International Journal of Pharmacology and Clinical Research (IJPCR)

IJPCR |Volume 3 | Issue 2 | July - Dec - 2019
www.ijpcr.net

Research article *Clinical research*

ISSN: 2521-2206

Evaluation of antihyperlipidemic activity of *achillea millefolium* flower extract on dexamethasone induced hyperlipidemia in rats

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ABSTRACT

The antihyperlipidemic activity in dexamethasone-induced hyperlipidemia in male Wistar rats, with the extracts of *Achillea millefolium.* The Extract showed to able to manage hyperlipidemia induced by high-fructose diet, reducing serum levels of total cholesterol and triglycerides, without signs may be indicated change in hepatic and renal function, suggesting that Extract is safe in the evaluated conditions. The hypolipidemic activity of natural products can be correlated to the presence of flavonoids due to their properties of inhibiting cholesterol biosynthesis and absorption and modifying the activity of lipogenic and lipolytic enzymes, leading to reduced lipid metabolism, as observed in hyperlipidemic rats treated with Extract which showed important reduction in the levels of total cholesterol and triglycerides. Other molecules clever to decrease the serum level of cholesterol are saponins, also present in Extract. It is very interesting that Extract was able to reduce both serum level of cholesterol and total triglycerides.

Keywords: Antihyperlipidemia, *Achillea millefolium*, Total cholesterol, Triglycerides

INTRODUCTION

Importance of hyperlipidemia

Hyperlipidemia is a broad term which is also called hyperlipoproteinemia, is a common disorder in developed countries and is the major cause of coronary heart diseases. It results from abnormalities in lipid metabolism or plasma lipid transport or a disorder in the synthesis and degradation of plasma lipoproteins. The term "dyslipidemia" now a days is increasingly being used to describe abnormal changes in lipid profile, replacing the old term hyperlipidemia. Hyperlipidemia means abnormal increase in fat levels of blood. These fats include cholesterol and triglycerides. These are important for our body to function, but when their levels are high they, can cause heart disorders. Hyperlipidemia is manifested as hypercholesterolemia and hypertriglycerlomia. Hypercholesterolemia is the most common hyperlipidemia. The lipids that are involved in hypercholesterolemia are cholesterol, an essential component of cell membrane and a precursor of steroid hormone synthesis and triglycerides are important energy source, they are transported in blood as lipoproteins. The consequence of hyperlipidemia is to cause atherosclerosis, thus the risk of coronary heart diseases and strokes. The risk of heart diseases in future also depends on many other factors that influence the health of a person's

level of cholesterol, blood vessels and blood circulation [1-4].

MATERIALS AND METHODS

Collection, Identification and Extraction of Medicinal plants

This plant was collected from various areas in bulk. This plant was authenticated at Government Brennen College, Thalassery, Kerala. Collected plants were shade dried for 15 days and they were coarsely powdered using a pulverizer.

The pulverized plant materials were taken up for extraction using hydro alcohol in the proportion of 30:70. The extraction was carried out by cold percolation method. The extracts were then dried in vaccum and they were stored in desiccator and subsequently to a refrigerator.

IDENTIFICATION OF PHYTOCHEMICAL CONSTITUTENTS

Preliminary phytochemical tests

Preliminary phytochemical tests were done by the methods described by usual procedures mentioned in Trease and Evans (1958) [67] and also as specified in the book of Practical Pharmacognosy (Kokate, 2000) [68]. The details of the same are provided below.

Methanolic extracts of flowers of *Achillea millefolium* was subjected to qualitative tests for the identification of various active constituents.

Chemicals

The solvents used for the study were obtained from SD Fine Chemicals India Ltd. and were of laboratory grade. Gemfibrozil was purchased from Pharpharma Ltd, dexamethasone and all other chemicals used in the present study were purchased from Merck, India and of analytical grade. *Achillea millefolium plant* flowers were collected in Warangal, Telangana, India.

Animals

Male albino wistar rats weight about 180-230g obtained and animal used for the study. They were housed, under standard laboratory conditions at room temperature (21°C±2°C) and relative humidity of 55-60%.they were fed with standard pellet diet and water *ad libitum*. The study protocol was approved by the Institutional Animal Ethical Committee (IAEC) College of pharmacy.

Acute toxicity studies

The acute toxicity of the *Achillea millefolium* of was evaluated in rats using the up and down procedure. Rats of either sex (three females and three males, weight: 150-200 g, received methanolic extract of *Achillea millefolium* starting at 2 g/kg orally by gavage. The animals were observed for toxic symptoms continuously for the first 3 hours dosing, finally, the number of survivors was noted after 24 h and these animals were then maintained for further 10 days with observations made daily.

