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Antihypercholesterolemic Role of Ethanolic Extract of Jamun (*Syzygium cumini*) Fruit and Seed in Hypyercholesterolemic Rats

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Abstract: *Syzigium cumini* or *Eugenia jambolana* usually known as 'jamun', is extensively consumed in several regions of India for the management of different diseases. The present investigation was carried to assess the antihyperlipidaemic characteristics of ethanolic extract of *Syzigium cumini* seed and fruit in hypercholesterolemic rats. Ethanolic extracts were obtained through conventional solvent extraction to evaluate their efficiency for improving the blood lipid profile. High cholesterol diet containing 1.5% of cholesterol was fed to the normal rats to raise their lipid profile *i.e.* cholesterol, low density lipoprotein and triglycerides. Then diet containing 3% extract was fed to the rats. Serum analysis showed that cholesterol, low density lipoproteins(LDL) and triglycerides were reduced in hyperlipidaemic rats and maximum reduction was up to 9.32, 11.46 and 7.09%, respectively and increase in high density lipoproteins (HDL) was 2.62%, due to nutraceutical seed extract ($Nutraceutical_{SE}$) diet. The nutraceutical fruit extract ($Nutraceutical_{FE}$) diet resulted in less reduction in the respective traits as compared to $nutraceutical_{SE}$ diet. Our research exhibited that jamunhas good protective role against hypercholesterolemia.

Key words: Ethanolic · Jamun · Hyperlipidaemic Rats · Cholesterol · Serum

INTRODUCTION

Plants are being widely used in the treatment of different disorders by traditional physicians in different countries over many centuries [9]. Drugs from plants are being used in the form of powder, herbal formulations, paste and tinctures [4]. A new term nutraceutical have been introduced since 1989. A nutraceutical is food or part of food that provides health benefits including the treatment as well as prevention of disease. Nutraceuticals are gained from plant sources for the benefit of mankind [2]. Jamun (*Syzygium cumini)* is one of the potential plants which are being used in treatment of several diseases in India for many decades [17]. Jamun belongs to family Myrtaceae. Other botanical names of jamun are *Eugenia jambolana* and *Eugenia cumini*. There are different common names in different regions such as jamun, jambul, jamblang, Indian blackberry and java plum [16]. In addition to dietary use, it is also used for treatment of wide range of diseases in sub-continent regions especially in India. It has been stated that different parts of the jamun have antidiabetic, antioxidant, anti-microbial, anti-bacterial, free radical scavenging, anti-diarrheal, gastroprotective, anti-inflammatory and antihyperlipidaemic activities [14]. The disease preventive activity of jamun is due to presence of antioxidant compounds like flavonoids and phenolic contents [8]. Oxidative stress has its role in inducing diabetes and atherosclerosis is a major disorder linked with diabetes. Jamun has potential to keep the blood cholesterol at its normal level. It has been stated that the *Syzygium cumini* seed holds numerous biologically active ingredients such as gallic acid, flavonoids, ellagic acid, saponins, glycosides and triteripenoids. These bioactive moieties are potentially useful in preventing chronic disorders like diabetes and hypercholesterolemia [7]. The present study

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was conducted to evaluate the hypocholesterolemic effect of jamun fruit and seed in normal and hypocholesterolemic rats.

MATERIALS AND METHODS

Raw Material: Jamun fruit was procured from the local market and subjected to sample preparation. Sprague Dawley rats used in the efficacy trials were acquired from National Institute of Health (NIH) Islamabad. Diagnostic Kits used were from Sigma-Aldrich, Bioassay (Bioassays Chemical Co. Germany) and Cayman Chemicals (Cayman Europe, Estonia).

Preparation of Jamun Extracts: Fruits were washed and pulp was separated from seed. Both seed and pulp were sun dried. Extracts were obtained using aqueous ethanol solvent (50% v/v) according to the method of Arun *et al*. [3]. Both fruit and seed powder were taken in flasks and solvent was added in it. Afterwards, flasks were covered with aluminum foil and were put into orbital shaker for 45 minutes. After removing flask the extracts were collected by squeezing the flask material through muslin cloth. Moreover, these extracts were used in diet for the treatment of hypercholesterolemia.

