ISSN: 0975-8232

IJPSR (2010), Vol. 1, Issue 7 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received 23 March, 2010; received in revised form 02 June, 2010; accepted 14 June, 2010

BLOOD CHOLESTEROL LOWERING EFFECT OF *ADENANTHERA PAVONINA* SEED EXTRACT ON ATHEROGENIC DIET INDUCED HYPERLIPIDEMIA RATS

Vettae Gounder Maruthappan *[1] and Kumaravelu Sakthi Shree [2]

Unit of Endocrinology, Department of Zoology, Bharathiar University[1], Coimbatore, India
PG and Research Centre, Department of Zoology, Govt. Arts College (Autonomous)[2], Coimbatore, India

Keywords:

Adenanthera pavonina,
atherogenic diet,
Blood cholesterol,
Histopathology,
Biochemistry

Correspondence to author:

Vettae Gounder Maruthappan

Unit of Endocrinology
Department of Zoology
Bharathiar Univeristy
Coimbatore (TN) – 641 046
India

Email: zoomaruthu@yahoo.co.in

ABSTRACT

The effect of *Adenanthera pavonina* (AP) seed extract on the blood cholesterol level of atherogenic diet rats was evaluated. Twelve adult male Wistar rats divided into 3 groups of 4 animals each were used and were treated orally with normal saline (Control group), atherogenic diet (Positive control) and 200mg/kg of AP seed extract (treatment) over a 4-week period. The present study was undertaken to assess body weight, total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), high density lipoprotein (HDL) and very low density lipoprotein (VLDL). They were significantly lower in the extract treated groups compared to the control group. Phytochemical screening showed that the extract contained cardiac glycosides, tannins, saponins, alkaloids and flavonoids. Cyanogentic glycosides and anthraquinones were absent. Histopathological examination showed that the extract did not cause any significant lesion changes in the liver, kidney and even the testes. The study showed that *Adenanthera pavonina* (AP) seed extract have the potential to cause a blood cholesterol lowering effect. The serum biochemistry changes may suggest that the extract has a tonic effect on the kidneys and the liver and these organs play central role in drug metabolism. Absence of significant lesion in the kidney, liver and testes may indicate that the plant is safe for medicinal use.

INTRODUCTION: Hyperlipidemia is the most prevalent indicator for susceptibility to atherosclerotic heart disease. It is characterized by abnormally elevated lipid (triglyceride and cholesterol) and lipoprotein (LDL-c, VLDL-c) levels in the blood [1]. Hypertension or high blood pressure is a common disorder which if not effectively treated, results in a greatly increased probability of coronary thrombosis, strokes and renal failure [2]. This is supported by an abundance of congruent result from genetic, epidemiological, experimental animal studies and clinical trials that the presence of high plasma lipid cholesterol increases the incidence of coronary heart diseases (CHD) [3]. Treatment of blood cholesterol reduces cardiovascular risk and this has been a major focus of campaigns aimed at reducing cardiovascular mortality and morbidity [4].

Adenanthera pavonina is endemic to Southeast China and India, with first reports being recorded in India. The tree has been introduced throughout the humid tropics. It has become naturalized in Malaysia, Western and Eastern Africa and most island nations of both the Pacific and the Caribbean. It belongs to the family Leguminosae and subfamily Momosoideae [5]. In terms of medicinal uses, the seeds have been found to be effective in treating cardiovascular diseases in pregnancy. The ground seeds are used to treat boils and inflammatory reactions. Decoction of leaves is used to treat gout and rheumatism [6].

A methanol extract of seeds *Adenanthera pavonina* was evaluated for pharmacological effects in animal models. The study demonstrated the blood cholesterol lowering effect of *Adenanthera pavonina.* Acute toxicity studies revealed that the extract produced reduced motor activity. The LD_{50} value of the extract was found to be 1.40 g/kg.[7] Studies have reported that presence of cardiac glycosides in the methanol extract of *Adenanthera pavonina* seeds [7], although there is no known study on the cardiovascular effect of the extract. This study is therefore designed to determine the presence of cardiac glycosides in *Adenanthera pavonina*, hence assess the phytochemical constituents of the seeds of this plant, evaluated the effect of the methanol extract obtained from the seeds on the blood cholesterol and then assesses the safety or otherwise of this extract in experimental animals.