EXPERIMENTAL DESIGN

Animal

- Total number of animal : 6
- Sex : Male
- Strain : Albino wistar rats
- Body weight : 180-230 gm

Surfactant administration

- Hyperlipidemic agent : dexamethasone
- Route of administration : Sub cutaneous

Drug administration

- Vehicle : 1% CMC
- Route of administration : Oral
- Drug dose : gemfibrozil 10 mg/kg
- Extract : 200mg/kg

DEXAMETHASONE-INDUCED HYPERLIPIDEMIA MODEL

Glucocorticoid hormonal level elevation induces the plasma lipid concentration but varies from species to species. Few synthesis of triacylglycerol in the liver is stimulated by the injection of glucocorticoid in rats and consequently may lead to the accumulation of fatty liver. The stimulation of the TG production could lead to increased secretion of VLDL. Increasing VLDL secretion has been reported when dexamethasone is injected for several days in rats. The increase in TG level induces imbalance in lipid metabolism leads to hyperlipidemia. Similarly, dexamethasone

treatment in newborn rats for 4 days showed widespread increase in serum lipids.

Six animals were grouped and used for evaluating the effect of antihyperlipedemic activity of *Achillea millefolium* extract at the dose level of 200mg/kg.

ANALYTICAL PROCEDURE

Estimation of plasma cholesterol level in rats

In this study the enzymatic; cholesterol oxidase-peroxidase (CHOD-POD) method was used. Cholesterol kit based on cholesterol oxidase (COD) and peroxidase (POD) enzymes were used along with the chromogen 4-aminoantipyrine and phenol. This method is one step, simple and rapid.

Estimation of plasma triglyceride level in rats

In this study the enzymatic; glucose oxidase-peroxidase (GOD – POD) method with NEthyl –N Sulfopropyl-N-Anisidine (ESPAS) was used.

Estimation of serum HDL- c level in rats

In this study the enzymatic; cholesterol oxidase-peroxidase (CHOD – POD) method was used.

Estimation of Serum LDL-Clevel in rats

Using the data obtained including total cholesterol, triglycerides and HDL cholesterol, the LDL cholesterol levels were calculated using the empirical equation.

Serum LDL Cholesterol = Total Cholesterol – (Triglycerides/5 + HDL – C)

Estimation of serum VLDL- c level in rats

Using the data obtained including triglycerides, the VLDL cholesterol levels were calculated using empirical equation. Serum VLDL –C = Triglycerides/5

Calculation of atherogenic index

Atherogenic Index (AI) = log (TG/HDL-C)

Free radical scavenging activity by DPPH method

DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of the plant extract. Scavenging of DPPH radical is related to the inhibition of lipid peroxidation. DPPH is usually used as a substance to evaluate the antioxidant activity [51-56].

Free radical scavenging activity ABTS Method

The decolorization of the $ABTS^{+}$, through measuring the reduction of the radical cation as the percentage inhibition of absorbance at 736nm. $ABTS^{+}$ was generated by incubating $ABTS^{+}$ chromophore through the reaction. The presence of specific chemical compounds in the extracts of *Achillea millefolium* may inhibit the potassium persulfate activity and hence reduced the production of $ABTS^{+}$.

Statistical evaluation

The values are expressed in mean ± SEM. One way ANOVA followed by Tukey's multiple comparison Test was used to analyse the effect of different doses of drugs when compared to control, with the help of Graph Pad Instat software, version 3.01. $P<0.05$ is considered as significant.

RESULT AND DISCUSSION

Phytochemical screening

The phytochemical screening results revealed that the after which it was observed whether the alkaloids were present due to absence of turbidity formation. The colour not changed from violet to blue or green in some samples indicated the absence of steroids. An interface with a reddish brown coloration was formed in the absence of carbohydrates as negative result. Red coloration identifies the presence of flavonoids (Shinado's test). A colour change was observed in the test tube, which indicated in the presence of tannins.

