

## Screening of Antihyperlipidemic Activity of Flavonoid Obtained From Leaves of *Andrographis Paniculata*

Nagarathana P.K.M.<sup>1\*</sup>, Sahu Satya Narayan<sup>2</sup>, Sridhar Bommineni<sup>3</sup> and Sah Dipendra Kumar<sup>4</sup>

<sup>1,2,3,4</sup>Karnataka College of Pharmacy, Thirumenahalli, Bangalore, Karnataka, India

### Abstract

**AIM:**The aim this study was to evaluate the anti-hyperlipidemic activity of leaves of *Andrographis paniculata* in rats.

**METHOD:** Hypercholestermia was induced by giving coconut oil 25%, cholesterol 1%, and cholic acid 0.5%. Theatherogenic diet and the treatment were given simultaneously for 26 days. On the 27th day, blood was collected by retro orbital puncture for the analysis of serum triglycerides, cholesterol, HDL, VLDL and glucose. Rats were treated with flavonoid of *Andrographis paniculata* (100, 200 mg/kg;po;/day/26 days) and atorvastatin(10 mg/kg;ip;day/30days) which served as standarddrug.

**RESULT:***Andrographis paniculata* treated group showed significant decrease in LDL-cholesterol, total cholesterol, triglycerides, AST, ALT, ALP and increase in HDL-cholesterol, Albuminandtotalprotein.

**CONCLUSION:** From result, it was concluded that flavonoid of leaves of *Andrographis paniculata* shows antihyperlipidemic activity.

### Keywords

*Antihyperlipidemic, Andrographis paniculata, Isoproterenol, Cholesterol*



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## INTRODUCTION

Atherosclerosis is a disease of the vessel wall involving lipid accumulation, chronic inflammation, cell death, and thrombosis that causes heart disease and stroke. The WHO has estimates that perhaps 80 % of world population rely upon traditional medicine of their primary health care needs, and a major part of this therapy involves the use of the plant extracts or their active principle<sup>1</sup>. Ayurvedic diagnostic methods of these diseases and treatment also did not progress as well as in conventional medicine. Allopathic hypocholesterolic drugs are available at large in the market but the side effects and contraindications of these drugs have marred their popularity. Recently herbal hypolipidaemics have gained importance to fill the lacunae created by the allopathic drugs<sup>2</sup>. The use of traditional medicine is widespread and plants still present a large source of novel active biological compounds with different activities, including anti-inflammatory, anti-cancer, anti-viral, antibacterial and cardioprotective activities. Herbal drugs such as *Allium sativum*, *Commiphora mukul*, *Boswellia serrata*, *Emblica officinalis*, *Terminalia arjuna*, *Trigonella foenum-graecum*, *Ocimum sanctum*, *Withania*

*somnifera* and *Zingiber officinale* hold great promise for development and use as novel strategies in prevention and treatment of atherosclerosis. Antioxidants are substances that delay the oxidation process, inhibiting the polymerization chain initiated by free radicals and other subsequent oxidizing reactions<sup>3</sup>. They provide protection against chronic diseases, including cancer and neurodegenerative diseases, inflammation and cardiovascular disease<sup>4</sup>. Adverse conditions within the environment, such as smog and UV radiation, in addition to diets rich in saturated fatty acids and carbohydrates, increase oxidative damage in the body. Antioxidants may play a role in these health promoting activities<sup>5</sup>. The numerous beneficial effects attributed to phenolic products<sup>6</sup> have given rise to a new interest in finding botanical species with high phenolic content and relevant biological activity.

*Andrographis paniculata* (family, Acanthaceae) grows widely in many Asian countries, such as China, India, Thailand and Sri Lanka and has a long history of therapeutic usage in Indian and Oriental medicine<sup>7,8</sup>. The herb is official in Indian Pharmacopoeia<sup>9</sup> as a predominant

constituent of at least 26 Ayurvedic formulations.

Previous investigations on the chemical composition of this well studied herb showed that it is a rich source of 20 - oxygenated flavonoids<sup>10,11,12,13</sup>.

The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences. For instance: flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and they showed their antiallergic, anti-inflammatory, anti-microbial and anti-cancer activities<sup>14</sup>

## MATERIALS AND METHODS

### Collection of plant materials

The leaves of *Andrographis paniculata* were collected from Thirupati forest region Thirupati District, Andhra Pradesh, INDIA in the month of July 2011. This plant species were authenticated by Prof. M. Madhava Chetty, Botanist, Department of Pharmacognosy and Photochemistry (Padmavathi mahila kalasala) the voucher specimen was deposited in the institutional museum.

