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Anti-hyperlipidemic activity of methanol extracts of three plants of *Amaranthus* in triton-WR 1339 induced hyperlipidemic rats

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ABSTRACT

Objective: To investigate the anti-hyperlipidemic activity of methanol extracts of leaves of three plants of *Amaranthus*. **Methods:** In this study, the anti-hyperlipidemic effects of three plants of *Amaranthus* were evaluated by using normal and triton-WR 1339 induced rats at the dose of 200, 300 and 400 mg/kg *p.o.* The serum harvested was analyzed for total cholesterol, triglycerides, high density lipoprotein and low density lipoprotein. **Results:** It was found that all the three plants at 400 mg/kg dose showed significant anti-hyperlipidemic effect ($P<0.01$), whereas 300 mg/kg dose is less significant in the entire parameters used for evaluation of anti hyperlipidemic effect ($P<0.05$). **Conclusions:** Methanol extracts of *Amaranthus caudatus*, *Amaranthus spinosus*, *Amaranthus viridis* showed significant anti-hyperlipidemic effect and this study provides the scientific proof for their traditional claims.

1. Introduction

Cardio vascular diseases are leading cause of death in both industrialized and developing nations. Disorders of lipid metabolism, following oxidative stress are the prime risk factors for initiation and progression of these diseases[1]. The current anti-hyperlipidemic therapy includes principally statins and fibrates, the former corrects the altered blood lipid profile by inhibiting the bio-synthesis of cholesterol and the latter acts by enhancing the clearance of triglyceride rich lipo proteins. The investigation on plant drugs will be a useful strategy in the discovery of new lead molecules eliciting improved activity by regulating the different mechanisms maintaining the lipid metabolism and thus can be used in treating hyperlipidemia of varied etiology[2]. Traditional system of medicine like Ayurveda, Unani and Chinese prescribe numerous herbal drugs for cardio vascular disorders. Recently herbal hypolipidemics have gained importance to fill the lacunae created by the allopathic drugs.

The *Amaranthus* plants are spread throughout the world, growing under a wide range of climatic conditions and they are able to produce grains and leafy edible vegetables. *Amaranthus caudatus* (*A. caudatus*) Amaranthaceae was traditionally used to cure piles, fever, diuretic, leprosy. Methanolic extract of *A. caudatus* showed antinociceptive, antipyretic[3], hepatoprotective activity[4], and *in vitro*-amylase inhibition and antioxidant activity[5]. *Amaranthus spinosus* (*A. spinosus*) Amaranthaceae, is an annual herb, native to tropical America and found as a weed in cultivated lands as well as follow lands throughout India. *A. spinosus* showed chemoprotective, *in vivo* antioxidant activity[6] and *in vitro* alphaamylase inhibition in oxidative stress in diabetic rats[7]. *Amaranthus viridis* (*A. viridis*) Amaranthaceae is an branched glabrous herb, distributed in all tropical countries. The traditional uses are anti-inflammatory, diuretic, anti ulcer, laxative, anti leprotic and asthma[8]. Methanol extract of *A. viridis* showed *in vitro* anthelmintic property[9], hepatoprotective and antioxidant activity[10].

A. caudatus, *A. spinosus* and *A. viridis* have been used to reduce the elevated lipid profile in Indian traditional system

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of medicine. The leaves were selected to investigate the anti hyperlipidemic activity. However there is lack of scientific report, so our aim is to provide scientific validation for traditional claims.

2. Materials and methods

2.1. Collection of plant material and extraction

The fresh leaves of *A. spinosus*, *A. viridis* and *A. caudatus* were collected from GKVK, Agricultural University, Bangalore, and were authenticated by the taxonomist Dr. Rajanna, GKVK, Bangalore. The voucher specimen (PESCP-26, 27, 28) was deposited in college herbarium. The leaves of *A. spinosus*, *A. viridis* and *A. caudatus* were shade dried and coarsely powdered. The coarse powder (60 g each) was subjected to extraction with methanol by soxhlet apparatus and extracts were concentrated using rotary evaporator under reduced pressure (yield-4.8, 4.4, 4.6 % w/w) and then was stored in a refrigerator at 4 ℃, until use for the biological testing and phytochemical screening.

