HYPOLIPIDEMIC EFFECT OF AMARANTHUS CAUDATUS L. IN TRITON WR-1339 INDUCED HYPERLIPIDEMIC RATS.

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Summary

This study was designed to determine the effect of methanolic and aqueous extracts of Amaranthus caudatus (A. caudatus) leaves (Amaranthaceae) on serum lipid profile changes in normal and Triton WR-1339 induced rats with view to elucidate its possible effects on cardiovascular diseases induced hyperlipidemia. To determine hypocholesterolemic activity in normal rats, the adult wistar rats were divided into 8 groups of 6 animals each, normal control, standard (Atorvastatin), methanolic and aqueous extracts of A. caudatus were treated at doses 200, 300 and 400 mg/kg body weight. The treatment period lasted for 8 days. Blood samples were collected from retro orbital puncture. The serum harvested was analysed for triglycerides and total cholesterol. The two extracts of A. caudatus were evaluated in Triton induced hyperlipidemic rats. Wistar rats were divided into 9 groups of 6 animals each, normal control, triton control, standard (Atorvastatin), methanolic and aqueous extracts of A. caudatus were treated at doses 200, 300 and 400 mg/kg bodyweight. Blood samples were collected from retro orbital puncture at 0 hr, 24 hr and 48 hr. The serum harvested was analysed for triglycerides, total cholesterol, HDL, LDL. Results showed that the methanolic extract of A. caudatus at 400 mg/kg bodyweight significantly (P<0.01) reduced the level of total cholesterol and triglycerides in normal rats. Methanolic extract of A. caudatus showed significant decrease (P<0.01) in the level of total cholesterol, triglycerides, LDL and increase in level of HDL at 400 mg/kg p.o. after 24 hr and 48 hr in Triton induced hyperlipidemic rats whereas aqueous extract of A. caudatus showed significant decrease (P<0.05) only in the level of triglycerides at 400 mg/kg p.o. after 24 hr and 48 hr. Hence the results of this study suggested that leaves of methanolic extract of Amaranthus caudatus possesses anti hyperlipidemic activities.
Introduction

Atherosclerosis, referred to as a “silent killer”, is one of the leading causes of death in developed countries and is on the rise in developing countries like India[1]. The American Heart Association has identified the primary risk factor associated with atherosclerosis as elevated levels of cholesterol and triglycerides in the blood. Therefore therapists consider the treatment of hyperlipidemia to be one of the major approaches towards decelerating the atherogenic process[2]. The ideal approach to prevent or to treat atherosclerosis and CVS complications is to target the lipid profile of hyperlipidemic patients using lipid lowering drugs or improving the diet. Traditional system of medicine like Ayurveda, Unani and Chinese prescribe numerous herbal drugs for Cardiovascular disorders. However number of herbal drugs are still to be evaluated pharmacologically.

*Amaranthus caudatus* (A.caudatus)Linn, Amaranthaceae, commonly known as chulai in Hindi, is a herbaceous annual growing to 15-100cm height. It was once nearly as important a food as maize and beans in central and south America. Nutritionally Amaranth grain has 2-3 times higher biological value than common cereal grains while containing substantially higher levels of protein with 2-3 times higher lysine content[3]. In India *A.caudatus* was traditionally used to cure kidney stones, stomach pain, leprosy, fever, piles[4]. The leaf has also been used as tea for relieving pulmonary conditions. In south Africa leaf is used as an abortifacient[5]. Recent studies have demonstrated variety of important and unique nutraceutical type applications for grain Amaranth[6]. Including its applications to lower blood serum cholesterol level[7]. The reports have shown that *A.caudatus* grain lowers blood glucose and serum cholesterol level[8]. Hence an attempt was made to investigate the leaves of *A.caudatus* for hypolipidemic effect in normal and Triton induced hyperlipidemia in rats. However, the literature indicates that there is no scientific evidence to support hypolipidemic effect of leaves of *A.caudatus*.

Materials and methods

Collection and extraction: *A.caudatus*. L (Amaranthaceae) was collected from GKVK, Agricultural University, Bangalore, were authenticated by Dr. Rajanna, the taxonomist of the university, voucher specimen was deposited in the herbarium of the Pharmacognosy Department, PES College Of Pharmacy, Bangalore, Karnataka, India, with reference number MAC- 2008/26.