***In Vivo* Studies:** For efficacy trials, forty rats were acquired from National Institute of Health (NIH), Islamabad and kept in animal room of National Institute of Food Science and Technology, University of Agriculture Faisalabad for the period of 60 days. Initially, some rats were sacrificed to get baseline values. The study was carried out in two categories separately. Study I comprised of rats fed on normal diet, whereas in study II, high cholesterol diet was administrated to raise their cholesterol level for 30 days. Furthermore, study I and study II comprised of 3 groups of rats fed on normal diet and diet containing seed and fruit extracts. Diet was consisted of 3% extracts including other ingredients. During entire experimental period, the animal room was maintained at a temperature and relative humidity of 23±2 °C and 55±5%, respectively, with 12:12 hrs light: dark cycle. At termination of animal study, the effect of control and nutraceutical diets were evaluated on the selected parameters including lipid profile through Microlab-300, Merck, Germany.

Serum Lipid Profile: Serum lipid profile of rats; cholesterol, high density lipoproteins (HDL), low density lipoproteins (LDL) and triglycerides were measured by following their respective protocols. Serum cholesterol level of rats was measured using CHOD-PAP method following the protocol of Kim *et al*. (2011). High density lipoprotein (HDL) and low density lipoproteins (LDL) in serum samples were calculated by method as mentioned by Alshatwi *et al.* (2010). Triglycerides in serum sample were estimated by liquid triglycerides (GPO-PAP) method as illustrated by Kim *et al.* (2005).

RESULTS AND DISCUSSION

Cholesterol: Mean squares for serum lipid profile (Table 1) showed a significant difference in cholesterol levels in both studies as a function of diets while interval behaved significantly in study II. Results were found non-significant with respect to intervals in study I while interaction behaved non-momentously in all the studies. Means for cholesterol levels are presented in Table 2. In study I, the highest value for cholesterol (83.15±3.32 mg/dL) was measured in control diet group trailed by nutraceutical$_{FE}$ (79.05±3.16 mg/dL) and nutraceutical$_{SE}$ (77.97±3.12 mg/dL) diet groups. During 60 days of model feeding trial, an increase in cholesterol level from 82.62±3.31 to 83.63±3.34 mg/dL was observed in control group. Nutraceutical diets containing jamun extract caused significant decline in serum cholesterol levels with a decrease from 80.89±3.21 to 77.35±3.09 mg/dL in nutraceutical$_{FE}$ and 80.45±3.22 to 75.55±3.02 mg/dL in nutraceutical$_{SE}$ group. In study II comprising of hypercholesterolemic rats, maximum reduction 92.14±3.68 mg/dL was recorded in nutraceutical$_{SE}$ group trailed by the nutraceutical$_{FE}$ (93.77±3.75mg/dL) and control (100.18±4.01 mg/dL). During the 60 days trial, the control group showed an increase in serum cholesterol from 0 day (98.07±3.92 mg/dL) to 60th day (102.03±4.08 mg/dL). Nevertheless, decrease in the cholesterol level was reported on the provision of extract containing diets. The maximum reduction from 96.95±3.87 to 87.98±3.51 was observed in rats administered on nutraceutical$_{SE}$ diets followed by nutraceutical$_{FE}$ from 97.63±3.90 to 89.93±3.59 mg/dL. It is obvious from Fig. 7 that the maximum diminishing tendency was observed in study II followed by study I. The highest reduction in cholesterol levels was 9.32 and 7.15% in nutraceutical$_{SE}$ and nutraceutical$_{FE}$ groups in the hypercholesterolemic rats (study II). Study I exhibited minimum decline *i.e.* 4.37 and 6.09% from nutraceutical$_{FE}$ and nutraceutical$_{SE}$ diets.

The results of the present study showed hypolipidemic effects of jamun seed extract which are in line with the findings of Sharma *et al.* [14], they studied

the relationship of lipid indices with the glycemic parameters on rabbits. Total lipids were reduced up to 10.7% in mild diabetic and 11.4% in severe diabetic rabbits. A comparative assessment regarding the antihyperlipidemic properties of jamun seed was conducted [7]. The results revealed that extract of jamun seed encompasses better ability to reduce cholesterol up to 57%. The results obtained are corroborated with earlier research findings of various researchers [6, 10, 11]. According to Prince *et al.*, [6] alcoholic extract of jamun seed caused up to 57.89% reduction in cholesterol levels of type II diabetic subjects. It was concluded from twelve days trial that jamun fruit lowered the cholesterol level up to 5.5% [10]. Furthermore, it was reported a reduction in serum cholesterol levels up to 30.62% on consumption of jamun seed extract [11].

