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# ANTIHYPERLIPIDEMIC AND ANTIOXIDANT ACTIVITY OF AERIAL PARTS OF *SWERTIA CHIRATA* (BUCH-HAM) IN POLOXAMER 407 INDUCED HYPERLIPIDEMIC RATS

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**ABSTRACT**

Hyperlipidemia is considered to be one of the greatest risk factors contributing to the prevalence and severity of cardiovascular diseases. The present study was undertaken to evaluate antioxidant and antihyperlipidemic effect of ethanolic extract of aerial parts of *Swertia chirata* (EESC). Although the herb *Swertia chirata* is ascribed with many therapeutic effects its antihyperlipidemic potential was unexplored. The study comprised of preliminary phytochemical investigation, antioxidant activity (by 2,2-diphenyl-1-picrylhydrazyl and thiobarbituric acid reactive substances assay) and *in vivo* antihyperlipidemic activity of EESC. Oral administration of extract, at different doses of 100, 200, 400mg/kg body weight in rats, dose dependently inhibited serum total cholesterol, triglycerides, low density lipoprotein, very low density lipoprotein and increased high density lipoprotein levels. The treated group showed significant reduction in levels of hepatic cholesterol, hepatic triglycerides, and tissue malondialdehyde. And also showed elevation in catalase and reduced glutathione levels in dose dependent manner when compared to disease control group. Treatment also showed to decrease atherogenic indices which are powerful indicators of the risk of heart disease. The Atorvastatin was used as standard antihyperlipidemic drug in this study.

**Key words:** Antihyperlipidemia, *Swertia chirata*, Cholesterol, Atherogenic indices.

## INTRODUCTION

Hyperlipidemia is characterized by elevated levels of serum total cholesterol, low density lipoprotein, very low density lipoprotein and decreased high density lipoprotein. Such elevated serum level of triglycerides, cholesterol, LDL and decrease levels of HDL are major risk factors for the premature development of cardiovascular disease like atherosclerosis, hypertension, coronary heart disease etc. It is also an important risk factor in the initiation and progression of atherosclerotic impasse (Sikarwar M, Patil M, 2012). Also, the increase formation of reactive oxygen species is one of the most influencing factors for the initiation of atherosclerosis and development of cardiovascular diseases. The main aim of treatment in patients with hyperlipidemia is lowering of LDL levels thereby reducing the risk of developing ischemic heart disease or the occurrence of further cardiovascular and cerebrovascular diseases (Anbu J *et al.*, 2011).

Although synthetic hyperlipidemics have been found effective in lowering elevated serum low-density lipid levels, they have been found to cause many side effects. e.g. statins, main class of antihyperlipidemics they are basically enzyme inhibitors, so it is likely that they may be inhibiting other critical enzymes in body. Also, the other agents like Niacin, Fibrates, Cholesterol absorption inhibitors, bile acid sequestering resins shows the adverse effects like flushing, pruritis, GI Irritaion, exacerbation of gout, impairment in the absorption of fat soluble vitamins. And also they are devoid of antioxidant property. But herbal treatment for hyperlipidemia has fewer side effects, is relatively cheap and most of the

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herbs have antioxidant potential. Moreover, the natural products having dual property of antihy perlipidemic and antioxidant activities can better manage the hyperlipidemia associated disorders which are usually accompanied by increased oxidative stress (Mahmud ZA *et al*., 2011). Recent study has shown that medicinal plants intake results in an increase of antioxidant enzymes activity and HDL cholesterol, and a decrease in malondialdehyde, which may reduce the risk of heart disease (Chenni A *et al*., 2007).

Considering all the above factors, it is worthwhile investigating plant drug which have been in existence though traditional system of medicine and believe to have wide biological activities, higher safety margin than synthetic drug. Hence studying the activity of the herb, which is claimed to possess antioxidant, antihyperlipidemic activities suggesting its usefulness in the treatment of hyperlipidemia may pave way for newer and better therapies.

