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Evaluation of Antihyperlipidemic Activity of Protocatechuic Acid in Alloxan Induced Diabetic Dyslipidemia in Rats

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ABSTRACT

Protocatechuic acids (PCA), a phenolic compound present in plants vegetables and fruits and is benzoic acid derivative has reported to have hypolipidemic activity. In present study PCA was evaluated for its antihyperlipidemic activity in diabetic animals. In the study hyperlipidemia in wistar male rats was induced by administration of Alloxan Monohydrate (150mg/kg, i. p.). Administration of PCA significantly lowered the serum lipid as well as glucose levels & hepatic antioxidant activities. These results suggested that the antihyperlipidemic activity of PCA might be due to increase in hepatic LDL receptors or may be due to its effect on enzyme level involved in synthesis and metabolism of cholesterol. Its antioxidant activity could be due to sequestering of iron or may be due to scavenging of free radicals. Thus, it can be concluded that PCA exhibits antihyperlipidemic, hypolipidemic and antioxidant activity could prove beneficial in the treatment of various disorders associated with the hyperlipidemia.

Keywords: Protocatechuic acid, Antihyperlipidemic, Dyslipidemia, diabetes.

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INTRODUCTION

Today in most of the developed and developing countries, hyperlipidemia and thereby atherosclerosis is the leading cause of cardiac mortality [1]. Hypercholesterolemia is one of the important risk factors for coronary heart disease (CHD) & diabetic cardiovascular complications. Recently, the corporate world has demonstrated an increased evidence of hyperlipidemia, in spite of the availability of various anti-hyperlipidemic agents, there is increase in the incidence of CHD and risk of congestive heart failure (CHF). Thus, there is still considerable interest in the evaluation of new anti-hyperlipidemic agents (synthetic and herbal) [2] with low frequency of side effects for this problem [3].

Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them [4]. Human diets of plant origin contain many hundreds of compounds which cannot be considered as nutrients, but appear to play important role in the maintenance of health. These substances are called nutraceuticals. Special interesting nutraceuticals of food include the polyphenols, phytoestrogens, phytosterols, phytates and polyunsaturated fatty acids, and so on [5].

Phenolic compounds are ubiquitous in edible vegetables, fruits and nuts and it is estimated that an average of 1–2 g/day of these components may be consumed in a human diet [6]. Protocatechuic acid (PCA) a simple phenolic acid, one of the major benzoic acid derivatives is a constituent of apples, green and black tea, vegetables and fruits and also naturally present in many Chinese herbal medicines such as *Salvia miltiorrhiza* (Danshen) and *Hibiscus sabdariffa L.* [7] *Alpinia (A.) oxyphylla.* [8]. A high level of this compound was found in the extract from the rind of *Citrus reticulata* Blanco [6].

PCA has reported to have hypolipidemic activity [9] that it lowers normal lipids levels in animals, also have antihyperglycemic activity [10] considering these effects of PCA the present study was aimed to evaluate PCA for its antihyperlipidemic activity in alloxan induced dyslipidemic animals.

MATERIALS AND METHODS

Reagents and kits

The kits for estimation of serum total cholesterol, HDL-C, LDL-C, triglycerides were purchased from Biolab Diagnostics, Tarapur, India. The alloxan monohydrate and triton X-100 were purchased from Sigma Aldrich. Cholesterol and corn oil were purchased from Research Lab, India.

Drug

The PCA was suspended in 1% w/v CMC and given orally. Atovastatin and alloxan monohydrate (150mg/kg, i. p.) were dissolved in saline.

Animals

Thirty male Wistar albino rats (150-200 gm) were used in the study. They were maintained at 25 ± 2° C and relative humidity of 45 to 55% and under standard environmental conditions (12 h light: 12 h dark cycle). The animals had free access to food (Amrut feeds Sangli, India).and water *ad libitum.* The experiments were conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India and approved by Institutional Animal Ethical Committee (DYPIPSR/IAEC/09-10/P-01) dated 25th Feb.2010.

Dose selection

Three doses of PCA viz. 25, 50, 100 were selected based upon the reported activities of PCA [11].

PROCEDURE

Evaluation of antihyperlipidemic activity of protocatechuic acid in alloxan induced diabetic dyslipidemia in rats.