Low Density Lipoprotein (LDL): Mean squares in Table 1 revealed that serum LDL levels were significantly affected by diets and intervals. Nevertheless, study interaction explicated non-significant influence. Means for study I (Table 3) indicated that LDL value was 31.45±1.22 mg/dL in control group that was reduced to 30.03±1.17 and 29.37±1.14 mg/dL in nutraceutical$_{FE}$ and nutraceutical$_{SE}$ groups, correspondingly. However, with the passage of time LDL increased non-significantly from 31.27±1.21 to 31.62±1.23 mg/dL in control, whereas in nutraceutical$_{FE}$ and nutraceutical$_{SE}$ groups there was a reduction from 31.04±1.17 to 29.10±1.13 mg/dL and 30.53±1.19 to 28.29±1.10 mg/dL, respectively.

LDL value for control group (study II) was 48.95±2.03 mg/dL that momentously decreased in nutraceutical$_{SE}$ and nutraceutical$_{FE}$ groups to 44.80±1.82 and 45.64±2.49 mg/dL, respectively. Maximum reduction was noticed in rats fed jamun seed extract (nutraceutical$_{SE}$ group) from 47.62±2.89 to 42.17±1.89 mg/dL. The Fig. 8 depicts the percent reduction in LDL level of rats fed on nutraceutical$_{SE}$ and nutraceutical$_{FE}$ diets. In study I, 6.24 and 7.31% reduction was observed by feeding nutraceutical$_{FE}$ and nutraceutical$_{SE}$ diets, respectively. Whereas, 8.97% decline was observed in rats fed with Nutraceutical$_{FE}$, while 11.46% reduction was recorded by the provision of nutraceutical$_{SE}$ diets in the hypercholesterolemic group (study II).

Table 1: Effect of diet and interval on serum lipid profile of rats in different studies

Studies	SOV	df	Cholesterol	HDL	LDL	Triglycerides
Study I	Diet (A)	2	113.975*	11.3701NS	16.9892**	70.1229*
	Intervals (B)	2	23.093NS	1.0176NS	10.1292*	6.6717NS
	AxB	4	12.033NS	0.1076NS	2.5181NS	8.6436NS
	Error	36	23.715	6.7855	2.8022	18.3801
Study II	Diet (A)	2	270.887**	32.4802NS	72.1284**	157.341**
	Intervals (B)	2	135.513*	0.7167NS	68.0927*	22.064NS
	AxB	4	64.308NS	1.0652 NS	17.6350NS	23.364NS
	Error	36	33.716	11.1983	9.6297	21.170

* = Significant

** = Highly significant

NS = Non-significant

Study I: Normal rats

Study II: Hypercholesterolemic rats

Table 2: Effect of diet and intervals on serum cholesterol (mg/dL) of rats in different studies

		Study intervals (Days)			
Studies	Diet	0	30	60	Means
Study I	Control	82.62±3.31	83.20±3.32	83.63±3.34	83.15±3.32a
	Nutraceutical$_{FE}$	80.89±3.21	78.92±3.15	77.35±3.09	79.05±3.16b
	Nutraceutical$_{SE}$	80.45±3.22	77.72±3.10	75.55±3.02	77.90±3.12b
	Means	81.32±3.25a	79.94±3.19ab	78.84±3.15b	
Study II	Control	98.07±3.92	100.44±4.02	102.03±4.08	100.18±4.01a
	Nutraceutical$_{FE}$	97.63±3.90	93.76±3.75	89.93±3.59	93.77±3.75b
	Nutraceutical$_{SE}$	96.95±3.87	91.49±3.65	87.98±3.51	92.14±3.68b
	Means	97.55±3.90a	95.23±3.81ab	93.31±3.73b	

Means carrying same letters do not differ significantly

Table 3: Effect of diet and intervals on serum LDL (mg/dL) of rats in different studies

		Study intervals (Days)			
Studies	Diet	0	30	60	Means
Study I	Control	31.27±1.21	31.47±1.22	31.62±1.23	31.45±1.22[a]
	$Nutraceutical_{FE}$	31.04±1.17	29.96±1.18	29.10±1.13	30.03±1.17[b]
	$Nutraceutical_{SE}$	30.53±1.19	29.29±1.15	28.29±1.10	29.37±1.14[b]
	Means	30.94±1.20[a]	30.24±1.17[ab]	29.67±1.15[b]	
Study II	Control	48.13±2.16	49.05±2.65	49.67±2.88	48.95±2.03[a]
	$Nutraceutical_{FE}$	47.87±2.15	45.49±2.17	43.58±1.56	45.64±2.49[b]
	$Nutraceutical_{SE}$	47.62±2.89	44.60±1.34	42.17±1.89	44.80±1.82[b]
	Means	47.87±2.69[a]	46.38±1.96[ab]	45.14±2.03[b]	