EXPERIMENTAL METHODS:

Collection and authentication of plant material: The seeds of *Adenanthera pavonina* were collected from the Bharathiar University compound, Coimbatore, India. They were authenticated at the Taxonomist of Department of Botany, Bharathiar University, where the voucher specimen was deposited at the herbarium.

Sample preparation and extraction: Seeds were air dried and powdered with an electronic blender. Sample was put in a conical flask and then one liter of methanol was added. It was stirred continuously and was kept for about 72 hours. It was ten filtered and the filtrate was dried with a rotatory evaporator to yield 3.95% of the extract.

Phytochemical screening: Standard phytochemical methods were used to test for the presence of saponins, alkaloids, tannins, anthraquinones, cardiac glycosides, cyanogenetic glycosides and flavonoids [8, 9, 10, 11, 12].

Saponins: About 1 g of the powdered sample was boiled with 10 ml of distilled water for 10 minutes. The samples were filtered while hot, cooled and the following tests were carried out.

- **Frothing**: 2.5 ml of the filtrate was diluted to 10 mLs with water and was shaken vigorously for 2 minutes. Frothing observed indicates a positive test.
- **Emulsification**: 2.5 ml of the filtrate was shaken vigorously for 2 minutes with a few drops of olive oil. An emulsified layer indicates a positive test.

Alkaloids: About one gram of the powdered sample was extracted with 10 ml of 10% hydrochloric acid by boiling for five minutes on a water bath. The extract was filtered and the pH of the filtrate was adjusted to about 6 by adding a few drops of dilute ammonia solution and tested with litmus paper after which few drops of Dragendorff's, Mayer's and Wagner's reagent were added separately to aliquots of the filtrate in the test tubes. A reddish brown, cream and reddish brown precipitate respectively indicates a positive test.

Tannins: About 1 g of the powdered sample was boiled with 10 ml of the distilled water for five minutes, filtered while hot and a few drops of ferric chloride reagent was added to the filtrate. A red coloration indicates a positive test.

Anthraquinones: 1 g of the powdered sample was extracted with 10 ml of 10% sulphuric acid, containing traces of ferric chloride solution for 15 minutes. It was filtered while hot and the cooled filtrate was extracted while hot with 2 equal volumes of chloroform. The presence of a rose pink color in the aqueous layer indicates a positive test.

Cardiac Glycosides: 1 g of the powdered sample was extracted with 10 ml of 80% alcohol for 5 minutes on a water bath; the extract was filtered and diluted with an equal volume of distilled water. A few drops of lead acetate solution was added, shaken, and filtered after standing for a few minutes. The filtrate was extracted with aliquots of chloroform. The combined chloroform extracts were divided into two portions and Keller Killiani and Kedde tests were carried out on them.

Keller Killiani test: The extract was evaporated to dryness and 3 ml of ferric chloride reagent was added to the cooled residue in a clean test tube. 2 ml of concentrated sulphuric acid was gently poured down the side of the test tube. A purple or reddish brown ring at the interface and green color in acetic acid layer indicates a positive test for 2-de-oxy sugar.

Kedde test: The dry residue was mixed with 1 ml of 2% 3, 5- dinitrobenzoic acid in ethanol. The solution was made alkaline with 5% sodium hydroxide. A brown purple color indicates a positive test for the presence of unsaturated lactone ring.

Cyanogenetic glycosides: 0.5 g of the powdered sample was placed in three different test tubes A, B, C. Test tubes A and B were mixed with water with a suspended moist sodium picrate paper in the neck of the tube, trapped by means of cork. Test tubes B and C were kept at room temperature while test tube A was placed on a boiling water bath. At the end of about half an hour, a change in color of the sodium picrate paper indicates a positive test.