The male wistar rats were divided into seven groups consisting of six animals each. The diabetes was induced to all groups except NC by alloxan monohydrate (150mg/kg i. p.), rats having blood glucose level above 250mg/dl were included for further study. The treatment schedule followed for individual group was as described below:

NC: served as normal control, and received vehicle i.e. distilled water only
DC: received alloxan monohydrate 150 mg/kg, i. p.
Glib: gliblencamide 0.20 mg/kg p. o. o. d.
Std: Served as hyperlipidemic standard and received atorvastatin 10 mg/kg p. o. o. d.
PCA25: Served as test and received PCA at the dose of 25mg/kg p. o. o. d
PCA50: Served as test and received PCA 50mg/kg p. o. o. d
PCA 100: served as test and received PCA at the dose of 100mg/kg p. o. o. d.

The rats received respective treatments once daily for consecutive six weeks. The experiment was carried out for 42 days. On the 22nd & 43rd day, the animals were anesthetized and blood was withdrawn from retro-orbital plexus. The serum was separated and estimated for various lipid parameters such as cholesterol, triglycerides, LDL, HDL & glucose using commercially available kits (Biolabs diagnostics, Tarapur, India). At the 43rd day animals were sacrificed, the aorta was isolated for the histopathological observation and liver was removed, processed and homogenized in Tris buffer (10 mM, pH 7.4) at a concentration of 10% (w/v). The homogenates were centrifuged at 10,000 × *g* at 4°C for 20 min, using Remi C-24 high speed cooling centrifuge. The clear supernatant was used for the assays of lipid peroxidation and endogenous antioxidant enzymes like catalase, SOD and GSH [12].

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Statical Analysis

p<0.05 was considered as significant. Results were analysed by one way ANOVA followed by Dunnett's test.

RESULTS

Effect of PCA on serum total cholesterol levels

Alloxan 150mg/kg, i. p. significantly (P<0.0001) increased the cholesterol levels in inducing control group. Treatment with PCA 25, 50,100 mg/kg significantly (P<0.05, P<0.01) reduced the elevated cholesterol levels at the 22nd 43rd day respectively. Cholesterol levels of the atorvastatin treated group were also significantly (P<0.001) lowered at 22nd as well as 43rd day. (Fig. 1)

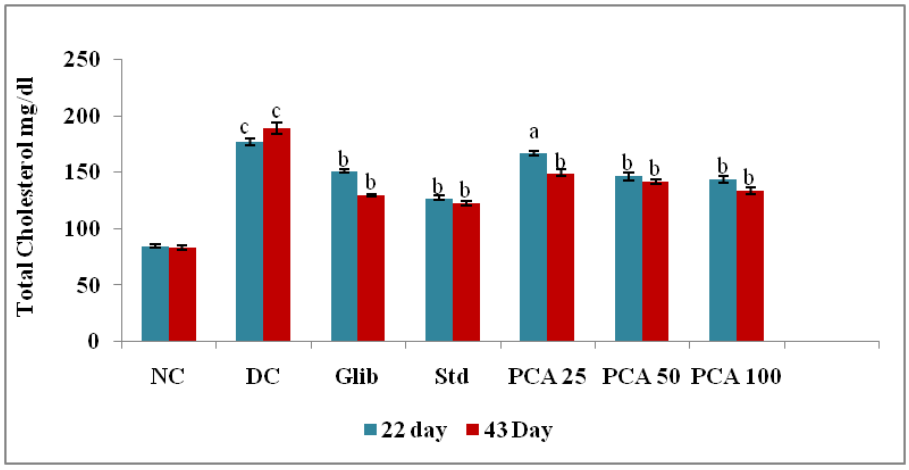

Fig.1. Effect of protocatechuic acid on total cholesterol levels in alloxan induced diabetic dyslipidemia model in rats

Values are expressed as Mean ± SEM. (n=6), ANOVA followed by Dunnett test. c p<0.0001. Student's t test when compared with Normal Control; a p<0.05,b p<0.01 when compared with DC-control. NC;-Normal-control: DC:-Diabetic control Glib: glibenclamide (0.20 mg/kg p. o.) Std:- Atorvastatin (10 mg/kg/day, p.o.), PCA-25, 50, 100:_Protocatechuic Acid 25, 50, and 100 mg/kg, p. o. respectively.