Study I: Normal rats

Study II: Hypercholesterolemic rats

Table 4: Effect of diet and intervals on serum Triglycerides (mg/dL) of rats in different studies

		Study intervals (Days)			
Studies	Diet	0	30	60	Means
Study I	Control	66.92±2.62	69.39±2.71	68.30±2.73	68.20±2.69[a]
	$Nutraceutical_{FE}$	65.57±2.67	64.45±2.63	63.56±2.59	64.52±2.63[b]
	$Nutraceutical_{SE}$	65.95±2.63	64.29±2.57	62.96±2.51	64.40±2.57[b]
	Means	66.14±2.64	66.04±2.64	64.94±2.61	
Study II	Control	77.42±3.09	78.93±3.15	79.95±3.19	78.76±3.15[a]
	$Nutraceutical_{FE}$	77.94±3.11	75.45±3.01	73.46±2.93	75.61±3.02[ab]
	$Nutraceutical_{SE}$	75.04±3.01	72.1±2.884	69.72±2.78	72.20±2.88[b]
	Means	76.8±3.07[a]	75.49±3.01[ab]	74.37±2.97[b]	

Study I: Normal rats

Study II: Hypercholesterolemic rats

Table 5: Effect of diet and intervals on serum HDL (mg/dL) of rats in different studies

		Study intervals (Days)			
Studies	Diet	0	30	60	Means
Study I	Control	35.74±1.42	35.85±1.43	35.93±1.44	35.84±1.43
	$Nutraceutical_{FE}$	36.92±1.47	37.26±1.50	37.54±1.51	37.24±1.48
	$Nutraceutical_{SE}$	37.05±1.48	37.46±1.49	37.80±1.53	37.43±1.49
	Means	36.57±1.46	36.85±1.47	37.09±1.48	
Study II	Control	38.16±1.56	37.95±1.55	37.50±1.55	37.88±1.55
	$Nutraceutical_{FE}$	38.69±1.54	39.17±1.56	39.56±1.58	39.14±1.56
	$Nutraceutical_{SE}$	40.27±1.61	40.85±1.63	41.32±1.65	40.81±1.63
	Means	39.37±1.57	39.65±1.58	39.89±1.59	

Study I: Normal rats

Study II: Hypercholesterolemic rats

One of the researchers group, conducted an animal modeling trial on hypercholesterolemic rats which were fed on jamun fruit extract. The results showed a decrease in serum LDL cholesterol up to 13.76% for jamun fruit extract [10].The present results are also in line with those obtained by Sharma *et al.* [13], estimated 25.5 and 25.1% reduction in mild and severe diabetic rabbits, respectively. According to Sharma *et al.* [12], flavonoids rich fraction of jamun seed extract considerably reduced the hyperlipidemia in diabetic rats. Jamun seed extract reduced serum LDL up to 27.3% in mild diabetic rats and 37.6% in severe diabetic rats. Later, Sampath *et al.* [11] investigated the hypolipidemic potential of jamun pulp in diabetic male rats and observed up to 70% reduction in serum LDL.