The plant Swertia chirata, aboriginal to Himalayas in India, Nepal and Bhutan, has been used for millennia, to cure variety of ailments and diseases. The chief bioactive constituents of plant are xanthones, flavanoids, iridoids, secoiridoids glycosides, which plays momentous role in its biological activities like antidiabetic, anti-inflammatory, hepatoprotective, antioxidant, antipyretic, antimalarial, analgesic, anticarcinogenic, antibacterial, antiviral, gastroprotective, antileishmanial, anthelmentic. Its medicinal usage is declared in American and British pharmacopoeias, Indian Pharmaceutical codex and in different conventional systems of medicines like Ayurveda, Unani and Sidha. Various studies have successfully done to prove its medicinal uses which are traditionally mentioned. One of the functions of this herb mentioned in various ayurvedic literature is Cholagogue (Gogate VM, 2000; Joshi SG, 2004) meaning to promote the flow of bile which is the major route for the excreation of cholesterol. Also, the herb contains chemical constituents such as mangiferin (Miura T *et al*., 2001) a xanthone and swertiamarin (Vaidya H *et al*., 2009) a secoiridoid glycoside have proven antihyperlipidemic activity when isolated from other sources (Phoboo S *et al*., 2011). These two clues guide us to study the probable antihyperlipidemic activity of this herb.

## MATERIALS AND METHODS

### Plant material

The dried aerial parts of *Swertia chirata* (2kg) were purchased from Yucca Enterprises, Wadala, Mumbai, in month of July. The plant was identified and authenticated by Dr. Vinayak Naik, Senior research Scientist, N. P. Botany. Piramal Life Sciences, Mumbai. Voucher specimen (PHL/6524) was deposited for further reference.

### Preparation of crude extract

Dried aerial parts of *Swertia chirata* were powdered and passed through 40 mesh sieve. Dried powder (400g) was subjected to cold maceration with 12% ethanol for 48Hr with continuous stirring. The extract was concentrated under vacuum in rotary evaporator at temperature of 50±0.5°C and dried completely by freeze drying to get free flowing powder. The yield of extract was 22.5g. For dosing, the extract was uniformly suspended in 0.5% Carboxy Methyl Cellulose (CMC) dissolved in water and administered orally (*p.o*)

### Preliminary phytochemical screening

The ethanolic extract of aerial parts of *Swertia chirata* was subjected to preliminary phytochemical screening to check the presence of flavonoids, tannins and phenolic compounds, alkaloids, glycosides, terpenoids, steroids, carbohydrates and proteins by using their respective chemical tests (Khandelwal KR, 2008).

### Experimental Animals

Male Sprague Dawley rats weighing 150 to 200gms were purchased from Bharat serum, Thane. The animals were housed under standard environmental conditions (temperature 22 ± 2ºc; humidity 60 ± 4%) with 12 hrs light/ dark cycle at Animal house of Bharati Vidyapeeth's college of Pharmacy, Navi Mumbai, India. The control and experimental animals were provided food and drinking water *ad libitum*. The present animal experiment was approved by Animal ethics committee (IAEC/PR/2012/02). All the procedures were conducted in accordance with CPCSEA guidelines.

### *In vitro* antioxidant activity

Estimation of DPPH radical scavenging activity (Liu J *et al*., 2013)

DPPH radical scavenging activity was determined as described by Mensor *et.al.*Sample stock solution of extract in ethanol (1mg/ml) was diluted to final concentration of 100, 200, 400, 600, 800, and 1000µg/ml in ethanol. 1ml of 0.3mM DPPH in ethanol solution was added to 2.5 ml of sample solutions of different concentrations and was allowed to react for 30 minutes at room temperature (R.T). After 30 minutes the absorbance values were measured at 518nm. Ascorbic acid was used as standard.

The percentage antioxidant activity (AA) was calculated using the following formula:

$AA\%=100- \{[(Abs_{sample}-Abs_{blank}) \times 100] \div Abs_{control}\}$

Ethanol (1.0ml) plus plant extract solution (2.5ml) was used as blank. Methanol (2.5ml) plus 0.3mM DPPH solution in methanol (1.0ml) was used as negative control.

