PREVENTIVE ROLE OF *CYPERUS ROTUNDUS* RHIZOMES EXTRACT ON AGE ASSOCIATED CHANGES IN GLUCOSE AND LIPIDS

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Summary

Cyperus rotundus rhizomes have been used traditionally as home remedy for various ailments. In the present study, the preventive role of ethanolic extract of *Cyperus rotundus* rhizomes (CRRE) was investigated on age-associated changes in glucose and lipids in young and aged rats. Male albino rats of Wistar strains were divided into four groups: Group I – control young rats; Group II – young rats treated with CRRE (500mg/kg body weight) orally for 30 days; Group III – aged control rats and Group IV – aged rats treated with CRRE (500mg/kg body weight) orally for 30 days. Age associated increase in serum glucose, total cholesterol, triglycerides, LDL cholesterol, VLDL cholesterol and a decrease in HDL cholesterol was observed in aged rats when compared to young rats. Administration of CRRE to aged rats prevented the age associated changes on glucose, total cholesterol, triglycerides, LDL cholesterol and VLDL cholesterol. HDL cholesterol level was found to be increased significantly in both young and aged rats after treatment with CRRE. These finding demonstrated that CRRE normalized the age associated altered levels of glucose and improved lipid profile status in aged rats thereby decreasing the risk factors for diabetes mellitus and cardio vascular diseases associated with advancing age.

Key words: Aging, Cardiovascular diseases, Diabetes, Dyslipidemia, *Cyperus rotundus* rhizomes, Herbal medicine

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Introduction

Aging is a multifactorial biological process, which is accompanied by a general decline in biochemical and physiological functions that leads to the decreased ability of an individual to respond to a wide range of stresses or challenges and increased susceptibility to age associated degenerative diseases and death. Although epidemiological studies have discovered that lipid levels, diabetes, sedentary lifestyle and genetic factors are risk factors for coronary disease, hypertension, congestive heart failure, and stroke, the quintessential cardiovascular diseases within our society, advancing age unequivocally confers the major risk. The incidence and prevalence of these diseases increase steeply with advancing age [1].

Aging increases total cholesterol and decreases phopholipids, leading to increased cholesterol-phospholipids molar ratios in hepatic mitochondria [2], brain [3] and cerebral synaptic membranes [4]. These age related changes in lipid composition in various tissues and organs are thought to account not only for the age-related accumulation of body fat, but also for age related cellular hypofunction [5]. Hypercholesterolemia increases the risk of cardiovascular diseases, which are the principal causes of mortality as well as morbidity. In addition, advancing age is also associated with impaired glucose tolerance, an increased prevalence of diabetes mellitus and cardiovascular disease [6]. Therefore reversing age related changes in glucose and lipid metabolism would help to maintain normal cellular function and prevent common diseases. Herbs or medicinal plants have high density of important nutrient such as minerals, vitamins, phytochemicals and natural antioxidant that can prevent chronic diseases such as cancer, arteriosclerosis, diabetes and improving the quality of life. The phytochemicals phenols, steroid, alkaloids, glycoside and diterpenoids have shown potential in the prevention and management of cardio vascular disorders [7].

Cyperus rotundus L. (Cyperaceae) is a medicinal plant distributed throughout India, Cyelon and most of the temporate countries. Rhizomes are home remedy for indigestin, spure, diarrhoea, and other intestinal problems of children [8]. The rhizomes are intellect promoting, nervine tonic, diuretic, antiperiodic, and traditionally used to treat diarrhoea, dysentery, leprosy, bronchitis, amenorohoea, dysmenorrhoea, renal and vesical calculi, ophthalmic disorders, blood disorders and general debility [9]. The rhizomes have been used in ancient medicine for use in several clinical conditions like fever and arthritis [10]. Pharmacological studies denote the rhizomes as analgesic, anti-inflammatory, antipyretic [11], antidiarrhoeal [12], antidiabetic [13], cytoprotective [14]. Besides, the methanolic extract of rhizomes has been found to inhibit nitric oxide and superoxide production in murine macrophage cell line [15]. The phytochemical investigation of *C. rotundus* rhizomes have revealed the presence of flavonol glycoside [16], polyphenol [17], saponin [18], β-sitosterol [9], vitamin C [19], sesquiterpenoids [20], and essential oil [21]. Our previous studies reports that CRRE contains polyphenol and *in vitro* free radical scavenging activities [22]. In view of its wide use and its chemical composition, the ethanolic extract of *C. rotundus* rhizomes was evaluated for its pharmacotherapeutic property on age associated altered levels of glucose and lipid profile in young and aged rats.

Methods

Collection and Identification of Plant

Fresh matured rhizomes of *C. rotundus* with their hairy root were collected locally from Thiruvaiyaru, Thanjavur District, Tamil Nadu, India, during the of months of September-November 2004. The collected rhizomes were identified and authenticated by Dr. M. Jegadeesan, Professor, Department of Environmental and Herbal Sciences, Tamil University, Thanjavur, Tamil Nadu. A Voucher specimen (R.N., 264) was deposited at the Herbarium. The rhizomes were shade dried at room temperature for 15 days and make in to small pieces and used for extraction.