The leaves (60 gms) shade dried, powdered, soxhlet extracted with methanol (400 ml). The extract was concentrated using rotary evaporator under reduced pressure, methanol extract (yield = 4.8%) maceration flask and added with sufficient quantity of purified water. Complete maceration takes place for about 24 hours, with occasional shaking during the first 6 hours. After 24 hours, the menstrum was collected and evaporated to obtain the dried extract (8.48 %) w/w was stored in a refrigerator at 4°C, until use for the biological testing and phytochemical screening.
Preliminary Phytochemical Screening: In order to determine the presence of phytoconstituents, a preliminary phytochemical study (colour reactions) with MEAV was performed\[^9\].

Drugs: Atorvastatin (Ajanta Pharma, India), Triton WR – 1339 (isoctyl – polyoxyethylene phenol)(Sigma Aldrich, USA) and all other chemicals used were of analytical grade. Methanol and aqueous extracts of *A.caudatus* was administered as Triton WR – 1339 was injected i.p. in saline solution. Atorvastatin administered orally in saline solution.

Animals: Throughout the experiment, experimental rats were processed in accordance with the instructions given by our institutional animal ethical committee CPCSEA\[^10\]. Healthy Wister Rats between 2-3 months of age and weighing 180-200g were used for the study. Rats were kept in standard polypropylene cage and maintained under standard laboratory conditions of temperature (25±1ºC), relative humidity (50±15%), 12hrs light-dark cycles, standard diet and water ad-libitum.

Acute Toxicity Studies\[^11\]: The acute oral toxicity study was carried out according to the guidelines set by OECD (Organisation for Economic Co-operation and development) guidelines No. 425. Healthy wistar rats (180-200 gms) were used for this study. The two doses of 2000 mg/kg p.o. and 5000 mg/kg p.o methanolic and aqueous extracts of *A.caudatus* were given to two groups containing 5 animals in each group. The treated groups were monitored for 14 days, for mortality and general behavior.

Hypocholesterolemic activity in normal rats\[^12\]: The rats were divided into 8 groups containing 6 animals each and were fasted overnight viz. Normal control received 2% Tween – 80(10 ml/kg), Standard Group, Atorvastatin(10 mg/kg), methanolic and aqueous extracts of *A.caudatus* treated groups(200, 300 and 400 mg/kg, p.o.). The treatments were given for 8 days. The blood samples were withdrawn from retro-orbital plexus under light ether anesthesia. The blood was subjected to centrifugation to obtain serum. Serum was analyzed for total cholesterol and triglycerides\[^13\].

Triton induced anti-cholesterolemic activity\[^14\]: Experimental rats fasted for 18 hrs, were provided water ad libitum. The rats were divided into 9 groups containing 6 animals each viz. Normal Control, received vehicle 2 % Tween 80(10 ml/kg), Triton control, received 200 mg/kg dissolved in 2% Tween 80(5ml/kg i.p.), Standard group, Atorvastatin, methanol and aqueous extracts of *A.caudatus*(200, 300 and 400 mg/kg p.o.), immediately after the administration of Triton WR-1339. In the following period of study (48 hr) animals had access only to water.

Biochemical Estimation: Blood samples were collected after 0, 24, 48 hr of Triton injection by retro-orbital puncture. Blood was immediately centrifuged (2500 rpm for 10 min) and serum was analyzed for total cholesterol, HDL, LDL and Triglycerides were determined\[^13\].

Results

Phytochemical screening: The percentage yield of MEAC and AEAC were found to be 4.1 % and 8.48 % w/w respectively. The methanol extract contained glycosides, saponins,
flavonoids, proteins, amino acids and carbohydrates and the aqueous extract contained glycosides, saponins, flavonoids, proteins, amino acids and carbohydrates.

**Acute toxicity Test:** The various observations showed the normal behavior of the treated rats. No toxic effects were observed at a higher dose of 5 g/kg body weight. Hence, there were no lethal effects in any of the groups till the end of the study.

**Effect on serum level of triglycerides and total cholesterol in normal rats:** At the end of 8 days of treatment, groups treated with MEAC showed significant decrease (P<0.01) level of triglycerides at 400mg/kg p.o. while MEAC at 200 and 300 mg/kg p.o and AEAC at all doses did not show significant decrease level of triglycerides. The standard Atorvastatin treated group showed significant (P<0.01) decrease level of triglycerides.