**Thiobarbituric acid reactive species (TBARS) assay**

Production of TBARS was determined using a modified method as described by Ohkawa *et.al.* The rats were sacrificed by overdose of $CO_2$inhalation. Livers were removed quickly and placed on ice. 10%w/v liver homogenates were prepared in ice cold 10mM Tris-Hcl buffer PH 7.4. The homogenates were centrifuged for 10 minutes at 1400g to yield a pellet which was discarded and supernatant was used for the assay. The homogenates (100ul) were incubated with or without 50ul of freshly prepared oxidants (5uM Sodium nitroprusside and 10uM ferrous sulphate) and different concentrations of the plant extract together with appropriate volume of deionised water to give a total volume of 300ul, at 37°C for 1hour. The colour reaction was carried out by adding 200,250 and 500ul each of 8.1% Sodium dodecyl sulphate (SDS), acetic acid (PH 3.4) and 0.6% TBA respectively. The reaction mixtures, including those of serial dilutions of 20uM MDA, were incubated at 97°C for 1 hour. The tubes were cooled and absorbance was measured at 532nm. Gallic acid was used as standard antioxidant (Olalye MT, Rocha JBT, 2007; Ohkawa H *et al*., 1979).

***In vivo* antihyperlipidemic activity**

Antihyperlipidemic activity of Ethanolic extract of aerial parts of Swertia chirata (EESC) was examined in Poloxamer-407 induced hyperlipidemic rats (Johnston TP, Palmer WK, 1993).

Male Sprague dawley rats were randomly divided into 6 groups as follows-

Group 1: Vehicle control group in which rats were daily administered vehicle i.e. 0.5% w/v sodium CMC suspension for 12 successive days.

Group 2: Diseases control group in which rats were administered with single dose of Poloxamer 407 (1gm/kg *i.p.*) on $13^{th}$ day.

Group 3: Standard control in which rats were daily administered Atorvastatin (10mg/kg) for 12 successive days.

Group 4: Test group of low dose in which rats were daily administered with low dose of EESC i.e. 100mg/kg for 12 successive days.

Group 5: Test group of medium dose in which rats were daily administered with low dose of EESC i.e. 200mg/kg for 12 successive days.

Group 6: Test group of high dose in which rats were daily administered with low dose of EESC i.e. 400mg/kg for 12 successive days.

**Blood sampling and Biochemical estimation**

Blood samples were withdrawn from retro-orbital plexus at 0hr, 24hr and 48hr after administration of test drug, and transferred directly into centrifuge tubes and allowed to clot at room temperature (R.T) for 20-25 minutes and centrifuged for 20 min. at 2000 r.p.m. The supernatant clear serum thus obtained was transferred carefully with help of micropipette into small test tubes for estimation. The serum concentration of Total cholesterol (TC), HDL-C, Triglycerides (TG), LDL-C were measured by standard procedures using an autoanalyzer (Erba Chem 7) with biochemical kits of Erba.. The LDL, VLDL, Atherogenic indices like atherogenic coefficient and cardiac risk ratio were calculated using the following formulae (Friedewald WT *et al*., 1972).

LDL = TC – (HDLC + TG/5)

VLDL = TG/5

Cardiac Risk Ratio (CRR) = TC/HDLC (Malaspina HB, 1981)

Atherogenic Coefficient (AC) = (TC– HDLC)/HDLC (Kayamori F, Igarashi K, 1994)

Atherosclerosis index(AI)= LDL/HDL (Mertz DP, 1980)

At the end of the experiment animals were sacrificed by inhalation of carbon dioxide. Livers were excised immediately, washed with ice cold saline and 10%w/v liver homogenates were prepared using phosphate buffer. Homogenates were centrifuged at 3500rpm for 15 minutes. Supernatant so obtained was used for determination of Hepatic cholesterol, Hepatic Triglycerides levels and various antioxidant parameters like lipid peroxidation, Reduced glutathione, Catalase.

**STATISTICAL ANALYSIS**

The data obtained was analysed by one way analysis of variance test (ANOVA) followed by Dunnett's test.

