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In Vitro Evaluation of Cholesterol Lowering Properties of Plant Extract, *Trigonella Foenum Graecum* L.

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Abstract**—** *Atherosclerosis is a disorder that causes the inner lining of arterial walls to accumulate lipids, cholesterol, and other chemicals. This restriction of blood flow results in high cholesterol, high blood pressure, high triglycerides, diabetes, obesity, and associated cardiovascular disorders. The current study sought to evaluate the ability of plant extract Trigonella foenum graecum ability to decrease cholesterol in vitro. Plant extracts were used at varying doses to block the activities of enzymes pancreatic lipase, glucose-6-phosphate dehydrogenase, and malic dehydrogenase. The extracts were then sequentially extracted using solvents including methanol, hexane, ethyl acetate, ethanol, and dichloromethane. According to the findings, the ethanol extract had the greatest significant hypolipidemic impact by reducing enzyme activities, and this was followed by the extracts of hexane, dichloromethane, and methanol. The ethyl acetate extracts showed the lowest hypolipidemic activity. These findings imply that the administration of several Trigonella foenum graecum plant extracts may have unique potential cholesterol-lowering qualities for reducing fat absorption via inhibiting pancreatic lipase.*

Keywords**— Fenugreek, pancreatic lipase, glucose-6-phosphate dehydrogenase, malic dehydrogenase, lipid metabolism.**

I. INTRODUCTION

The hardening of the arteries by an increase in plaque in the inner layer of the artery is known as atherosclerosis. Even though it may not always result in symptoms, high blood cholesterol is one of the primary indicators of risk for heart disease. It has been observed recently that include functional foods in diets might help prevent cardiovascular illnesses (Esposito *et al.*, 2004; Singh *et al.*, 2015; Jan Fedacko *et al.*, 2016). Water soluble fiber diets, including those containing spirulina, oats, and coriander, have been shown to have hypocholesterolemic effects. These diets have also been linked to a significant drop in serum total cholesterol and a decrease in low density lipoprotein without a corresponding reduction in high density lipoprotein (Salas-salvado *et al.*, 2008; Estruch *et al.*, 2013). Cholesterol levels can be influenced by several elements, such as genetics, nutrition and obesity. Maintaining dietary items regarded as nutraceutical functional foods—such as green vegetables, turmeric, peppers, soy products, coriander, bitter gourd, cumin, olive oil, mustard oil, fish, etc. is crucial for decreasing cholesterol. Low density lipoproteins (LDL) and very low density lipoproteins (VLDL) are decreased and high density lipoprotein (HDL) is elevated in the serum when soy, ginger, coriander, and spices are consumed (Lydie and Vilaume, 2001; Pavana *et al.*, 2007; Dhanapakiam *et al.*, 2008).

In many cardiac disorders, it may be advantageous to increase blood levels of high density lipoproteins while reducing levels of low and very low density lipoproteins. Curcumin causes rats that are both normal and hypocholesterolemic to excrete more bile acids in their feces. This suggests that the spice increases the liver's ability to produce bile as well as the process by which cholesterol is converted into bile salts (Dhanapakiam *et al.*, 2008). The effects of *Trigonella foenum graecum*

supplementation on several areas of lipid metabolism are poorly studied. The goal of this present work was to use in vitro studies to ascertain the impact of fenugreek on lipid metabolism in light of the significant consequences.

II. MATERIALS AND METHODS

Using distilled water, the gathered plant portion was carefully cleaned to get rid of any dirt or other contaminants. In order to preserve the vibrant green color of the plant components and to stop the breakdown of their active ingredients, they were dried at room temperature. After being dried and ground to a coarse texture, the plant material was sealed in a container that was airtight.

Extraction of *Trigonella foenum graecum* L.

A section of the *Trigonella foenum graecum* L. plants' leaves and stems were gathered from the Chithode neighborhood in the Erode District of Tamil Nadu, India. The stems and leaves of the plant were chopped into small pieces, shadow dried for three days, and then dried for six hours at 60 degrees Celsius in a hot air oven. The plant portions were then ground well into the fine powder using a mill, and the size of the particles ranges from 300 to 400 µm was screened using a sieving machine for future use. Using a Soxhlet device, 50 g of powdered materials were extracted using 95% (v/v) ethanol.

250 ml of solvents, including ethanol, methanol, ethyl acetate, hexane, dichloromethane, and water, were added to the flask's bottom. Using a mantle beneath reflex condensers, all of the solvents were heated for two hours. After the extraction process was finished, the solvents were evaporated using a rotary evaporator at 80°C. In order to prepare the samples for future examination, they were finally gathered, prepared in different concentrations (0.5, 1.0, 1.5, 2.0, and 2.5 mg/ml), and kept at 4°C (Padayappa *et al.*, 2020).