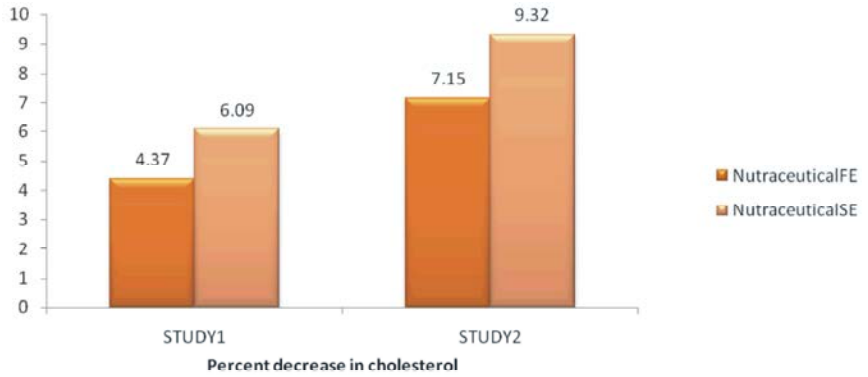

Fig. 1: Percent decrease in Cholesterol as compared to control

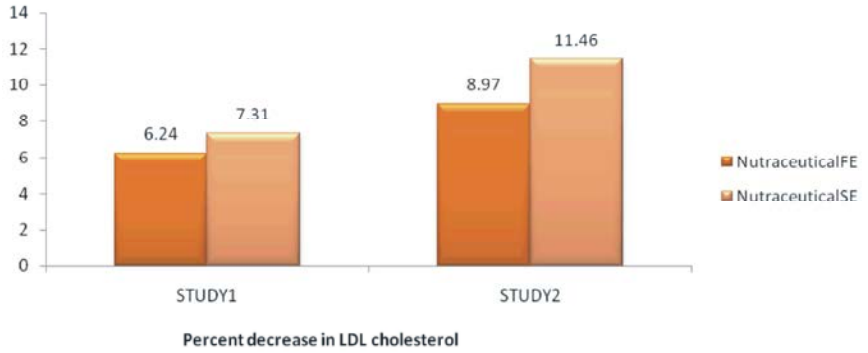

Fig. 2: Percent decrease in LDL Cholesterol as compared to control

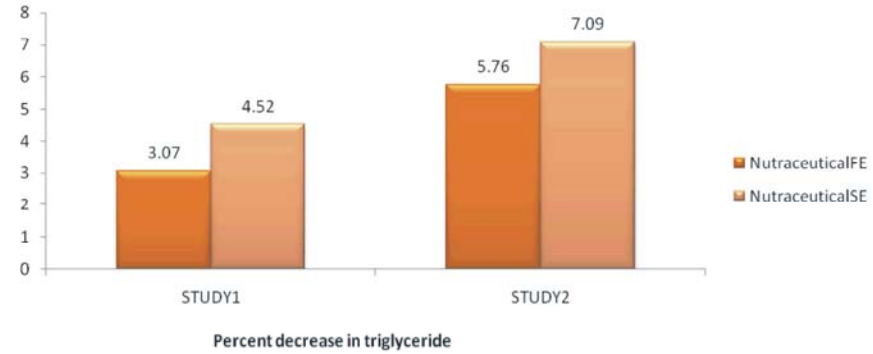

Fig. 3: Percent decrease in Triglycerides as compared to control

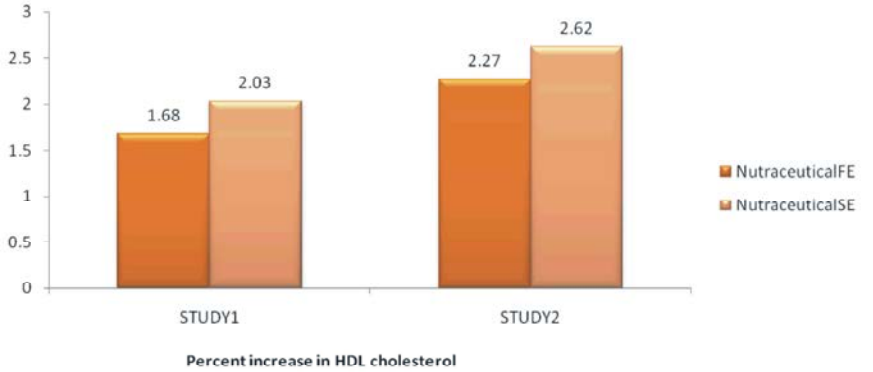

Fig. 4: Percent increase in HDL cholesterol as compared to control

Triglycerides (TG): Mean squares for the effect of diet have significantly changed triglycerides in all the studies, while interval was significant regarding study II and interaction has non momentous effect on this trait (Table 1). Mean values for triglycerides (Table 4) in study I for control, nutraceutical$_{FE}$ and nutraceutical$_{SE}$ groups were 68.20±2.69, 64.52±2.63 and 64.40±2.57 mg/dL, respectively. It was observed that triglycerides in control increased from 66.92±2.62 to 68.30±2.73 mg/dL, but in nutraceutical$_{FE}$ and nutraceutical$_{SE}$ groups there was a decrease from 65.57±2.67 to 63.56±2.59 mg/dL and 65.95±2.63 to 62.96±2.51 mg/dL, respectively. Similarly, in study II, mean values for control, nutraceutical$_{FE}$ and nutraceutical$_{SE}$ groups differed substantially *i.e.*78.76±3.15, 75.61±3.02 and 72.20±2.88 mg/dL, correspondingly. Maximum reduction from 75.04±3.01 to 69.72±2.78 mg/dL was noticed for triglycerides in nutraceutical$_{SE}$ group.