Flavonoids: A small quantity of the extract was dissolved in dilute sodium hydroxide and hydrochloric acid was added to the mixture. A yellow solution that turns colorless on addition of hydrochloric acid indicates the presence of flavonoids.

Anticholesterolemic effects of *Adenanthera pavonina* in rats: The animals used in this study were 12 male Wistar rats weighing between 200-250gms. They were maintained in the laboratory, Department of Zoology, Bharathiar University, Coimbatore, India. They were kept in rat cages and fed commercial rat feeds (M/s. Hindustan Lever Ltd., Bangalore) and allowed free access to clean fresh water in bottles *ad libitum*. The twelve animals were divided into 3 groups of 4 animals per group. While group A rats served as normal control, groups B served as positive control and C animals were administered with 200 mg/kg doses of the extract. The extract and distilled water were administered orally to the animals once daily for 28 days using stomach cannula. The animal experiments were approved by animal ethical committee of institute (722/02/a/CPCSEA dt. 04.12.2006).

Safety evaluation of the seeds of Adenanthera pavonina rats: The animals used in this study were 20 male Wistar rats weighing between 240-280gms. They were maintained in the laboratory, Department of Zoology, Bharathiar University, Coimbatore, India. They were kept in rat cages and fed commercial rat feeds (M/s. Hindustan Lever Ltd., Bangalore) and allowed free access to clean fresh water in bottles *ad libitum*. The twenty animals were divided into 4 groups of 5 animals per group. While group A rats served as control and received distillated water, groups B rats served as positive control and received atherogenic diet and C animals were administered with 200 mg/kg doses of the methanol extract. The extract was administered to the rats per once daily using stomach cannula for 21 days. The animal experiments were approved by animal ethical committee of institute (722/02/a/CPCSEA dated 04.12.2006).

Technique for Obtaining Serum Samples: Blood samples were collected by cervical decapitation from diethyl ether anaesthetized rats into clean non- heparinized bottles and allowed to clot. The serum was separated from the clot and centrifuged according to groups into clean bottles for biochemical analysis.

Determination of serum biochemical parameters: Serum total protein was measured using Lowry *et al*., method [13], total cholesterol and triglyceride was estimated by the method of CHOD-PAP and high density lipoprotein by the method of GPO-PAP. Low density and very low density cholesterol were calculated by using Friedwald formula.

Histopathology: The liver, kidney and testes of all the animals were fixed in 10% buffered formalin in labeled bottles. Tissues were processed routinely and embedded in paraffin wax. Sections 5 µm thick were cut, stained with haematoxylin and eosin and examined under the light microscope.

Statistical analysis: Results were subjected to the student's t-test and were considered significant at $P<0.05$ [14].

Results and Discussion: The phytochemical screening of the methanol extract of the seeds

of *Adenanthera pavonina* showed the presence of the following: tannins, alkaloids, saponins, flavonoids, and cardiac glycosides. The results however showed that anthraquinone and cyanogenetic glycosides were absent (Table 1). The results thus showed that the methanol extract of the seeds of *Adenanthera pavonina* is rich in cardiac glycosides, alkaloids, saponins, tannins, and flavonoids. Alkaloids usually have marked physiological action on animals. Saponins on the other hand are of great pharmaceutical importance because of their relationship to compounds such as the sex hormones, cortisones, diuretic steroids, vitamin D and cardiac glycosides.

