The Journal of Animal & Plant Sciences, 24(6): 2014, Page: 1618-1622
ISSN: 1018-7081

ANTI-HYPERCHOLESTEROLEMIC AND ANTI-ATHEROGENIC ACTIVITY OF *Terminalia chebula* FRUIT IN NORMAL AND CHOLESTEROL FED RABBITS

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ABSTRACT

Over the centuries plants have been effectively used as a potent source of traditional medicine. Present experiment was aimed to evaluate the hypocholesterolemic and anti-atherogenic effect of feeding *Terminalia chebula* powder (TCP) in rabbits. A total of (n=20) rabbits were assigned to four treatment groups with n=5 animals in each group. Experimental treatments received Normal Saline (NS); *T. chebula* powder (TCP), high fat diet (HFD) and high fat diet plus *T. chebula* powder (HFDTCP). Blood samples were taken on a weekly basis to diagnose the effects of test drug on serum Total Cholesterol (TC), LDL, HDL, Triglycerides (TG) and Atherogenic Index. All the parameters were measured in mg/dl except the atherogenic index measured in units. In HFD rabbits TC (362.59±6.00), LDL (262.59±6.12) triglycerides (258.7±2.90) and atherogenic index (13.50±1.20) was higher ($p < 0.05$), while HDL (25.73±1.09) was significantly reduced to an alarming level at the end of eight weeks. When HFD rabbits were treated with TCP for eight weeks, TC (132.60±4.93), LDL (89.13±2.75), Triglycerides (125.86±9.06) and atherogenic index (1.54±0.03) reduced significantly and increased ($p < 0.05$) the HDL (52.10±1.50). It was therefore concluded that that *T. chebula* powder can safely be used to reduce bad cholesterol (LDL) and to enhance good cholesterol (HDL).

Key words: Medicinal plants, *Terminalia chebula*, HDL, LDL, Hypercholesteremia, Atherogenic Index

INTRODUCTION

Sedentary life style has led to cardiovascular and hypertensive diseases. Oxidative stress, high blood cholesterol, decreased high density lipoproteins (HDL), increased low density lipoproteins (LDL), smoking and impaired glucose tolerance leads to hypertension (Bhosale, 2013). Sustained hypertension not only damages heart, kidney, blood vessels and brain but also leads to deaths following congestive cardiac failure, renal failure and stroke (Lamina and Okoye, 2012). Elevated levels of serum total cholesterol (TC) increase 1.9 and 1.8 fold the risk of coronary heart diseases in men and women respectively (Kannel, 1991). Drugs having the hypolipidemic and antioxidant properties are being used to treat hypertension. Mostly β blocker medicines are used; these drugs definitely reduce the serum cholesterol level by different ways but also have some side effects like, diarrhea, stomach cramps, nausea, vomiting, depression and hallucination (Gielen *et al*., 2006; Berglund and Andersson, 1981).

Terminalia chebula is a moderate tree used in traditional medicines belonging to the family combretaceae. Traditionally, *Terminalia chebula* have been used as medicine in Asia, Europe and Africa. The fruits have different names in different parts of the world; Haritaki, Harad, Hirada (subcontinent), Aralu (Sri Lanka), Zhang-Qin-Ge, Hezi (China), Harra Harro (Tibet), Myrobalane (Germany) and Myrobalan in dien (France) (SuryaPrakash *et al*., 2012).

Ayurvedic and homeo-medicines have been using this fruit to treat various diseases due to its broad spectrum antibacterial (Malekzadeh *et al*., 2001), antifungal and anti-stress properties (Bajpai *et al*., 2010; Singh and Kumar, 2013). There are reports that compounds present in this fruit can be used to cure cancer (Saleem *et al*., 2002). It has active pharmacological agents against viruses that helpe to cure different types of hepatitis (Kim *et al*., 2001). Its paste is reported to cure ulcer (Raju *et al*., 2009) and wounds (Choudhary, 2008). It has also been reported as an anticonvulsant (Singh *et al*., 2011), anti-mutagenic, detoxifier (Grover and Bala, 1992) having cardio protective (Dwivedi, 2007) radio protective (Jagetia and Baliga, 2002), and immune-modulatory (Mithraja *et al*., 2012) effects.

Here we used the fruit of *Terminalia chebula* in rabbits to report its efficiency to reduce serum TC, LDL, triglyceride and atherogenic index and increase in serum HDL. Further, it is reported that the use of this medicinal fruit is safe, reduces the oxidative stress and have no reported side effects.

MATERIALS AND METHODS

Preparation of Herb Powder: The fruit of *T. chebula* was identified and purchased from market, washed with water, air dried and ground along with the seeds.

Animals: The experiment protocols were approved by animal research committee, GC University, Lahore, Pakistan. Twenty male rabbits (*Oryctolagus cuniculus*) were used in the experiment. Their ages were between 60±10 days, with a mean body weight of 1.25±0.25 Kg. These animals were acclimatized for one week in the University animal house in standard conditions. Animals were fed on animal diet consisting of Bengal gram, Wheat, Maize, Carrot, water and green fodder in sufficient quantity during entire eight weeks of experimental period.