Fig.4 depicts highest percent decline in triglycerides for study II *i.e.* 5.76 and 7.09% for nutraceutical$_{FE}$ and nutraceutical$_{SE}$, respectively. However, study I also showed decline in triglyceride levels in groups; nutraceutical$_{FE}$ (3.07%) and nutraceutical$_{SE}$ (4.52%) in respective trials. The results obtained are in close agreement with previous finding of various researchers [11, 13, 15, 17]. Sharma *et al.* [13] reported significantly lower plasma triglycerides concentrations (13.3 and 17.1%) in ethanolic seed extract fed mild and severe diabetic rats at dose rate of 100 mg/Kg body weight. Recently, It was studied the hypocholesterolemic effects of jamun seed in diabetic rats treated with extract for a period of four weeks. The results showed that jamun seed [11].

Significantly reduced the triglyceride levels from 194±9 to 104±6 mg/dL. It was elucidated the hypolipidemic potential of extract of jamun pulp on diabetic rats and reported 33% reduction in serum triglyceride concentrations [17]. Sharma *et al.* [15] supported the present investigation of alleviation in serum triglycerides levels with jamun seed extract. They reported that jamun seed extract containing diets significantly reduced triglycerides as compared to control diet.

High Density Lipoprotein (HDL): It is obvious from the Table 1 that the mean squares for treatments, interval and interaction imparted non momentous effect on HDL concentration in study I and II. In study I (normal rats), maximum HDL level was observed in nutraceutical$_{SE}$ diet (37.43±1.49 mg/dL), trailed by nutraceutical$_{FE}$ (37.24±1.48 mg/dL) and control (35.84±1.43 mg/dL) diet groups (Table 5). During sixty days trial period, HDL level in control group increased from 35.74±1.42 to 35.93±1.44 mg/dL. Likewise, increasing trend was observed in the nutraceutical$_{FE}$ (36.92±1.47 to 37.54±1.51 mg/dL) and nutraceutical$_{SE}$ (37.05±1.48 to 37.80±1.53 mg/dL). Means of study II (hypercholesterolemic rats) also showed maximum HDL level of 40.81±1.63 mg/dL in nutraceutical$_{SE}$ diet group, whilst, nutraceutical$_{FE}$ and control group had HDL levels of 39.14±1.56 and 37.88±1.55 mg/dL, respectively. The HDL level in control group declined from 38.16±1.56 to 37.50±1.55 mg/dL. However, it increased for nutraceutical$_{FE}$ and nutraceutical$_{SE}$ groups from 38.69±1.54 to 39.56±1.58 mg/dL and 40.27±1.61 to 41.32±1.65 mg/dL, respectively.

It is evident from Fig. 10 that in study I and II, non-substantial increase in HDL level is observed. In study I the rats fed with nutraceutical$_{FE}$ diets showed 1.68% rise while the increase in rats fed with nutraceutical$_{SE}$ diets was 2.03%. Similarly, non-momentous trend was seen in case of hypercholesterolemic rats (Study II) 2.27 and 2.62% increase recorded by the provision of nutraceutical$_{FE}$ and nutraceutical$_{SE}$ diets, respectively. One of the researchers groups, Sharma *et al*. investigated the effect of jamun seed extract on high density lipoproteins in male albino rabbits [15]. They recorded significant increase in the HDL level (13%) in mild diabetic rabbits and 18% in severe diabetic rabbits supplemented with jamun seed extract diet. Earlier, It was assessed the effect of jamun fruit in the blood [10].

Lipid profile of diabetic patients. Fruit extract (90 ml/day) were provided to diabetic individuals for 8 days. They recorded a significant rise (3.7%) in HDL level concentration with a reduction in total cholesterol and LDL. Sharma *et al*. [12] explicated the effect of jamun seed extract on type II diabetic rats. The rats were given extract for 21 days. Significant increase in HDL (19.5%) was recorded at the termination of the study. According to findings of Sampath *et al.*, [11] consumption of jamun seed preparation significantly (50%) increased the serum HDL levels of diabetic rats.

CONCLUSIONS

It is concluded that variations in lipid profile during hypercholesterolemia reinstated near the normal levels by the treatment of jamun fruit and seed extracts. In this research results showed that seed extract exhibited better effects than fruit extract in lowering the blood lipids. Fruit and seed extracts restored all the disturbed parameters to normal state and their effects are similar to pharmaceutical medicines.

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