Preparation of Rhizomes Extract

Approximation 60 g of the rhizomes was placed in a soxhlet extractor and extracted with ethanol: water (7:3 ratio) for 72 h. The extract was concentrated *invacuo*, until the solvent was completely removed. The yield of ethanol extract was found to be 14.02 g. The extract was dissolved in distilled water at the time of use and given orally through gastric intubation to the experimental rats.

Animals and Experimental Protocol

Young (3-4 months, 120-150 g) and aged (22-24months, 380-410 g) Wistar male albino rats were used for the experiments. The rats were housed in polypropylene cages on a 12L:12D cycle. During the course of experiments, the temperature was maintained between $27^{o}C \pm 2^{o}C$. The animals were fed with commercially available pellet rat feed (Gold Mohur, M/s Hindustan Lever Ltd, Mumbai, India) and water *ad libitum.* The study protocol was carried out as per the rules and regulation of the institutional animals Ethics committee (IAEC). The animals were divided in to four groups, each group consists of six animals.

Group I : Young control rats.
Group II : Young rats received CRRE (500mg kg^{-1} b.wt/day) for 30 days.
Group III : Aged control rats.
Group IV : Aged rats received CRRE (500mg kg^{-1} b.wt/day) for 30 days.

Animals were kept starved overnight on the 30^{th} day. On the next day, after recording the body weight, animals were sacrificed by decapitation by making an incision on jugular vein to collect blood. The blood without anticoagulant was centrifuged at 6000rpm for 5 min for serum separation.

Determination of Glucose and Lipid Profile

Serum glucose was estimated by the oxidase method [23]. The total cholesterol was estimated by the method of Allain et al. [24]. Triglycerides was estimated by the method of Werner et al. [25]. HDL cholesterol was separated by adding phosphotungstic acid and

magnesium chloride to the fresh samples to precipitate other lipoproteins and the HDL cholesterol was estimated by the method of Allain et al. [24]. The concentration of LDL cholesterol was calculated by using the Friedwald formula [26] and VLDL cholesterol was calculated by dividing the triglycerides value (in mg/dl) by 5.

Statistical analysis

Values were expressed as mean ± standard deviation for six rats in the each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey's test for post-hoc multiple comparison tests. Statistical Package for Social Studies (SPSS) 9.0 version was used and $p<0.05$ was considered to be significant.

Results

The level of serum glucose of control and CRRE treated young and aged rats was illustrated in figure 1. The level of glucose was increased significantly (18.06%) in aged rats when compared to young rats. In aged rats, administration of CRRE reverted the increased level of serum glucose to near normal value of young control rats. In young rats, treatment of CRRE did not show any significant changes in serum glucose.

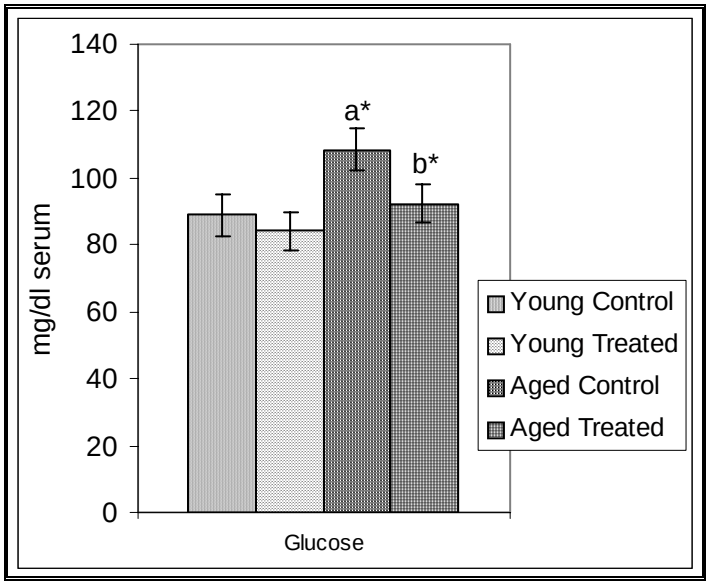

Figure 1. Effect of *Cyperus rotundus* rhizomes on serum glucose in young and aged rats. Each value is expressed as mean ± SD for six rats in each group. Superscript letters represent $p<0.05$ (Tukey's test). [a]As compared with young control, [b]As compared with aged control. [*]$p<0.001$.

The lipid profile status in serum of control and CRRE treated young and aged rats is given in Table 1. Serum of aged rats showed a significant increase in total cholesterol, triglycerides, LDL cholesterol and VLDL cholesterol and a decrease in HDL cholesterol as compare to young control rats. The increase was 41.20% for total cholesterol, 26.34% for triglycerides, 63.61% for LDL cholesterol and 26.37% for VLDL cholesterol. The decrease in HDL cholesterol was 31.91%. Administration of CRRE reverted the age dependent changes on serum lipids in aged treated rats to near normal value. In young rats, treatment of CRRE significantly increased HDL cholesterol level ($p<0.05$) and decreased LDL cholesterol ($p<0.05$) as compared to young control rats.