Effect of PCA on serum triglyceride levels

In the present study alloxan monohydrate induced diabetes in animals triglyceride levels were significantly (P<0.0001) elevated of inducing control group when compared with normal control group. Pretreatment with PCA 25, 50 and 100 mg/kg for 22 days significantly (P<0.05, p<0.01,) lowered the elevated triglyceride levels in comparison with diabetic control group. PCA 25, 50 and 100 mg/kg treated group significantly (p<0.01) reduced elevated triglyceride level after 43 days of treatment. Atorvastatin and glibenclamide treated group also showed significant (P<0.01) decrease in the elevated triglyceride (TG) levels at 22nd and 43rd day of treatment.

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Effect of PCA on serum HDL & LDL levels

Diabetes induced by alloxan monohydrate 150 mg/kg, i.p. significantly (P<0.0001) elevated the low density lipoprotein (LDL) while significantly (P<0.001) lowered the high density lipoprotein levels (HDL) of the inducing control group at 22nd and 43rd days in comparison to normal control group. (Fig. 2)

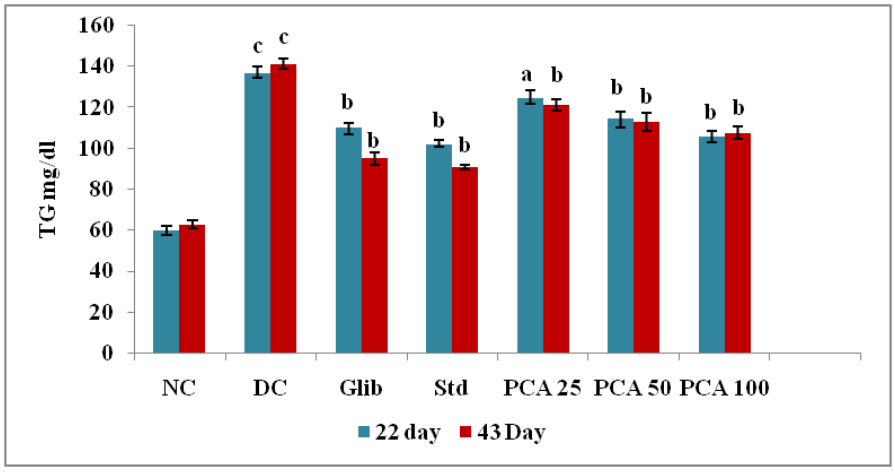

Fig. 2. Effect of protocatechuic acid on triglycerides levels in alloxan induced diabetic dyslipidemia model in rats
Values are expressed as Mean ± SEM. (n=6), ANOVA followed by Dunnett test. c p<0.0001. Student's t test when compared with Normal Control; a p<0.05,b p<0.01 when compared with DC-control. NC;-Normal-control: DC:-Diabetic control Glib: glibenclamide (0.20 mg/kg p. o.) Std:- Atorvastatin (10 mg/kg/day, p.o.), PCA-25, 50, 100:_Protocatechuic Acid 25, 50, and 100 mg/kg, p. o. respectively

The PCA 25, 50, 100 mg/kg treated groups showed significant (p<0.05, p<0.01) decrease in the LDL levels at 22nd day. The PCA 25, 50, 100 mg/kg treated groups also showed significant (p<0.01, p<0.01,) decrease in the LDL levels at 43rd day respectively when compared with diabetic control group. On the contrary, the HDL levels of PCA (50,100 mg/kg) treated groups were significantly (P<0.05, P<0.01) increased at 22nd day of treament respectively and PCA 25 mg/kg was nonsignificant. After 43 days of treatment with PCA 25, 50, 100 mg/kg showed significantly (p<0.01) restored HDL levels respectively as compared to diabetic control. Treatment with atorvastatin significantly (p<0.05, p<0.01) restored HDL and decreased LDL (p<0.01) levels at 22nd and 43rd day of treatment and glibenclamide (p<0.01,) also showed a significant (p<0.01) increase in HDL and decrease in LDL (p<0.05, p<0.01) levels at 22nd and 43rd day of treatment. (Fig. 3)

