4.5*
C. longa + milk	145 ± 2.5	150 ± 3.0**	155± 4.0**

Values are in mean±SD; *** P<0.001 as compared to pretreatment levels; **P< 0.01 as compared to pretreatment levels; *P< 0.05 as compared to pretreatment levels; # Not Significant as compared to pretreatment levels.

the stimulation of hexose cellular uptake and lipogenesis and the inhibition of lipolysis (22). The maximum fall observed during FBG and GTT studies was 17.7 and 27.9 % respectively with the dose of 200 $mgkg^{-1}$ of *C. longa* in normal rats. The hypoglycemic activity proposed is due to the inhibition of key enzymes involved in the gluconeogenesis and glucogenolysis pathway. The same dose showed a marked improvement in GTT of sub and mild diabetic animals by 34.9 and 35.8 % respectively. These falls are comparable with the fall of the synthetic drug Glipizide hence, the dose of 200 $mgkg^{-1}$ was identified as the most effective dose for the long term treatment of two week of severely diabetic animals and it reduced the level of FBG by 38.2 % and PPG by 44.5 % in case of severely diabetic rats. On the other hand blood glucose levels of its control group were increased significantly. This increment could be due to reduced glucose clearance apparently arising from a defect in glucose transport (23, 24).

Diabetes mellitus is usually associated with elevated serum lipid levels considered as risk factors for coronary heart diseases (25, 26). Lowering of these elevated levels either though drug or dietary therapy seems to be associated with a decrease in the risk of vascular disease (27). The present study of severely diabetic animals showed significantly declined levels of TC, LDL, and VLDL by 23.4, 24.9 and 50.2 % respectively and enhanced HDL cholesterol level by 28.4 % after two weeks treatment with *C. longa*. Many antihypercholesterolemic drugs do not decrease TG levels though TG plays an independent role in increasingly the risk of coronary heart disease (28, 29). However, this long-term treatment of *C. longa* for two weeks lowered the TG levels too by 50.3 %.

Moreover, the enhanced levels of hepatoprotective enzymes due to severe diabetes such as SGOT, SGPT, ALP and CRE were also reduced by 28.0, 34.6, 34.6, and 8.3% respectively as an additional advantage of this study. The *C. longa* powder together with milk decreased the glucose concentration in urine by 50 %, which is generally high in case of chronic diabetic cases (30). Other important parameters such as Hb, TP and bw, which are generally lower than their normal values in chronic diabetic cases, were also controlled by the treatment

of *C. longa* and managed to raise by 6.7, 8.6 and 3.4 % respectively.

A scientific and systemic exploration reveals the antidiabetic, hypolipidemic and hepatoprotective effects of *C. longa* freeze dried rhizome powder dissolved in milk which could be used as an effective and safe antidiabetic dietary supplement of high potential.

The study concludes that this first reporting of the scientific exploration of triple action potential of *C. longa* freeze dried rhizome powder dissolved in milk with special reference in type I diabetes models is not only interesting but very encouraging too for medicinal chemists as well as diabetic patients globally.

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