**RESULTS**

**Preliminary phyochemical screening:**

Preliminary phyochemical screening revealed that EESC showed the presence of alkaloids, glycosides, phenolic acids, flavanoids and tannins.

***IN VITRO* ANTIOXIDANT ACTIVITY**

**DPPH free radical scavenging activity**

Various concentrations of EESC (100-1000µg/ml) were tested. Percent antioxidant activity for 1000µg/ml of EESC and Gallic acid was found to be 90.1% and 98.02% respectively

**TBARS assay**

Various concentrations of EESC (100-1000µg/ml) were tested. Percent inhibition of Thiobarbituric Acid Reactive Substances (TBARS) formation for 1000µg/ml of EESC and Gallic acid was found to be 72.25% and 85.35% for Sodium nitroprusside induced lipid peroxidation and 67.81% and 79.80% for Iron sulphate induced lipid peroxidation respectively.

***In vivo* antihyperlipidemic effect**

**Effect of EESC on serum lipid profile**

The effect of EESC on serum lipid profile in Poloxamer 407 induced hyperlipidemic rats is depicted in table 1. After induction of hyperlipidemia (i.e. 24hr and 48hr after Poloxamer 407 administration) there was significant increase in serum TC, TG, LDL and VLDL levels when compared to basal values i.e. 0hr values. The peak increase in these levels was observed 24hr post induction with Poloxamer 407 administration. Administration of EESC showed significant ($p<0.01$) and dose dependent reduction in total cholesterol (183.2 ±5.50), Triglycerides (145.9±3.46), LDL (135.3 ±2.69) and VLDL (29.21 ±0.71) levels after 48 hr. whereas the HDL levels were found to increase in treatment group as compared to disease control group. The highest dose i.e. 400mg/kg EESC was found to be most effective. The atorvastatin (10mg/kg) was used as standard drug in the entire study.

**Effect of EESC on hepatic cholesterol and triglycerides**

EESC treatment (100/200/400mg/kg, *p.o.*) in Poloxamer -407 induced hyperlipidemic animals showed significant ($p<0.01$) inhibition of hepatic cholesterol and hepatic triglyceride when compared with diseases control group in a dose dependent manner as shown in Table 2. Hepatic total cholesterol was decreased by 93.36 and 96.74% by 400mg/kg EESC and Atorvastatin respectively. Hepatic triglycerides were decreased by 81.86% and 94.95% by 400mg/kg EESC and Atorvastatin respectively.

**Effect of EESC on liver enzyme assay**

**Lipid Peroxidation**

Treatment with EESC (100/200/400mg/kg, *p.o.*) significantly inhibited Poloxamer-407 induced elevation of tissue malondialdehyde in dose dependent manner (Table 2). EESC at a dose of 100mg/kg, 200mg/kg, 400mg/kg and atorvastatin 10mg/kg showed 26.02%, 70.93%, 87.63% and 91.19% inhibition in malondialdehyde respectively when compared with disease control group.

**Reduced Glutathione and Catalase**

Treatment with EESC (100/200/400mg/kg, *p.o.*) significantly elevated Poloxamer-407 induced depletion of tissue reduced glutathione and catalase in dose dependent manner (Table 2). EESC at a dose of 100mg/kg, 200mg/kg, 400mg/kg and atorvastatin 10mg/kg showed 17.91%, 56.71%, 76.12% and 95.52% elevation in reduced glutathione and 17.52%, 40.59% and 59.05% elevation in catalase levels respectively when compared with disease control group.

**Atherogenic Indices**

Atherogenic indices are powerful indicators of the risk of heart diseases. Higher the value, higher is the risk of developing cardiovascular disease and *vice versa*. The administration of EESC significantly reduced these indices the as compared to disease control group in dose dependent manner. The highest dose was found to be most effective as shown in Table 3.

