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doi:

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Effect of erythrina indica on stress induced alteration on lipid profile in rats

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ARTICLE INFO

Article history:

Received 15 August 2012

Received in revised form 15 September 2012

Accepted 17 December 2012

Available online 28 December 2012

Keywords:

Stress

Erythrina Indica

Hypolipidemic

Lipid Levels

Dyslipidemia

ABSTRACT

Objective: The study was undertaken to evaluate the effect of stress induced alteration on lipid profile in rats by different fractions (Petroleum ether, ethyl acetate and chloroform) of ethanolic extract of Erythrina Indica an indigenous plant used in ayurvedic medicine in India. **Methods:** The study was carried on albino rats (150–200g) of either sex, divided into four groups of 6 each. Group I served as control, Groups II, III and IV was treated with different fractions (Petroleum ether, ethyl acetate and chloroform) of ethanolic extract of Erythrina Indica 150mg/kg, p.o. in a single daily dose from day 1 to day 22. Physical stress of 5 hours swimming was given to all the groups on day 22. Blood samples were withdrawn in group I on day 0 (blank control) and on day 22 after stress (positive control). Blood samples were withdrawn in group II, III and IV on days 3,7,14 and 21 and on day 22 after stress. **Results:** All the blood samples were analyzed for total cholesterol (TC) HDL, cholesterol (HDLC) triglyceride (TG) by enzymatic method and LDL & VLDL cholesterol was calculated by on the basis of Friedwalds equation. After 21 days of treatment changes the serum lipid levels in rats insignificantly. In control group stress increased the lipid levels in rats significantly except HDL cholesterol which reduced insignificantly. When Erythrina indica treated rats were subjected to stress on day 22, their serum lipid levels increased significantly except HDL cholesterol which reduced insignificantly. **Conclusions:** study indicates that various fractions (Petroleum ether, ethyl acetate and chloroform) of the ethanolic extract of Erythrina Indica is effective in attenuating stress induced dislipidemia in rats.

1. Introduction

Modern life is full of hassles, deadlines, frustrations, and demands. In that many people affected by Stress, it is so commonplace that it has become a way of life. Stress is a normal physical response to events that make you feel threatened or upset your balance in some way[1]. Stressful stimuli may influence the onset and progression of a number of disorders in human being leading to hypertension, diabetes, stroke, cancer depression etc[2]. There are various reports that stress can change the level of certain hormones like insulin, cortisol and epinephrine[3–4]. These entire hormones affect lipid profile of the body to a great extent. In animal studies it was found that stress raises serum and tissue cholesterol level of rats on normal diet[5–6]. Last few decades the reputation of medicinal plants and its herbal remedies has increased globally due

to its therapeutic efficacy and safety. Bioactive richness of the active constituents of leaves of Erythrina Indica has promoted us to undertake the present study and the lipid profile of individuals showed that it's significantly reduce the lipid level (cholesterol and triglyceride) and remarkable stress induced alteration on lipid profile in rat.

2. Material and Method

2.1. Animal

Albino rats of either sex (150–200 g) were used. They were housed in plastic cages at an ambient temperature of 26±2°C and 45% to 55% relative humidity with a standard 12 h light or dark cycle. They had free access to food and water and were acclimatized for at least one week before experimentation. Each experimental group consisted of minimum of 6 animals. National Research Council guidelines for the care and use of laboratory animals were followed throughout the study.

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2.2. Preparation and fractionation of crude extracts

Crude extract was obtained through cold extraction process. The coarse powder was submerged in ethanol and allowed to stand for 10 days with occasional shaking and stirring. When the solvent became concentrated the alcohol content was filtered through cotton and then through filter paper (What man filter paper no. 1). Then the solvent was allowed to evaporate using rotary evaporator at temperature 40–45°C. Thus, the highly concentrated crude extract was obtained. That was then fractionated using petroleum ether, ethyl acetate and CHCl₃. The solvents of these fractions were evaporated by rotary evaporator and then dried under mild sun. The dried fractions of extract were then preserved in the freeze for the experimental uses^[7].

2.3. Drugs

Erythrina indica leaf extract was used in the present study. Erythrina indica (150mg/kg) was administered once daily, orally for 22 days in suspension form through a rat feeding cannula on empty stomach in the morning. Dose was extrapolated from human dose on body surface area basis^[7].