Induction of Hypercholesterolemia: Hypercholesterolemia was induced as described by Santoshkumar and Manjunath (2013) with minor modifications. Rabbits were fed edible coconut oil and Banaspati Ghee mixed together in the ratio of 2:3 v/v. In addition to the normal feed, rabbits were fed with a daily dose of 10 ml/kg/body weight orally to induce hypercholesterolemia.

Grouping and Treatment Schedules: The animals were divided into four groups, five animals in each group with different treatments as follows;

Normal Saline (NS) Group**:** This group was control fed on normal feed only.

Terminalia chebula Powder (TCP) Group**:** These rabbits were fed on normal diet and were orally administrated daily dose of 540 mg /Kg of *T. chebula* fruit powder (Santoshkumar and Manjunath, 2013).

High Fat Diet (HFD) Group: This group was fed on normal diet with a daily dose of 10 ml/Kg/body weight cholesterol as described.

High Fat Diet *Terminalia chebula* Powder (HFDTCP) Group: These rabbits received a daily dose of 10 ml/Kg/body weight cholesterol as well as 540 mg/Kg/ body weight of *T. chebula* powder with normal feed.

Dose Administration: The measured dose of *T. chebula* powder was mixed with distilled water in test tubes and administered orally using a syringe without needle as described by Pari and Umamaheswari (2000).

Serum Analysis: 2 ml blood sample was collected from ear vein on a weekly basis from all the animals. The collected samples were centrifuged at 3500 x g at 25 ^{o}C for 10 min and serum thus obtained was divided into aliquots and stored at -20^{o}C, till further analysis. Serum was analyzed for TC, LDL, HDL and Triglycerides using commercial kits obtained from Erba diagnostic Mannheim Gmbh, Germany as described by Nain *et al*. (2012)

Atherogenic Index: Atherogenic index was calculated using the formula described by Santoshkumar and Manjunath (2013);

$$\text{Atherogenic Index} = \frac{\text{Total Cholesterol} - \text{HDL}}{\text{HDL}}$$

Statistical Analysis: The normal distribution of data was evaluated by Kolmogorov Smirnov's test. Data are represented as mean ±S.D. Hypothesis testing methods included the one way analysis of variance (ANOVA) followed by Dunnette's comparison tests. Results with $p < 0.05$ were significant (Sokal and Rohfl, 1995). Data analysis was performed using statistical package SPSS (Version 13.0 SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

The present experiment was conducted to evaluate the effect of *T. chebula* powder supplementation on reduction in different forms of cholesterol in rabbits. Results of current investigation revealed that TCP reduced the serum TC level significantly ($p < 0.05$). The TC concentration was higher (125.79±3.58) in NS group when compared with the TCP rabbits (100.53±1.47) after eight weeks. At the end of experiment serum TC was higher ($p < 0.05$) in the HFD group (362.59±6.00) compared with HFDTCP group (132.60±4.93). This increase in serum TC was related to increase in cholesterol intake in the diet as well as duration of the exposure time to rabbits (Table 1). Our observations are in accordance with Santoshkumar and Manjunath (2013) who reported similar trends in TC concentration when albino mice were treated with *Emblica officinalis* (Amla). Reduction in the TC may be associated to the presence of phenolic compounds in *E. officinalis* and *T. chebula* (Kirakosyan *et al*., 2003). Phenolic compounds present in plants have identified as potent antioxidants agents (Kirakosyan *et al.*, 2003); their conjugated ring structure and hydroxyl groups scavenge superoxide ion (Robak and Gryglewski, 1988), nascent oxygen (Rafat Husain *et al*., 1987) and lipid peroxy radicals or stabilize the oxidative radicals (Liu *et al.*, 2008). Oxidative stress produced by reactive oxygen species (ROS) can results in diseases like cancer, diabetes and malfunctioning of liver, heart and eyes (Liu *et al*., 2008).

LDL in NS group was 66.32±1.72 after eighth weeks. There was an increase ($p < 0.05$) in LDL with increased intake of lipids in the food. LDL in HFD group started to increase may be in a feed dependent manner upto week eight (262.59±6.12). TCP have opposite effects on LDL, continuous use of this drug for eight weeks reduced ($p < 0.05$) the LDL to 89.13±2.75 level in HFDTCP group as shown in Table. It has been observed that TCP increased the serum HDL level while fats reduced the HDL (Table 1). HDL in HFD group (25.73±1.09) indicate the significant decrease while

HFDTCP group had significantaly high serum HDL (52.10±1.50) level after eight weeks.