Table 1. Effect of *Cyperus rotundus* rhizomes on Lipids in young and aged rats.

	Young		Aged	
	Control	Treated	Control	Treated
Total Cholesterol	102.15 ± 6.88	98.37 ± 6.49	173.74 ± 7.01 ^{a**}	116.39 ± 7.14 ^{b**}
Triglycerides	62.14 ± 4.12	59.74 ± 4.09	84.37 ± 4.17 ^{a**}	64.59 ± 4.04^{b**}
HDL cholesterol	43.39 ± 2.13	46.79 ± 2.01 ^{a*}	29.54 ± 2.19 ^{a**}	42.27 ± 2.04 ^{b**}
LDL cholesterol	46.33 ± 4.61	39.63 ± 4.57 ^{a*}	127.32 ± 6.14 ^{a**}	61.20 ± 3.03 ^{b**}
VLDL cholesterol	12.42 ± 0.84	11.94 ± 0.88	16.87 ± 0.79 ^{a**}	12.91 ± 0.83 ^{b**}

Each value is expressed as mean ± SD for six rats in each group. Lipids mg/dl serum. Superscript letters represent $p<0.05$ (Tukey's test). ^{a}As compared with young control, ^{b}As compared with aged control. $^{*}p<0.05$, $^{**}p<0.001$.

Discussion

The major cause of death worldwide currently continues to be cardiovascular disease, which affects aged men and women equally [27]. Aging is a primary risk factor for cardiovascular diseases development [28] and increased levels of oxidative stress [29]. Glucose is a substrate and an indispensable energy supplier, which supports cellular function. In the present investigation, an increase was observed in serum glucose in aged rats. The age associated increase in glucose level documented in our study is in accordance with earlier reports [30,31]. The administration of CRRE reverted the age associated increased level of glucose thereby reduces the risk for diabetes mellitus associated with advancing age.

In the present study, we also observed an age associated increase in the level of total cholesterol, triglycerides, LDL cholesterol, VLDL cholesterol and decrease in HDL cholesterol in serum of aged rats. The age related increase in the level of total cholesterol and triglycareide was found to be similar to those reported in previous studies in rats [5,32] and human [33]. It has been reported that the age related increase in cholesterol was caused by LDL+HDL fraction [34]. The elevated cholesterol especially LDL level plays a crucial role in arteriosclerosis and intimal lesions that progress from fatty streaks to

ulcerated plaques [35]. Arteriosclerosis is a progressive multifactorial disease of the arterial wall and it is considered the most frequent causes of cardiovascular diseases. In aged rats CRRE reverted the age associated increased level of total cholesterol and LDL cholesterol to the level of young control rats. This finding is consistence with previous finding showing that water and alcoholic extract of *C. rotundus* rhizomes exhibited cholesterol lowering effect in obesity induced animal [36]. The beneficial effect of cholesterol lowering activity of CRRE could be attributed to β-sitosterol and saponin that are well known for their cardioprotective properties by lowering the cholesterol [37,38]. Aging was found to decrease lipoprotein lipase activity in adipose tissue, which explains the higher plasma triacylglycerol levels in aged animal [39]. The increase of serum trigtlycerides in the aged rats was caused by an increase of VLDL. CRRE administration restored the age associated change in triglycerides level to the level of young control rats, and this changes was achieved by decreasing of VLDL triglycerol. The decrease in triglycerides level is an important finding because recent studies show that triglycerides are independently related with coronary heart diseases [40].

The crucial risk factor for coronary heart diseases induces a low level of HDL cholesterol. The association between a low level of HDL cholesterol and an increased risk of cardiovascular disease has been well established through epidemiological and clinical studies [41]. Since low HDL cholesterol plays a direct role in the athrogenic process and is acknowledged as a strong predictor of coronary heart diseases, therapeutic intervention to raise HDL cholesterol together with other risk factors is widely encouraged. HDL cholesterol exert part of its antiatherogenic effect by promoting the reverse cholesterol transport pathway, in which HDL cholesterol induces an efflux of excess accumulated cellular cholesterol and prevents the generation of an oxidatively modified LDL. Administration of CRRE increased the level of HDL cholesterol in young and aged rats. The significant increase in the level of HDL cholesterol further strengthens the fact that the rhizomes extract may be used to reduce the risk factor for cardiovascular diseases.

The beneficial effect exhibited by CRRE on age associated changes in serum glucose and serum lipid profile might contribute to the antioxidant and polyphenolic content of the rhizomes. Hence, the present study suggests the possible utility of *C. rotundus* rhizomes as therapeutic agent in reducing risk factor for age associated degenerative diseases diabetes mellitus and cardio vascular diseases.

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