2.4. Stress

A 5 hours swimming stress^[3, 8] was given to all the animals on day 22. This includes active swimming and immobility period. For swimming, a plastic tub (24" in height and 40" in diameter) half filled with tap water and maintained at room temperature of 28°C was used.

2.5. Blood Sample

The animals were anaesthetized with ether rapidly within 2 minutes according to stress free procedure. This does not cause stress to the animal. The blood sample was collected from retro bulbar plexus immediately after anaesthetization. Serum was separated and was kept at 4°C until use.

2.6. Observational Parameter

In this experimental study the following serum lipid levels were estimated. Total cholesterol (TC) and HDL cholesterol, Triglyceride (TG). LDL & VLDL cholesterol was calculated on the basis of Friedwalds equation^[9] LDL cholesterol = TC – (HDL + TG/5); VLDL cholesterol = TC – (HDL + LDL).

2.7. Procedure

Experiment was designed to study the effect of stress on lipid profile and effect of Erythrina indica on stress induced alteration on lipid profile in rats. For this experimental study rats were divided into four groups of 6 each. The different treatment scheduled was as follows:

Group I -- Control.

Group II – Petroleum ether fraction (150mg/kg body wt.)

Group III -- Ethyl acetate fraction (150mg/kg body wt.)

Group IV -- Chloroform fraction (150mg/kg body wt.)

In control group animals were treated with 0.5 ml normal saline (p.o.) daily from day 1 to day 22. Serum lipid levels were estimated on day 0 (blank control) and on day 22 after stress (positive control). Animals in group II was treated with Erythrina indica (150mg/kg p.o.) daily from day 1 to day 22. Serum lipid levels were estimated on day 3,7,14 and 21 and on day 22 after stress.

2.8. Statistical Analysis

Results were expressed as Mean + SEM and student 't' was applied for analysis of data. *P* value < 0.05 was considered as significant.

3. Results

Different fractions (petroleum ether, ethyl acetate and chloroform) of ethanolic extract of Erythrina indica (150mg/kg p.o.) and control groups animal – on normal diet shows TC from 72.6±0.98 to 73.2±0.02(NS), raised the HDLC from 25.84±0.62 to 24.84±0.62 (NS), TG from 44.81±1.81 to 44.13±0.29 (NS), the LDLC from 27.83±1.34 to 25.04±1.21 (NS), and VLDLC from 9.96±0.36 to 9.02±0.14 (NS) (Table 1 and Table 2) after stress in control group TC increased from 75±1.87 to 103.33 ±1.36, *P* < 0.001, HDLC reduced from 25.2±1.03 to 24.83 ±0.74,(NS). TG increased from 44.81±1.81 to 67.03 ±1.81, *P* < 0.001, LDLC increased from 27.83±1.34 to 53.09±1.14, *P* < 0.001 and VLDLC increased from 9.96±0.36 to 14.40±0.36, *P* < 0.001 (Table 1 and Table 2 Control Group). In control group stress increased the lipid levels in rats significantly except HDL cholesterol which reduced insignificantly. When Erythrina indica treated rats subjected to stress on day 22, their serum lipid levels increased significantly except HDL cholesterol which reduced insignificantly, after stress in Erythrina indica treated rats TC increased from 72.6±0.98 to 85.32±1.26, 73.6 ±0.08 to 85.52±1.20 , 73.2±0.02 to 84.32±1.24 *P* < 0.001, HDLC reduced from 25.84±0.62 to 25.27±0.10, 25.34±0.60 to 25.37±0.20, 24.84±0.62 to 24.17±0.60 TG increased from 44.42±0.69 to 47.93±0.41, 44.22 Different fractions (petroleum ether, ethyl acetate and chloroform) of ethanolic extract of Erythrina indica (150mg/kg p.o.) and control groups animal – on normal diet shows TC from 72.6±0.98 to 73.2±0.02(NS), raised the HDLC from 25.84±0.62 to 47.33±0.21, 44.13±0.29 to 46.93 ±0.81 *P* < 0.01, LDLC increased from 25.04±1.21 to 38.47±1.45 , 25.92±1.31 to 37.87±1.60 , 26.34±1.21 to 37.47±1.70, *P* < 0.001 and VLDLC increased from 9.22±0.14 to 10.59±0.13, 9.82±0.12 to 10.29±0.16, 9.02±0.14 to 9.59±0.12 . *P* < 0.01 (Table 1 and Table 2 Erythrina Indica Treated Groups), when compared to positive control serum lipid levels were significantly less in Erythrina indica treated rats except HDL cholesterol which reduced insignificantly (Table 2).