Effect of PCA on serum glucose levels

Alloxan (150 mg/kg , i. p.) significantly (p<0.0001) increased blood glucose levels in rats. PCA at the dose of 50, and 100 mg/kg showed significant (p<0.05, p<0.01) reduction in blood glucose level at 22nd day, while PCA 25 mg/kg, p. o. was nonsignificant. PCA at 25, 50, 100 mg/kg, p.o. significantly (p<0.01) decreased elevated blood glucose level at 43rd day when

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compared with diabetic control. The glibenclamide and atorvastatin treated group showed significant ($p<0.01$) decrease in glucose level at 22nd and 43rd day respectively when compared with diabetic control. (Fig. 4)

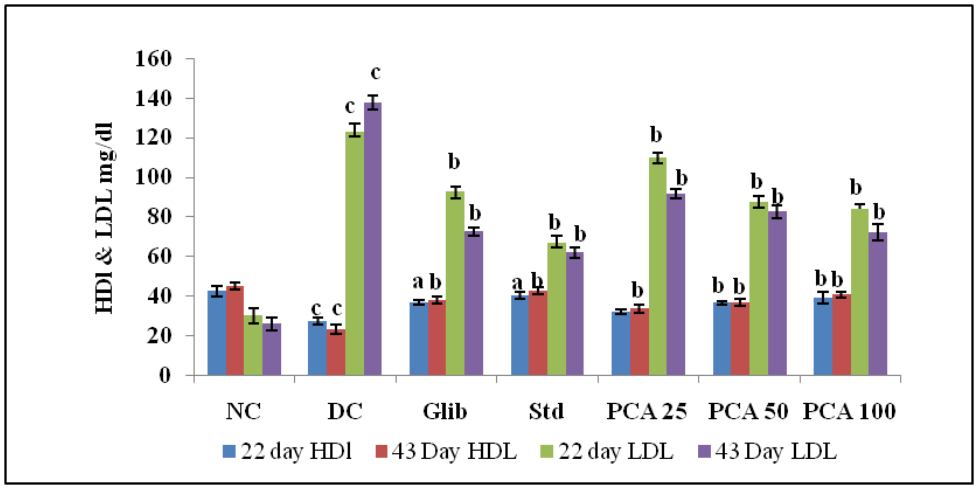

Fig. 3. Effect of protocatechuic acid on high density lipoprotein (HDL) & low density lipoprotein (LDL) levels in alloxan induced diabetic dyslipidemia model in rats (42 days).

Values are expressed as Mean ± SEM. (n=6), ANOVA followed by Dunnett test. c $p<0.0001$. Student's t test when compared with Normal Control; a $p<0.05$,b $p<0.01$ when compared with DC-control. NC;-Normal-control: DC:- Diabetic control Glib: glibenclamide (0.20 mg/kg p. o.) Std:- Atorvastatin (10 mg/kg/day, p.o.), PCA-25, 50, 100:_Protocatechuic Acid 25, 50, and 100 mg/kg, p. o. respectively.

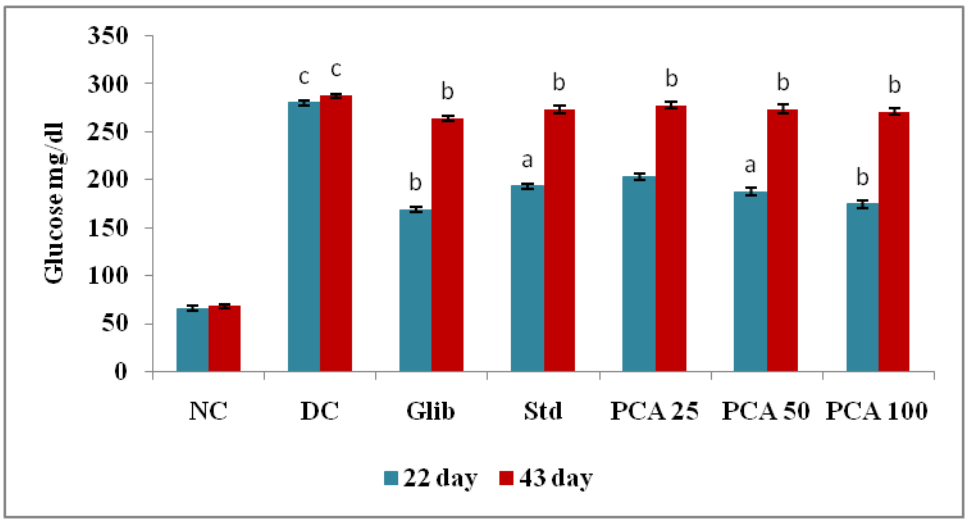

Fig.4. Effect of protocatechuic acid on glucose levels in Alloxan induced diabetic dyslipidemia model in rats.