The collected plant material was washed thoroughly with water to remove the adhering soil, mud, and debris. All old insect damage or fungus infected leaves, and flowers were removed. The leaves were dried in the shade at room temperature to a constant mass. The plant material was coarsely powdered into coarse powder a warring blender. The powder was stored in an airtight container and protected from light.

### Preparation of Extract

Powdered leaves, 100gm, were subjected to successive extraction in a soxhlet extractor using methyl alcohol. The extract obtained was concentrated in a rotary shaker and evaporated to dryness to get constant weight.

### Animals

In-house laboratory bred healthy male albino rats of Wistar strain weighing 150-220g were included for the study. Animals were housed in polypropylene cages on clean paddy husk bedding. Animals were maintained under controlled temperature at  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  with 12hr light/dark cycle. All animals had free access to food and water ad libitum.

## EXPERIMENTAL DESIGN

Male-Wistar rats were divided into the 5 groups each group have six animals.

Group1: Normal control

Group2: Hyperlipidemic control

Group3: Animals are treated with standard drug (atorvastatin) (10 mg/kg)

Group4: Animal are treated with test drug (100mg/kg)

Group5: Animal are rats treated with test drug (200mg/kg)

Atherogenic diet was suspended in coconut oil 25%, cholesterol 1%, and cholic acid 0.5%. The atherogenic diet and the treatment were given simultaneously for 26 days. On the 27<sup>th</sup> day, blood was collected by retro orbital puncture for the analysis of serum levels of total proteins, albumin, creatinine, glucose, urea, AST, ALT, Total cholesterol, HDL-C, LDL-C, VLDL-C and triglycerides. Body weight, food efficiency, liver weight and abdominal and liver fat of the rats<sup>15</sup>.

Statistical analysis were carried out by one way ANOVA method.

## RESULTS

In cholesterol diet treated group there was significant decrease in total protein, HDL-cholesterol, albumin levels ( $P \leq 0.001$ ) when compared to the control group.

Atorvastatin along with cholesterol diet treated group showed significant increase in total protein, HDL-cholesterol, albumin levels, ( $P \leq 0.01$ ) when compared to the cholesterol diet group.

Rats treated with cholesterol along with flavonoid of leaves of *Andrographis paniculata* (100mg/kg) showed significant increase in total protein, HDL-cholesterol, albumin levels ( $P \leq 0.05$ ) when compared to standard diet group.

Rats treated with cholesterol along with flavanoid of leaves of *Andrographis paniculata* (200mg/kg) shown significant increase in total protein, HDL-cholesterol, albumin levels ( $P \leq 0.001$ ) when compared to standard group.

In high cholesterol diet treated group there was significant increase in triglycerides level, LDL-cholesterol levels, VLDL-cholesterol levels, total-cholesterol levels, AST levels, ALT levels ( $P < 0.01$ ) when compared to the control group.

Standard group i.e., Atorvastatin and high cholesterol diet treated group showed significant decrease ( $P < 0.01$ ) in triglycerides, LDL-cholesterol levels, VLDL-cholesterol levels, total-cholesterol levels, AST levels, ALT levels when compared to high cholesterol diet group.

All rats treated with high cholesterol diet and flavonoid of leaves of *Andrographis paniculata* (100mg/kg, b.w. p.o.), respectively showed significant decrease ( $P<0.01$ ) in triglycerides, LDL-cholesterol levels, VLDL-cholesterol levels, total-cholesterol levels, AST levels, ALT levels when compared with standard group.

All rats treated with high cholesterol diet and flavonoid of leaves of *Andrographis paniculata* (200mg/kg, b.w. p.o) respectively showed significant decrease ( $P<0.05$ ) in triglycerides, LDL-cholesterol levels, VLDL-cholesterol levels, total-cholesterol levels, AST levels, ALT levels when compared with standard group. (Table 1)

**Table 1** Effect of flavonoid of leaves of *Andrographis paniculata* on plasma total cholesterol, TG, Total protein, Albumin, LDL, VLDL, HDL, AST, ALT levels in cholesterol diet (CD) induced hypercholesterolemia in rats