TABLE 1: PHYTOCHEMICAL SCREENING OF THE POWDERED SAMPLES OF THE SEEDS OF *ADENANTHERA PAVONINA*

TEST	OBSERVATION	INFERENCE
Test for saponins		
• Frothing	Frothing was observed.	Presence of saponins suspected (+)
• Emulsification	The formation of emulsified layer was observed.	Presence of saponins confirmed (+)
Test for alkaloids		
• Mayer's reagents	A cream precipitate was observed.	Alkaloids present (+)
• Dragendorff's reagent	A reddish-brown precipitate was observed.	Alkaloids present (+++)
• Wagner's reagent	A reddish-brown precipitate was observed.	Alkaloids present (+)
Test for tannins		
• A red color was observed	A red color was observed	Tannins present (+)
Test for anthraquinones	Absence of a rose color in the aqueous layer was observed.	Anthraquinone absence (-)
Test for cardiac glycosides		
• Keller-Killiani	A reddish-brown ring was observed at the interphase and a green color was observed in the acetic layer.	Cardiac glycosides present (+)
• Kedde test	A brown purple color was observed.	Cardiac glycosides present (+)
Test for Cyanogenetic glycosides	There was no change in color of the sodium	Cyanogenetic glycosides absent (-)
Test for flavonoids	A yellow solution that turned colorless on addition of hydrochloric acid was observed	Flavonoids present (+)

+ = present, +++ + = high concentration, - = absent

Tannins like alkaloids are substances which show protein precipitation and are related to the physiological effects of herbal medicines. Flavonoid containing plants have influence on arachidonic acid metabolism, thus could have anti-inflammatory, antiallergic, antithrombotic or vasoprotective effects. Cardiac glycosides are steroidal glycosides which exert a slowing and strengthen effect on the failing heart [15]. The implication of all these is that this plant is of great medicinal importance. The presence of cardiac glycosides in this plant has further confirmed its medicinal use as antihyperlipidemic agent. The presence of flavonoids in this plant is therefore evidenced of its anti-inflammatory properties. This therefore supports the earlier work of Olajide *et al* [7] that the plant has anti-inflammatory property.

The results of the effect of the methanol extract of the seeds of *Adenanthera pavonina* on the

blood cholesterol lowering effect of Wistar rats showed that the extract caused significant reduction of the mean body weight when compared with those of the control (Table 2). The study showed that the anti-hypercholesterolemia effect of this extract on the mean body weight is comparable to that of extract, an adrenergic antagonist, though at a very high dose of the extract.

The extract efficacy in treating hypercholesterolemia as well as most of its toxic effects results from nonselective beta blockade. It decreases blood cholesterol level as a result of decrease in cardiac output [16]. The presence of cardiac glycoside in this plant known to slow and strengthen a failing heart may have accounted for its antihypercholesterolemia effect. Besides, it is known that tannins have diuretic effect hence its presence in this plant may have contributed to the antihypercholesterolemic effect [16].

TABLE: 2 THE ANTICHOLESTEROLEMIC EFFECTS OF THE METHANOL EXTRACT OF THE SEEDS OF *ADENANTHERA PAVONINA*

GROUPS	BODY WEIGHT IN GM COMPARED WITH WEIGHT FIRST DAY
Normal Control (Saline)	+11.24
Positive Control (Atherogenic diet)	+46.23
Methanol extract of the seeds of *Adenanthera* (200mg/kg body weight)	- 21.72

Values are mean ± SD of six rats from each group

The results of the safety evaluation of the methanol extract of the seeds of *Adenanthera pavonina* on rats showed that the extract caused significant increase in the levels of total protein and globulin (Table 3). It is inferred that alteration in the plasma total protein is most often due to decrease in the quantity of albumin, which may be accompanied by a relative hyperglobulinaemia [17, 18]. In this study, all protein experienced an increase, suggesting that there is increased protein synthesis or mobilization. The increase in globulin level may indicate that the plant could have the potential to stimulate immune response by increasing antibody production (immunoglobins) [19], and this also could be responsible for its use as a medicinal plant.

Diseases associated with high TG levels (Diabetes mellitus, obesity, chronic renal disease, primary hyperlipoproteinemia) carry high risk of cardiovascular disorder (CVD) [20]. Hypercholesterolemia is reportedly the major risk factors in life style related diseases such atherosclerosis and related cardiovascular complications including cerebral paralysis and myocardial infarction [21].