Values are expressed as Mean ± SEM. (n=6), ANOVA followed by Dunnett test. C $p<0.0001$. Student's t test when compared with Normal Control; a $p<0.05$,b $p<0.01$ when compared with DC-control. NC;-Normal-control: DC:- Diabetic control Glib: glibenclamide (0.20 mg/kg p. o.) Std:- Atorvastatin (10 mg/kg/day, p.o.), PCA-25, 50, 100:_Protocatechuic Acid 25, 50, and 100 mg/kg, p. o. respectively.

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Effect of PCA on serum antioxidant levels

In alloxan induced diabetes there was significant increase hepatic catalase, SOD, GSH levels. There was significant ($p<0.01$) decrease in catalase, SOD, GSH levels in PCA 25, 50, 100 mg/kg treated groups when compared with diabetic control group. The LPO levels was decreased significantly ($p<0.01$) by alloxan monohydrate treatment while PCA 25, 50, 100 mg/kg treated group significantly restored the LPO levels when compared with diabetic control group. (Table1)

Table 1. Effect of protocatechuic acid on serum antioxidant levels in alloxan induced diabetic dyslipidemia model in rats.

Parameter/ Groups	Control	Hyperlipidemic	Glib	Standard	PCA25	PCA50	PCA100
GSH (µg of GSH/g of tissue)	70.72 ±1.64	24.37 ±1.28##	32.80 ±2.34*	38.90 ±2.55**	47.05 ±2.58**	50.21 ±1.30**	54.98 ±2.11**
SOD (units/ mg of tissue)	85.84 ±1.61	34.66 ±1.46##	67.49 ±1.48**	64.10 ±0.96**	68.64 ±1.49**	74.53 ±1.62**	79.37 ±0.85**
Catalase (µM of H_2O_2/g of tissue/min)	16.09 ±0.87	9.29 ±0.62##	14.27 ±0.57**	12.24 ±0.57*	12.86 ±0.62**	13.10 ±0.83**	14.04 ±0.81**
LPO (nM of MDA/ g of tissue)	3.79 ±0.27	9.09 ±0.59##	6.27 ±0.49**	6.73 ±0.50**	6.43 ±0.26**	5.74 ±0.34**	4.40 ±0.24**

DISCUSSION

The main causative factor for cardiovascular disorders and atherothrombotic diseases such as congestive heart failure, atherosclerosis, etc. is the disturbances occurring in lipid metabolism [13]. Though, there are a large class of antihyperlipidemic drugs used in the treatment, none of the existing ones available worldwide is fully effective, absolutely safe and free from side effects [14]. Hence, efforts are being made to find out safe and effective agents that may be beneficial in correcting the lipid metabolism and preventing further complications. The number of patients seeking alternate and herbal therapy is growing exponentially. Herbal medicines are the synthesis of therapeutic experiences of generations of practicing physicians of indigenous systems of medicine for over hundreds of years. Herbal medicines are now in great demand in the developing world for primary health care not because they are inexpensive but also for better cultural acceptability, better compatibility with the human body and minimal side effects [15].

In type 2 diabetes due to insulin resistance there is lipolysis and the adipocyte release FFA and inflammatory cytokines (IL 6 and TNF α) which interfere in the insulin signalling and with the ability of insulin to suppress lipolysis [16]. Filling the storage space from plasma glucose and triglycerides is insulin dependent. Insulin present in minimal concentration in