4. Discussion

Swimming and force water swimming in small laboratory animals has been widely used for studying the physiological changes and the capacity of the

Table 1

Serum Lipid Levels (mg %) Before Stress (0 Day)

Group	TC	HDLC Cholesterol	TG	LDLC Cholesterol	VLDL Cholesterol
Group-I(Control)	75±1.87	25.2±1.03	44.81±1.81	27.83±1.34	9.96±0.36
Group -II(Petroleum ether)	NS 72.6±0.98	NS 25.84±0.62	NS 44.42±0.69	NS 25.04±1.21	NS 9.22±0.14
Group-III (Ethyl acetate)	NS 73.6±0.08	NS 25.34±0.60	NS 44.22±0.69	NS 25.92±1.31	NS 9.82±0.12
Group -IV(Chloroform)	NS 73.2±0.02	NS 24.84±0.62	NS 44.13±0.29	NS 26.34±1.21	NS 9.02±0.14

Values are Mean ±SEM Statistical analysis = Student T Test. n=6; * P <.05, ** P <.01

*** P <.001, when compared to before stress (same group), @ P <.001 when compared to control group after stress; NS = Not significant

Table 2

Serum lipid levels (mg %) After stress (Day 22)

Group	TC	HDLC Cholesterol	TG	LDLC Cholesterol	VLDL Cholesterol
Group-I (Control)	*** 103.33±1.36	NS 24.83±0.74	*** 67.03±1.81	*** 53.09±1.14	*** 14.40±0.36
Group -II(Petroleum ether)	***@ 85.32±1.26	NS 25.27±0.10	**@ 47.93±0.41	***@ 38.47±1.45	**@10.59±0.13
Group-III (Ethyl acetate)	***@ 85.52±1.20	NS 25.37±0.20	**@ 47.33±0.21	***@ 37.87±1.60	**@ 10.29±0.16
Group -IV (Chloroform)	***@ 84.32±1.24	NS 24.17±0.60	**@ 46.93±0.81	***@ 37.47±1.70	**@ 9.59±0.12

Values are Mean ±SEM Statistical analysis = Student T Test. n=6; * P <.05, ** P <.01

*** P <.001, when compared to before stress (same group), @ P <.001 when compared to control group after stress; NS = Not significant

organism in response to stress. Swimming is not always a simple exercise stress because emotional factors are difficult to eliminate[10]. Change in lipid levels to stress is rather contradictory and may depend on situational, environmental and inter individual factors. Stress affect hypothalamic–pituitary–adrenal axis and affect various neurotransmission system like dopamenergic, cholinergic, 5–Hydroxydryptaminergic gabaminergic and benzodiazepine. Various hormone secretion also altered by stress like CRH, GH, Insulin, epinephrine and cortisol. The physiological mechanism of stress induced changes in lipid levels remains largely unelucidated. It appears like that the hypothalamic–pituitary–adrenal (HPA) axis contributes to the stress induced cholesterol changes. Stress increased serum lipid levels significantly in the entire group (i.e. control and Erythrina indica treated) except HDL cholesterol which reduced insignificantly. When the rise in serum lipid levels after stress in group II, III, and IV (Erythrina indica treated rats) were compared with positive control (Table 2) the rise in serum lipid levels in Erythrina indica treated rats were significantly less than control rats, except HDL cholesterol which reduced insignificantly. It can be concluded that Erythrina indica (150mg/kg p.o.) is effective in attenuating stress induced dislipidemia in rats. The present study is a preliminary attempt in evaluating the stress induced alteration on lipid profile using Erythrina indica leaf extract. Further phytochemical and pharmacological investigation is warranted in this direction for establishing its detailed mechanism of action and for substantiating its traditional and folk claims.

Conflict of interest statement

We declare we have no conflict of interest.

Acknowledgements

The authors are thankful to Saastra College of Pharmaceutical Education and Research, Vels University (VISTAS) and its management for providing research facilities and encouragement and to our friends those who helped us to complete this research.

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