Inhibition of pancreatic lipase activity

The inhibition activity of pancreatic lipase was used to evaluate the ability of plant extracts to decrease cholesterol (Puneeth *et al.*, 2016). 1 ml of reaction mixtures is comprising one unit of lipase enzyme and 100 ml of phosphate buffer (pH 7.2) containing 0.5% Triton-X-100 was incubated separately with plant extracts. P-nitrophenyle butyrate (5 mM in acetonitrile) was included to initiate the enzyme activity, which was measured at 340 nm. Percentage activity was used to convey the results, and a control was kept in place.

Inhibition of glucose-6-phosphate dehydrogenase activity

By tracking the decrease of NADP at 340 nm, glucose-6-phosphate dehydrogenase activity was determined (Xu *et al.*, 2005). 1 ml of a reaction mixture comprising 0.5 units of enzyme, 50 ml of tris HCl (pH 7.4), and 0.1 ml of glucose-6-phosphate was incubated individually with plant extracts. At 340 nm, the enzyme reactions were monitored after 0.15 mM NADP was added. The control was appropriately kept up to date, and the percentage activity of the findings was reported.

Inhibition of malic dehydrogenase activity

By tracking the decrease of NADP 340 nm, malic dehydrogenase activities were discouraged (Kong *et al.*, 2001). Plant extract was incubated separately in 1 ml of tris HCl (100 mM, pH 7.4) include L-malate (10 mM), $MnCl_2$ (2 mM), and 0.5 unit of malic dehydrogenase enzyme. At 340 nm, the enzyme reactions were monitored after 2.0 mM NADP was added. The control was appropriately kept up to date, and the percentage activity of the findings was reported.

Statistical analysis

Each value was displayed as mean ± SD. Two-way ANOVA was used to evaluate the statistical significance ($p<0.05$), and then a two-tailed Student's t-test was performed.

III. RESULTS AND DISCUSSION

The pancreas secretes the digesting enzyme pancreatic lipase, also referred to as triacylglycerol acylhydrolase. The hydrolyzing enzymes convert dietary fat molecules into triglyceride (Peter Nuhn, 1990). The results of this experiment demonstrated that the activities of pancreatic lipase, glucose-6-phosphate dehydrogenase and malic dehydrogenase were greatest in the ethanol extract meanwhile the lower activities were showed in ethylacetate extract. The pancreatic lipase is more active against short-chain glycerides than long-chain glycerides. According to Yasaman and Sharma (2018), pancreatic lipase plays a crucial part in the mechanism of various medications that are recommended for decreasing cholesterol. One important lipolytic enzyme that catalyzes the breakdown of dietary triglycerides is pancreatic lipase (Lowe, 2002). Pancreatic lipase, which is secreted by pancreatic acinar cells, releases fatty acids that are absorbed in the small intestine and enter the peripheral circulation as chylomicrons. Additionally, lipase inhibition decreases fat absorption by interfering with fat hydrolyses, which decreases the utilization of ingested lipids (Birari and Bhutani, 2007; Seyedan *et al.*, 2015).

The enzyme glucose 6-phosphate dehydrogenase catalyzes the production of fatty acids and cholesterol by converting G6P to 6-phosphogluconolactone and generating NADPH from $NADP^+$. Despite being necessary for lipogenesis, not much has been identified about the possible function of G6PD in adipocytes for energy balance, including glucose and lipid metabolism. The other extracts shown mild and negligible effects on the inhibition of glucose-6-phosphate dehydrogenase, but the ethanol extracts significantly reduced the enzyme's activity at 2.0% concentration (14.00±0.88), resulting in a hypolipidemic impact.

The degree of diabetes generally corresponds with a reduction in the serum activities of malic dehydrogenase (MDH) and glucose 6 phosphate dehydrogenases (G6PDH) (Xu *et al.*, 2005). These enzymes' reduced activity is linked to hyperglycemia. Diminished MDH activity leads to a decreased malate/succinate shuttle mechanism, which modifies the energy metabolism dependent on carbohydrates (Arai et al., 2003). The samples employed in this investigation had varying degrees of impact on the MDH activity in vitro (Table 1). G6PDH activity, however, significantly decreased. It's interesting to note that the remaining samples had no discernible impact on the in vitro activity levels. Since glycolytic enzymes constitute the ultimate energy generation machinery, their activity levels in hepatocytes are hard to change. However, these activity levels are reduced under diabetes circumstances, necessitating the use of alternative glucose-utilizing pathways.