2.2. Preliminary phytochemical screening

The methanol extracts of *A.spinosus*, *A.viridis* and *A. caudatus* were screened for the presence of various phytoconstituents[11].

2.3. Animals

Healthy wistar rats between 2-3 months of age of either sex and weighing 180-200 g were acclimatised to the laboratory at temperature (25 ± 1) ℃, relative humidity (50 ± 15) %, 12 h light - dark cycles, kept in standard polypropylene cages of maximum 2 animals each, given standard diet (Kamadhenu Enterprises, Bangalore) and water ad-libitum in accordance with the instructions given by Institutional Animal Ethical Committee, CPCSEA[12].

2.4. Acute toxicity studies

Methanol extracts of *A. spinosus*, *A. viridis* and *A. caudatus* were studied for the acute oral toxicity according to the guideline No. 423 set by Organization for Economic Cooperation and Development (OECD)[13]. Healthy wistar rats (150 - 180 g) were used for the study. The two doses of 2 000 mg/kg (*p.o.*) and 5 000 mg/kg (*p.o.*) of the test samples were given to two groups containing 5 animals in each group for three plants. The treated groups were monitored for 14 days, for mortality and general behaviour. The extract was devoid of any toxicity in rats when given in dose up to 5000 mg/kg by oral route. Hence, for further studies 200, 300 and 400 mg/kg doses of extract were selected.

2.5. Hypolipidemic activity in normal rats

Animals in the normal control group received normal saline 10 mL/kg orally. Standard group received Atorvastatin at 10 mg/kg orally. The test group of animals were treated with the methanol extracts of *A. caudatus*, *A. spinosus*, *A. viridis* at predetermined therapeutic doses of 200, 300 and 400 mg/kg *p.o.* The treatments were given for 8 days. The blood samples were withdrawn from retro-orbital plexus. All the lipid profile parameters were determined. Total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides were analysed from serum[14].

2.6. Triton induced anti-hyperlipidemic activity

Experimental rats fasted for 18 h were provided water ad-libitum. The rats were divided into 11 groups containing 6 animals each *viz.* normal control group (NC-Vehicle) received 2% vehicle tween-80 at 5 mL/kg orally, triton control group (TC-Vehicle) received triton-200 dissolved in 2% Tween-80 at 5 mL/kg *i.p.*, Standard group received atorvastatin at 10 mg/kg orally.Methanol extracts of all the three plant groups received three doses of 200, 300 and 400 mg/kg *p.o.* All the treatments were given immediately after injection of triton WR-1339 except normal control group. In the following period of study (48 h) animals had access only to water.

2.7. Biochemical estimation

Blood samples were collected after 0, 24 and 48 h of triton injection by retro-orbital puncture. Blood was immediately centrifuged (2 500 rpm for 10 min) and serum was analysed for total cholesterol, triglycerides, HDL and LDL[14].

3. Results

3.1. Phytochemical screening

The percentage yield of *A. caudatus*, *A. spinosus*, *A. viridis* were found to be 4.6%, 4.8% and 4.4% w/w, respectively. The methanol extract of three plants contained glycosides, saponins, flavonoids, proteins, amino acids and carbohydrates.

3.2. Acute toxicity test

The acute oral toxicity test showed the normal behaviour of the treated rats. No toxic effects were observed at a higher dose of 5 g/kg bw. Hence there were no lethal effects and indicated that it may have a reasonable safety margin with regards to acute toxicity.

3.3. Hypolipidemic activity in normal rats

The groups treated with methonal extract of *A. caudatus* (MEAC), methonal extract of *A. viridis* (MEAV) showed