fasting blood is released within 1 to 2 min of the beginning of each meal and peaks at 30 to 60 min, parallel to the increasing concentrations of circulating nutrients. It would appear that the primary role of insulin is to clear the circulation either in anticipation of, or in response to, incoming nutrients, the result being conversion of circulating nutrients to glycogen and or triglycerides. The storage capacity for glycogen is limited to the liver and muscle tissues. Hence the primary storage area for excess nutrients is the adipose tissue. When the adipocytes are full, they can no longer store triglycerides. This results in elevation of plasma triglycerides concentration. As the triglycerides concentration increases, the liver tries to help by diverting some of the nutrients to cholesterol synthesis indirectly by providing acetyl coenzyme A. This increases the total cholesterol level in the blood and leads to hyperlipidemia in insulin deficiency [17]. Visceral obesity and increased intra-abdominal fat have been shown to precede development of insulin resistance. Increasing insulin resistance is proposed to be the precursor for 2 distinct events. The first is an increase in postheparin hepatic lipase activity, which is responsible for hydrolysis of TG and phospholipids in LDL and HDL particles. This increase in the degradation of the core material and surface remodeling of both classes of lipoproteins would lead to smaller and denser LDL particles and significant reduction in HDL2, the big buoyant subclass of HDL particles. The second is an increase in production of apolipoprotein B, leading to an increase in the synthesis and secretion of TG-containing very-low-density lipoprotein (VLDL) cholesterol particles. TG from TG-rich particles are exchanged for cholesteryl esters from HDL and LDL particles by the action of cholesteryl ester transfer protein. The TG enriched HDL and LDL particles are the preferred substrate particles for hepatic lipase, which hydrolyzes the TG core of the particles, leading to smaller and more dense HDL and LDL particles. Visceral obesity may contribute to this lipoprotein phenotype by accelerating this pathway. It has been suggested that increased intra-abdominal fat leading to insulin resistance induces synthesis and secretion of TG-containing VLDL particles. The increase in TG-rich lipoproteins as donor particles can facilitate TG enrichment of LDL and HDL particles. More likely, increased intra-abdominal fat content leads to an increase in hepatic lipase activity, resulting in extensive hydrolysis of the TG core of HDL and LDL particles as the main abnormality in the metabolic syndrome. Increased hepatic lipase activity has been closely associated with increased small, dense LDL and decreased HDL2. This appears to be the major contributor to the generation of small, dense LDL and reduction of HDL2 particles in the metabolic syndrome. Increased levels of small, dense LDL particles, elevated TG, and decreased HDL cholesterol are highly interrelated and occur in a cluster [18]. This mechanism along deficiency of insulin fails to activate lipoprotein lipase and therefore leads to hypertriglyceridemia.

Atherosclerosis is one of the major risk factors for coronary heart disease and it is widely recognized that the oxidative modification of human low density lipoprotein (Ox-LDL) may play an important role. Excess LDL in the artery wall due to hypercholesterolemia can undergo oxidative modifications. The oxidative modification hypothesis of atherosclerosis predicts that low-density lipoprotein (LDL) oxidation is an early event in atherosclerosis and that Ox-LDL contributes to atherogenesis. The oxidation of lipids in human atheromata lesions and production of excess reactive oxygen species (ROS) including superoxide anion (O_2 −), hydrogen peroxide (H_2O_2), and peroxynitrite (ONOO−), are the evidences for the presence of Ox-LDL in vivo. Ox-LDL has a number of potentially pro-atherogenic activities contributing to important

clinical manifestations of coronary artery disease such as endothelial dysfunction and plaque disruption. Antioxidative substances are believed to suppress the onset and development of atherosclerosis. The flavonoids and phenolic compounds have also been shown to have antioxidant effects. Thus, substances which combine antioxidant and hypocholesterolemic activities are expected to be effective in preventing the formation and progression of atherosclerosis [19]. So the study was undertaken to screen PCA for its antihyperlipidemic and antioxidant activity.

The PCA also significantly reduced elevated lipid and glucose levels in alloxan induced diabetic rats at 22nd and 43 day, also showed significant antioxidant activity when compared with diabetic control animals. According to Kannel et al., an ideal drug is one, which raises HDL-C along with the lowering of LDL-C. The biochemical estimations shown that the protocatechuic acid significantly increased the protective HDL-C level and decreased the atherogenic LDL and VLDL levels. The cholesterol-lowering effect of PCA could be due to an increased excretion of cholesterol and bile acids through fecal sterol excretion or due to inhibition of cholesterol synthesis pathway. The possible mechanism of PCA may involve increase of HDL-C, which is attributed to the mobilization of cholesterol from peripheral cells to the liver by the action of Lecithin Cholesterol O-acyltransferase (LCAT) [20]. CAT enzyme is involved in the transesterification of cholesterol, the maturation of HDL and the flux of cholesterol from cell membranes into HDL. The activity of enzyme tends to decrease in diet-induced hyperlipidemia [20].