T. chebula also reduced ($p < 0.05$) the triglycerides, triglycerides in HFD group was 258.7±2.90 and when this group was fed with TCP, serum triglycerides were reduced to 125.86±9.06. Likewise, there was significant decrease in atherogenic index in HFDTCP group. A high level of serum triglycerides is related to several diseases. Atherosclerosis occurs when endothelium doesn't function properly and can't keep a balance among thrombosis and fibrinolysis. Further, the recruitment of inflammatory cells into the vascular wall is also impaired leading to the plaque formation in the arteries when there are high levels of LDL and TC in the blood resulting in vasoconstriction and coronary heart diseases (Levine *et al*., 1995). TCP improved the functioning of endothelium and reduced the chances of atherosclerosis by an unknown mechanism significantly reducing the atherogenic index. Overall effects of TCP in all treatment groups can be seen and analyzed in Fig. 1. All these findings also confirm the reports of Tappia *et al*. (2013) when they studied some alternative therapies for the reduction of cholesterol and cardiovascular diseases.

High LDL (bad cholesterol) levels leads to severe heart disease especially by the formation of fatty deposits resulting in the artery blockage (Podrez, 2013) while HDL (good cholesterol) removes these fatty deposits by transporting the excessive cholesterol to liver for its safe disposal (Colpo, 2005). Hypertriglyceridemia is a major reason for heart attack in those patients who already have higher levels of serum LDL and lower levels of HDL. The negetive effects of hypertriglyceridemia can be reduced simply by controlling the HDL levels in the blood because hypertriglyceridemia can't cause coronary heart diseases if there is high serum HDL concenration (Austin *et al*., 1998).

Table 1. Effect of *Terminalia chebula* Powder on Lipid profile of rabbits after eight weeks

	Total Cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	Triglyceride (mg/dl)	Atherogenic Index
NS Group	125.79±3.58	66.32±1.72	48.23±1.10	87.52 ±2.96	1.60 ±0.12
TCP Group	100.53[a]±1.47	40.39[a]±2.12	64.35[a]±2.20	63.88[a]±5.21	0.56[a] ±0.07
HFD Group	362.59±6.00	262.59±6.12	25.73±1.09	258.7±2.90	13.50±1.20
HFDTCP Group	132.60[b]±4.93	89.13[b]±2.75	52.10[b]±1.50	125.86[b]±9.06	1.54[b] ±0.03

[a]: $p < 0.05$ [a] when compared to the normal, while [b]: $p < 0.05$[b] when compared to the cholesterol group, n=5

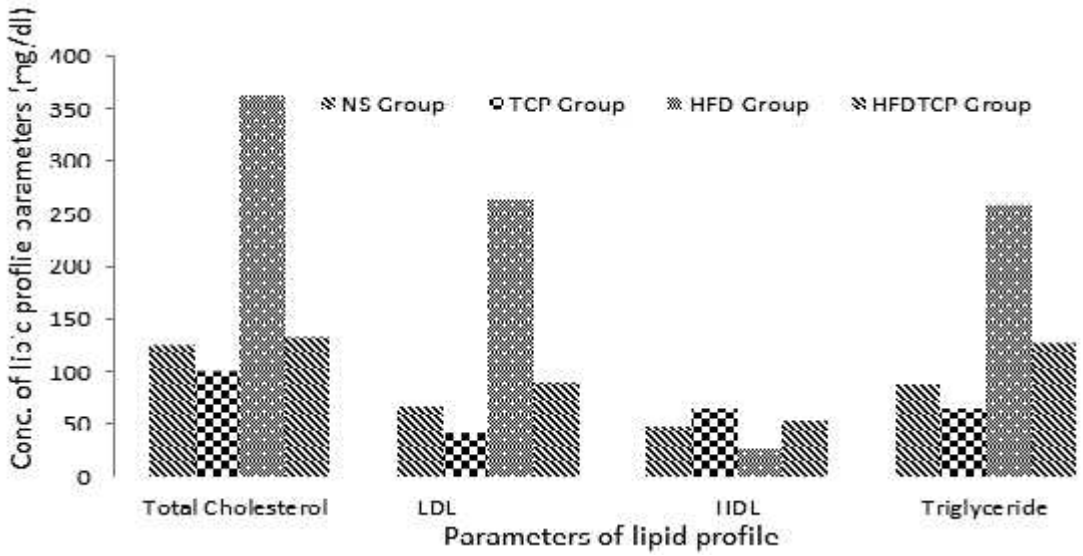

Figure-1: Serum lipid profile parameters in the treatment groups at the end of 8th weeks

Present study clearly indicates that *T. chebula* powder not only reduce the TC and resulting oxidative stree to protect the vital organs but also reduces the bad cholesterol, improves the functiong of endothelium and ultimately reduces the coronary heart disease (Donato *et al*., 2007). TCP have some natural agents which reduce serum LDL and enhance serum HDL. Mostly statin (HMG-CoA reductase inhibitors) medicine is used to reduce the LDL in hypercholesteremic patients which is also very effective against the atherosclerotic plaque. It

has some side effects like muscle pain or muscle weakness; nausea, constipation, or diarrhea; liver damage and kidney damage (Sugerman *et al*., 2013) but *T. chebula* powder is very safe to be used to reduce bad cholesterol without any reported side effects. *T. chebula* fruit is cost effective, easily accessible and have lots of beneficial effects other than the reduction of high blood cholesterol. There are no known details about its molecular mechanism so; further research is required to unveil the molecular basis for cholesterol regulation by *T. chebula* fruit.

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