The results of the safety evaluation of the methanol extract of the seeds of *Adenanthera pavonina* on rats showed that the extract caused significant decrease in the levels of serum cholesterol and triglyceride levels. It is shown that atherogenic diet elevates serum triglyceride levels essentially by preventing its uptake and clearance by inhibiting catabolizing enzymes like lipoprotein lipase (LPL) and lecithin cholesterol acetyl transferase (LCAT) [22].

TABLE 3: EFFECTS OF METHANOL EXTRACT OF THE SEEDS OF *ADENANTHERA PAVONINA* ON THE SERUM CHEMISTRY OF RATS

PARAMETERS	NORMAL CONTROL	POSITIVE CONTROL	200 MG/KG BODY WEIGHT
Total protein (g/dL)	6.0±0.40	8.2±0.34	7.4±0.26*
Albumin (g/dL)	4.6±0.30	6.3±0.20	5.3±0.42*
Globulin (g/dL)	3.5±0.20	5.7±0.12	4.5±0.35*
Total cholesterol (g/dL)	36.2±0.35	48.4±0.26	40.5±0.31*
Triglycerides (g/dL)	25.1±0.92	37.3±0.45	30.4±0.83*
HDL-c (g/dL)	16.12±0.98	18.64±0.96	17.79±0.45*
LDL-c (g/dL)	28.14±0.12	56.74±1.58	43.31±1.64*
VLDL-c (g/dL)	7.78+1.35	14.87+1.45	11.56+0.01*

Values are mean ± SD of six rats from each group; * $P<0.05$ show significant when compare with positive control

Diseases associated with high TG levels (Diabetes mellitus, obesity, chronic renal disease, primary hyperlipoproteinemia) carry high risk of cardiovascular disorder (CVD) [20]. Hypercholesterolemia is reportedly the major risk factors in life style related diseases such atherosclerosis and related cardiovascular complications including cerebral paralysis and myocardial infarction [21]. The results of the safety evaluation of the methanol extract of the seeds of *Adenanthera pavonina* on rats showed that the extract caused significant decrease in the levels of serum cholesterol and triglyceride levels.

It is shown that atherogenic diet elevates serum triglyceride levels essentially by preventing its uptake and clearance by inhibiting catabolising enzymes like lipoprotein lipase (LPL) and lecithin cholesterol acetyl transferase (LCAT) [22]. Apparently the methanol extract of the seeds of *Adenanthera pavonina* is able to reduce the inhibition on LPL and LCAT activity making triglycerides available for uptake and metabolism by tissues. It is clear that the aqueous extract of the seeds of *Adenanthera pavonina* is relatively more anti-hypercholesterolemic than antitriglyceridemic. Atherogenic diet induced hypercholesterolemia has been related to its ability to alter the physico-chemical properties of lipoproteins and thereby prevent their uptake by liver for clearance [23]. The methanol extract of the seeds of *Adenanthera pavonina* has been shown to facilitate catabolism of LDL through its hepatic receptors in atherogenic diet treated rats.

The methanol extract of the seeds of *Adenanthera pavonina* is effective in minimizing atherogenic diet induced increase in HDL-c as well as decrease in non-HDL-c (LDL + VLDL) suggesting promotion of decreased catabolism of non-HDL-c by the hepatic tissue. Similar conclusion has also been drawn by Pande and Sonal Dubey[2] [4] in their study on hypocholesteremic activity of *Sphaeranthus indicus* on atherogenic diet induced hyperlipidemia. The hypolipidemic efficacy of the methanol extract of the seeds of *Adenanthera pavonina* is also substantiated by the calculated cardiovascular risk factor.

Histopathological changes due to the methanol extract of the seeds of *Adenanthera pavonina* was also embarked upon. Liver, kidneys, and testes from the experimental animals were harvested and processed for histology. The results showed that in all the organs examined none showed any visible lesion indicating that the doses used were not high enough to cause any toxic change. This may then indicate that this plant is relatively safe for use as a medicinal herb.