All the results obtained indicate potential of PCA as antihyperlipidemic, antihyperglycemic, antioxidant agent. This activity may be either due to increase in hepatic LDL receptor or may be due to action on the one of the enzyme system involved in synthesis, metabolism and/or excretion of the cholesterol [9].

The mechanism of antioxidant action of plant phenolics among them phenolic acids has usually been attributed to OH scavenging activity. On the other hand, some results indicate that phenolic acids (including PCA) may sequester iron from reacting with H2O2 to generate OH, rather than directly scavenging OH. The protective effect of these compounds should be attributed to their capability to bind iron [8].

CONCLUSION

The PCA showed significant antihyperlipidemic, antioxidant & antihyperglycemic activity against alloxan induced diabetic dyslipidemia. Overall, we can conclude that the PCA can be a better hope for development of an effective and safe agent for present pharmacotherapy to control hyperlipidemia and related complications.

Abbrevations: PCA- protocatechuic acid, TC-total cholesterol, TG- triglyceride, HDL- high density lipoprotein, LDL- low density lipoprotein, AI- atherogenic index.

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REFERENCES

[1] Jain S, Kathiravan M, Somania R, Shishoo C. Bioorganic & Medicinal Chemistry 2007; 15:4674–4699.
[2] Balaraman R, Komal M, Amin A, Bafna P, Gulati O. J Ethnopharmacol 2003; 86 :191–195.
[3] Birgitte C, Molgaard P, Zhao Y. J Ethnopharmacol 1995; 46: 125-129.
[4] Grover J, Yadav S, Vats V. J Ethnopharmacol 2002; 81: 81-100.
[5] Seon-Min J, Yong- Bok P, Myung-Sook C. Clin Nutr 2004; 23:1025- 1034.
[6] Violetta K, Hanna S, Wanda B. Toxicol 2005; 216: 24–31.
[7] Shui G, Dan G, Tian-Qing L, Xue-Hu M, Zhan-Feng C. Toxicol in Vitro 2009; 23: 201–208.
[8] Shui G, Yong-Ming B, Bo J, Li-Jia. Eur J Pharmacol 2006; 538: 73–79.
[9] Tamura A, Fukushima M, Shimada K, Han KH, Sekikawa M, Watanabe S, Nakano M, Matsumoto M, Chiji H. J Nut Sci Vitaminol (Tokyo) 2004; 50(1): 13-8.
[10] Harini R, Pugalendi KV. J Basic Clin Physiol Pharmacol 2010; 21(1): 79-91.
[11] Chuen-Lan L, Jin-Ming W, Chia-Yih C, Ming-Tzong C, Tsui-Hwa T. Food Chem Toxicol 2002; 40: 635-641.
[12] Balaraman R, Bafna PA, Kolhapure SA. J Ethnopharmacol 2004; 94: 135–141.
[13] Siegel JM. Behav Brain Sci 2005; 1; 82-83.
[14] Ghatak A, Asthana OP. Indian J Pharmacol 1995; 27: 14–29.
[15] Rang HP, Dale MM. Text book of pharmacology. 5th ed. New Delhi:Churchill living stone; 2003 p. 493 (vol 5).
[16] Satoskar RS, Bhandarkar SD, Rage NN. Insulin and oral antidiabetic drugs. In: Satoskar RR, Pharmacology and Pharmacotherapeutics. Mumbai, Popular Prakashan; 2007: pp. 885.
[17] Poothullil J. Neurosci Biobehav Rev 1993; 17: 85-89.
[18] Amir F Ayyobi, John D. Am J Cardiol 2003; 92: 27J–33J.
[19] Alicia S, Mickey C, Dewey D, Sheryl L, Dennis A, Edward M. Pharmacy and herbal medicine in the US Social Science & Medicine 1999; 49: 279-289.
[20] Khanna A, Riziv F, Chander R. J Ethnopharmacol 2002; 82:19-22.
[21] Zulet MA, Barber A, Garcin H, Higueret P Martinez JA. J Am Coll Nutr 1999; 18(1): 36-42.