Ono *et al.* (2006) assessed the effect of ethanol and aqueous extracts of *Nelumbo nucifera* leaf on pancreatic lipase inhibition using both in vitro and in animal investigations. The in vivo findings showed that the plasma triacylglycerol levels in the group of rats injected with the plant extract were much lower than those of the control group, and that these levels dramatically increased an hour after the lipid emulsion was orally administered. Kurihara *et al.* (2003) investigated the ability of *Cyclocarya paliurus* water extract (such as leaves) to decrease pancreatic lipase activity. The plant extracts inhibited the activity of pancreatic lipase. Moreover, the extract (250 mg/kg) reduced the plasma triacylglycerol levels in mice that were administered 5 mL/kg of lard and olive oil.

In an in vitro experiment, Kim and Kang (2005) used tributyrin as a substrate and a continuous-monitoring pH-Stat method to assess the lipase inhibitory effect of aqueous and ethanol extracts of different sections of 19 selected medicinal plants. Compared to orlistat, the two plant extracts that had the highest efficacy were *Illicium religiosum* and *Juniperus communis Thuja orientalis*, *Pyrus pyrifolia* and *Euonymus alatus* were shown to have pancreatic lipase activity.

Using a radioactive technique, Sharma *et al.* (2005) examined the antilipase activity of various portions of 75 medicinal plants from various families. Methanolic extracts from the whole portions of three plants such as *Setaria italica*, *Orixa japonica*, and *Eriochloa villosa* exhibited the strongest in vitro antilipase activity of all the extracts that were studied. Kwon *et al.* (2003) examined the inhibitory impact of *Dioscorea nipponica* methanol extract on pancreatic lipase activity. Lipase activity was lowered by the plant extracts in a dose-dependent manner. When compared to orlistat, a popular drug at different levels, the extract effectively inhibited pancreatic lipase at a dose of 10 µg/mL (IC50), resulting in a 50% drop in enzyme activity.

IV. CONCLUSION

Pancreatic lipase, glucose-6-phosphate dehydrogenase and malic dehydrogenase that the administration of several *Trigonella foenum graecum* plant extract were evaluated for their in-vitro hypolipidemic qualities different combinations plant extracts. Research indicates that significant biological impacts of fenugreek and that the effectiveness of enzyme activities.

Table 1: Inhibition activities of pancreatic lipase, glucose-6-phosphate dehydrogenase and malic dehydrogenase at different concentrations of plant extracts

Solvents	Concentrations	Pancreatic lipase	glucose-6-phosphate dehydrogenase	malic dehydrogenase	P value
Methanol	0.5	33.42±1.04	35.11±0.09	32.90±0.67	0.00011*
	1.0	31.55±1.09	32.56±1.04	29.45±0.74	
	1.5	27.74±0.08	29.98±1.22	26.89±0.40	
	2.0	26.89±1.53	25.55±0.93	24.65±0.99	
Hexane	0.5	28.55±0.25	34.04±1.98	32.00±1.93	0.00001*

	1.0	26.98±0.10	31.88±2.87	28.92±1.25	
	1.5	23.49±1.49	29.54±0.41	27.12±1.99	
	2.0	22.23±0.09	26.03±0.66	25.76±1.64	
Ethyl acetate	0.5	40.90±0.98	41.00±0.45	42.78±0.42	0.000115*
	1.0	36.44±0.12	39.81±0.01	40.54±0.20	
	1.5	34.15±0.15	36.91±0.20	38.83±0.31	
	2.0	31.75±0.10	34.23±0.29	35.51±0.92	
Ethanol	0.5	18.68±0.89	20.00±0.30	21.21±0.58	0.000425*
	1.0	16.00±0.22	19.78±0.12	20.12±0.57	
	1.5	13.33±0.98	17.72±0.09	18.33±0.97	
	2.0	11.97±0.19	14.00±0.88	16.12±0.13	
Dichloromethane	0.5	30.23±0.76	35.28±0.43	33.77±0.78	0.000198*
	1.0	28.23±0.11	32.12±0.54	30.22±0.91	
	1.5	27.09±0.67	29.68±0.19	27.64±0.11	
	2.0	24.02±0.35	28.90±0.72	25.23±0.87	
Fenofibrate (Standard)	1.0	26.02±1.10	26.15±1.52	25.10±1.20	0.000488*
	1.5	24.10±1.60	24.01±1.08	23.18±1.45	
	2.0	22.08±1.22	23.12±0.95	21.02±1.16	
	1.0	19.40±1.05	20.15±1.24	20.96±1.10	

Two way ANOVA

* Significance (P< 0.05)

** Insignificance (P>0.05)

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