Treatment group	TG	TP	ALB	TC	LDL	VLDL	HDL	AST	ALT
Normal control	72.98±0.344***	6.13±0.039*	4.63±0.00***	205.1±1.628**	180.2±1.56**	14.59±0.68*	51.57±0.64**	17.60±1.720	13.94±0.023* **
HCC	122.1±1.081***	3.190±0.27***	2.05±0.15***	265.5±1.120***	235.6±1.13**	24.42±0.21***	28.07±1.74** *	49.01±2.347	54.91±1.387* **
Atorvasta tin10mg/kg	79.55±1.135***	6.96±0.25***	3.51±0.11***	217.5±4.23***	191.2±4.07***	15.90±0.22***	44.87±1.83** *	29.90±1.44	31.16±0.641 2
AP 100mg/kg + HCD	94.21±1.127**	5.46±0.08***	2.76±0.05***	245.6±1.41***	219.5±1.48**	18.86±0.18***	35.75±0.6389***	40.63±1.53	51.30±0.7245***
AP 200mg/kg + HCD	84.87±1.056**	6.025±0.072**	4.29±0.09***	208.3±0.984*	181.6±1.53*	17.32±0.22***	40.40±0.215 9*	19.52±2.07	23.16±1.002* **

Data was analysed using one way ANOVA followed by Dunnett's t test. \*\*\* $P<0.001$ , \*\* $P<0.01$ , \* $P<0.05$ .n = 6

## DISCUSSION

In this study, rats fed with High Cholesterol Diet (cocktail) containing 700ml peanut oil and 300ml lard oil, 100g cholesterol, 30g propylthiouracil and 100g cholic acid was given orally 1ml/100g body weight Cholic acid acts by increasing cholesterol absorption by its emulsifying property and

concomitant suppression of cholesterol  $7\alpha$ -hydroxylase activity that results in decreased cholesterol excretion. The use of propylthio-uracil is to create hypothyroidism. Cholesterol feeding has been often used to elevate serum or tissue cholesterol levels to assess the hypercholesterolemia- related

metabolic disturbances in animals. Cholesterol feeding alone however does not affect the serum triglyceride (TG) level. It is assumed that a high level of saturated fat in addition to cholesterol is required to significantly elevate serum TG level in rat model. Rats are generally considered to be resistant to atherogenesis, although lesions have been produced by heroic measures. The high cholesterol diet (CD) was given for 26 days along with hydroalcoholic extract of leaves of *Andrographis paniculata*. Cholesterol diet (CD) treated group showed significant increase in total cholesterol, triglycerides, LDL-c ( $265.5 \pm 1.120$ ,  $122.1 \pm 1.081$ ,  $235.6 \pm 1.133$ ) levels respectively whereas HDL-c ( $28.07 \pm 1.742$ ) level showed significant decrease when compared to the control TG, TC, LDL-c ( $72.98 \pm 0.344$ ,  $205.1 \pm 1.628$ ,  $180.2 \pm 1.565$ ) group. On 27th day, atorvastatin treated group showed significant decrease in total cholesterol, triglycerides, LDL-c ( $217 \pm 4.24$ ,  $79.55 \pm 1.13$ ,  $191.2 \pm 4.07$ ) levels where as HDL-c ( $46.79 \pm 0.894$ ), level was significantly increased when compared to the CD treated group. On 27th day, rats treated with pure compound of leaves of *Andrographis paniculata* (100mg/kg, b.w. p.o) treated group showed significant

decrease in total cholesterol, triglycerides, LDL-c ( $245.6 \pm 1.41$ ,  $94.21 \pm 1.127$ ,  $219.5 \pm 1.48$ ) levels respectively whereas HDL-c ( $35.75 \pm 0.638$ ) level showed significant increase when compared to the CD ( $28.07 \pm 1.742$ ) treated group. And the results were comparable with that of the standard drug Atorvastatin. On 27th day, rats treated with high CD and flavonoid of leaves of *Andrographis paniculata* (200mg/kg, b.w. p.o) treated group showed significant decrease in total cholesterol, triglycerides, LDL-c ( $208.3 \pm 0.98$ ,  $84.87 \pm 1.056$ ,  $181.6 \pm 1.53$ ) levels respectively whereas HDL-c ( $40.40 \pm 0.21$ ) level was significantly increased when compared to the cholesterol diet treated group. And the results were comparable with that of the standard drug Atorvastatin. Histopathological study was done there was increase in the size of the tunica intima in all CD treated groups in aorta of all rats. There was reduction in the thickness of the wall of aorta in flavonoid of leaves of *Andrographis paniculata* (100gm/kg, 200mg/kg).

## CONCLUSION

From the above experimental studies it was concluded that *Andrographis paniculata* at

two different doses (100mg/kg and 200mg/kg) showed dose dependent anti-atherosclerotic activity.

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