The MEAC and AEAC treated groups showed significant decrease (P<0.01) level of cholesterol at 400mg/kg p.o. The standard Atorvastatin 10mg/kg treated group showed significant (P<0.01) level of cholesterol in normal rats.

**Effect of MEAC and AEAC on lipid profile in triton induced hyperlipidemic rats:**

**Effect on serum level of triglycerides:** The MEAC and AEAC treated groups showed significant decrease (P<0.01) in the level of triglycerides at 400mg/kg p.o after 24hr and 48 hr while at 200 and 300mg/kg p.o did not show significant decrease in serum triglycerides level. (Fig1)

![Fig.1 Effect of *Amaranthus caudatus* on serum triglyceride level in Triton WR-1339 induced hyperlipidemic rats.](image)

Values are expressed as mean± SEM. n= 6 Significant values were compared with * P<0.05 and ** P<0.01 triton control vs all groups
Effect on serum level of total cholesterol: The groups treated with MEAC showed significant decrease (P<0.05) at 400mg/kg.p.o after 24 h and 48 h. (Fig2)

Fig.2 Effect of *Amaranthus caudatus* on serum cholesterol level in Triton WR-1339 induced hyperlipidemic rats.

Values are expressed as mean± SEM. n= 6 Significant values were compared with * P<0.05 and ** P<0.01 triton control vs all groups

Effect on serum level of HDL: The MEAC groups showed significant increase (P< 0.01) at 400mg/kg p.o. and (P<0.05) at 300mg/kg p.o. level of HDL. The AEAC treated groups showed significant increase (P<0.05) at 400mg/kg p.o after 24h and 48 h. (Fig3)

Fig.3 Effect of *Amaranthus caudatus* on serum HDL level in Triton WR-1339 induced hyperlipidemic rats.

Values are expressed as mean± SEM. n= 6 Significant values were compared with * P<0.05 and ** P<0.01 triton control vs all groups
Effect on serum level of LDL: The MEAC group showed significant decrease (P<0.01) at 400mg/kg p.o and AEAC treat group showed significant decrease (P<0.05) at 400mg/kg p.o. (Fig4)

Effect of Atorvastatin on lipid profile: Atorvastatin (10mg/kg. p.o) standard drug treated group showed significant decrease (P<0.01) in the level of triglycerides, total cholesterol and LDL after 24h and 48h and significant increase (P<0.01) in the level of HDL after 24h and 48h

Fig.4 Effect of Amaranthus caudatus on serum LDL level in Triton WR-1339 induced hyperlipidemic rats.

Values are expressed as mean± SEM. n= 6 Significant values were compared with * P<0.05 and ** P<0.01 triton control vs all groups

Discussion

Systemic administration of Triton WR 1339 in fasted rat induced hyperlipidemia. The maximum plasma triglyceride and total cholesterol were reached at 20h followed by declined to normal values\[15\]. The plant constituents like steroids, flavonoids, saponins are reported to possess lipid lowering activity. The plant steroids reduce the absorption of cholesterol and thus increase fecal excretion of cholesterol. Flavonoids augment the activity of lecithin acyl transferase (LCAT) which regulates blood lipids. LCAT plays role in the incorporation of cholesterol in to HDL (this may increase the level of HDL). Several studies have showed that increase in HDL is associated with decrease in cardiovascular diseases\[16\]. It is possible that active principles contained in A. caudatus, such as flavonoids and phytosterols interfere simultaneously with various biochemical pathway of lipids\[17\].
In present study, there was decrease in triglyceride and cholesterol in extract treated group. *Amaranthus caudatus* L. may act by inhibiting cholesterol synthesis and increase excretion of cholesterol, which may probably be due to presence of steroids and saponins. *Amaranthus caudatus* extract also increased the level of HDL which may be probably due to presence of flavonoids in extract\[18,19,20,21\].

Hence it can be concluded that *Amaranthus caudatus* has significant anti-hyperlipidemic effect owing to its ability to reduce level of total cholesterol, triglyceride, LDL and increasing HDL level. Further experimentation needs to be done with regard to fraction of extract, isolation and characterization to explore active constituents responsible for anti-hyperlipidemic activity and to elucidate the possible biochemical mechanism.

References