REFERENCES:

1. Anuradha CV and Ravikumar P: Restoration on tissue antioxidants by fenugreek seeds (*Trigonella Foenum Graecum*) in alloxan-diabetic rats. Indian J Physiol Pharmacol 2001; 45:408-420.
2. Rang HP, Dale M .M and Ritter J.M: Pharmacology, 4th edition Churchill Livingstone, London, 1999.
3. Bopanna KN, Bhagyalakshmi N, Rathod SP, Balaraman R and Kannan J: Cell culture derived *Hemidesmus indicus* in the prevention of hypercholesterolemia in normal and hyperlipidemic rats. Ind J Pharmacol 1997; 29:105-109.
4. Elsik M and Krum H: Hypertension: How long to go? Australian Prescriber, 2007; 30(1):6.
5. Balogun AM and Fetuga BL: Fatty acid composition of seed oils of some members of the Leguminosae family. Food Chem 1985; 17(3):175-182.
6. Burkill HM: The useful plants of West Tropical Africa. Vol. 2. Royal Botanical Gardens, Kew, 1994.
7. Olajide OA, Echianu CA, Adedapo ADA and Makinde J.M: Anti-inflammatory studies on *Adenanthera pavonina* seed extract. Inflammopharmacol, 2004;12(2):196-202
8. Earl JK and Warren MS: Chemical composition of plant tissue. Biochemists Handbook. Redwood Press, London, 1961.
9. John W: Alkaloid survey. Encyclopaedia of Chemical Technology. University Press, New York 1963.
10. Felgils F: Anthraquinone, Ascorbic acid. Stop tests in organic analysis. Elsevier Press, Amsterdam, 1975.
11. Evans WC: Trease and Evans Text book of Pharmacognosy. Cambridge University Press, London, 1989.
12. Ghani A: Medicinal plants of Bangladesh. The Asiatic Society of Bangladesh, Dhaka, Bangladesh, 1998.
13. Lowry OH, Rosebrough NJ, Farr AL and Randall RI: Protein measurement with folin-phenol reagent. J Biol Chem, 1951; 193:265-275.
14. Essex-Sorlie D: Medical Biostatistics and epidemiology. 1st ed., Lange Medical Books, 1995.
15. Evans WC: Trease and Evan Pharmacognosy. 15th ed., W.B. Saunders Co. Ltd. Philadelphia, 2002.
16. Katzung BG: Basic and clinical pharmacology. 9th ed., the McGraw- Hill Companies, Inc, 2004.
17. Duncan JR, Praise KW and Mahaffey EA: Veterinary Laboratory Medicine (Clinical Pathology) Lowa State University Press, Ames, 1994.
18. Coles EH: Veterinary Clinical Pathology, 4th ed., W.B. Saunders Company, Philadelphia, 1986.
19. Puri A, Saxena R, Saxena RP and Saxena KC: Immunostimulant agents from *Andrographis paniculata*. J Natural Prod, 1993; 56(7):995-999.
20. Ford ES, Giles WH and Dietz WH: Prevalence of the metabolic syndrome among US adults: Findings from the third National Health and Nutrition Examination Survey. JAMA 2002; 287:356-359.
21. Marie C, Vladimir: Effects of high-fat and *Chlorella vulgaris* feeding on changes in lipid metabolism in mice. Biologia Bratislava, 2001; 56:661.
22. Campillo JE, Torres MD, Dominguez E, Romeroa A and Perrez C: *Ficus carica* lead administration reduces hypertriglyceridemia in streptozotocin diabetic rats. Diabetologia, 1994; 37; 213.
23. Byers SO, Friedman M and Sugiyama T: Triton hypercholesterolemia cause or consequence of augmented cholesterol synthesis. Am J Physiol, 1963; 204:1100-1102
24. Pande VV and Sonal Dubey. Antihyperlipidemic activity of *Sphaeranthus indicus* on atherogenic diet induced hyperlipidemia in rats. Int J Green Pharmacy 2009; 2:159-161.