**DISCUSSION**

Hyperlipidemia is the major risk factors for the premature development of cardiovascular disease like atherosclerosis, hypertension, coronary heart disease etc. It is also an important risk factor in the initiation and progression of atherosclerotic impasse. Several studies reveal that an increase in HDL cholesterol and decrease in TC, LDL cholesterol and TG is associated with a decrease in the risk of ischemic heart diseases (Ghule BV *et al*., 2009). In the present study we have investigated *in vitro* antioxidant activity of EESC by DPPH radical scavenging and TBARS assay. Also the effect of EESC on serum lipid profiles, hepatic TC, TG, enzyme levels (reduced glutathione, lipid peroxidation, and catalase) and atherogenic indices was evaluated in Poloxamer-407 induced hyperlipidemic rats.

Oxidation is one of the destructive processes in which it breaks down and damages the various molecules. Oxidative stress is a causative factor that links hyperlipidemia with pathogenesis of atherosclerosis (Yang X, 2010). Hypercholesterolemia leads to increased production of oxygen free radical i.e. reactive oxygen species (ROS). These attack and induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA (Rajani GP & Ashok P, 2009). ROS exerts its cytotoxic effect by causing oxidation of lipids i.e. lipid peroxidation which results in formation of MDA. Lipid peroxidation is crucial step in pathogenesis of several diseases like atherosclerosis, inflammatory bowel diseases and others (Mylonas C, Kouretas D, 1998). Decrease in lipid peroxidation leads to reduction in arterial wall cholesterol content and thus decrease in atherosclerosis caused by hyperlipidemia. Determination of lipid peroxidation by TBARS assay provides an indirect measurement of antioxidative deficit (Oh PS *et al*., 2006). The antioxidant activity of EESC was evaluated by both DPPH and TBARS assay. From the results of both these assays, it seems that EESC possesses radical scavenging, antioxidant activity. Further EESC also decreased the extent of lipid peroxidation and increased the levels of antioxidant enzymes i.e. catalase and reduced glutathione in liver tissue in dose dependant manner.

The *in vivo* antihyperlipidemic activity was evaluated on Poloxamer 407 induced hyperlipidemic rats. Poloxamer 407 is a nonionic surfactant and is nontoxic to cellular membranes, was used successfully to induce

hyperlipidemia in previous studies. It causes these effects by activating HMG- CoA and inhibiting lipoprotein lipase activity (Subramaniam S *et al*., 2011). Poloxamer 407 has been utilized in the hyperlipidemic model due to its convenience, reproducibility, and lack of undesirable underlying pathological conditions (Kim HY *et al*., 2008). Also this model has shown relatively low species differences between experimental animals (Subramaniam S *et al*., 2011). Therefore this model has chosen for evaluating antihyperlipidemic activity of EESC with its three dose levels i.e. 100, 200, 400mg/kg of body weight.

The result of the *in vivo* study revealed that EESC when administered orally to the poloxamer induced hyperlipidemic rats causes sharper and more significant decrease in TC, TG, LDL and VLDL levels. Also it showed significant increase in total cholesterol levels of experimental group on both $24^{th}$ and $48^{th}$ hr after poloxamer i.p. injection. Atorvastatin has also been included in the study in order to understand how far EESC's activity is comparable to that of standard drug. Highest dose of EESC was found to be most effective.

EESC also decreased atherogenic indices which are Atherogenic coefficient, Cardiac risk ratio and Atherosclerosis index. Atherogenic indices are powerful indicators of the risk of heart disease: the higher the value, the higher the risk of developing cardiovascular disease and *vice versa*. In this study, we observed that the EESC significantly reduced atherogenic indices. According to Usoro *et al*. lower atherogenic index is protective against coronary heart disease (Ikewuch CJ, Ikewuchi CC, 2010).

## CONCLUSION

EESC lowered serum and liver TC and TG content, lowered serum LDL, VLDL and increased HDL levels, inhibited formation of lipid peroxidation product, increased antioxidant activity, lowered atherogenic indices thereby repressed development of atherosclerosis and other cardiovascular diseases. The current study indicates that EESC possesses hypolipidemic and antioxidant potential and these beneficial effects may be because of its phytochemical constituents including Mangiferin and Swertiamarin.

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