Table No.2. Phytochemical screening of *Achillea millefolium* Flower

S.No	Phytoconstituents	Presence
1.	Tannins	-
2.	Alkaloids	+
3.	Steroids	-
4.	Glycosides	-

5.	Flavonoids	+
6.	Carbohydrates	-
7.	Saponins	+

Dexamethasone induced hyperlipidemia in rats

Rats treated with Extract showed decreased serum levels of total cholesterol, LDL and triglycerides, compared to control hyperlipidemic rats. The HDL level of extract treated groups were constantly decreased when compared to normal and control group of animals. Similar results were observed for the standard drug of gemfibrozil used as positive control which have more potent hypolipidemic activity when compared to control and 200mg/kg of extract shown equipotent hypolipidemic action. Observed HDL levels indicated that the 200mg/kg treated animals observed were increased dose when compared to standard drug. Which are justified in the Table.No.3 & Fig. No:10.

Extract 200 mg/kg treated groups were showed decreased serum levels of VLDL, altherogenic index Phospholipids and free fatty acids were respectively, compared to control hyperlipidemic rats. Which are clarified in the Table.No.4 & Fig. No.11.

Table No.3. Effect of Hydroalcoholic extracts against Dexamethasone induced hyperlipidemia in rats

Group	Dose	Total Cholesterol (mg/dl)	Total TG (mg/dl)	HDL Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)
I	Control (Normal)	65.1±1.352	63.84 ±1.77	38.67 ±1.689	13.7 ±0.333
II	Dexamethasone (10 mg/kg) S.C	117.84±1.687	151.84±1.667	26.17±0.307	54.34 ±1.687
III	Dexamethasone (10 mg/kg) S.C+ gemfibrozil (10mg/kg) P.O	71.51±1.352	67.34±0.764	34.34±0.421	21.67±0.33
IV	Dexamethasone with Extract (200 mg/kg)	77.04±1.45	70.43±0.35	26.14±0.57	23.10±0.42

All the values were represented as mean±SEM. All the data were statistically analyzed by one-way **ANOVA** followed by Dunnett's test and values P.

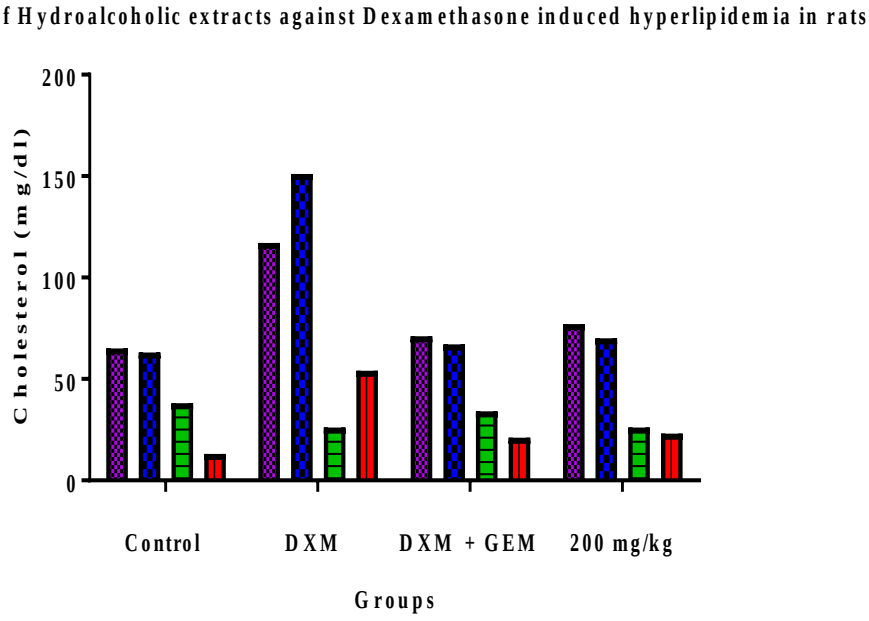

Figure No.10. Dexamethasone induced hyperlipidemia in rats

Table No.4. Effect of Hydroalcoholic extracts against Dexamethasone induced hyperlipidemia in rats

Group	Dose	VLDL Cholesterol (mg/dl)	Atherogenic index	Phospholipids (mg/dl)	Free fatty acids (mg/dl)
I	Control (Normal)	13.17 ±0.307	1.66	92.74 ±166	20.38 ±0396
II	Dexamethasone (10 mg/kg) S.C	38.74 ±1.542	4.51	132.2±2.983	35.2 ±0.152
III	Dexamethasone (10 mg/kg) S.C+ Gemfibrozil (10mg/kg) P.O	18.17 ±0.307	2.22	95.38 ±1.55	22.63 ±0.223
IV	Dexamethasone with Extract (200 mg/kg)	36.88±0.45	3.9	77.34±0.75	29.77±0.75

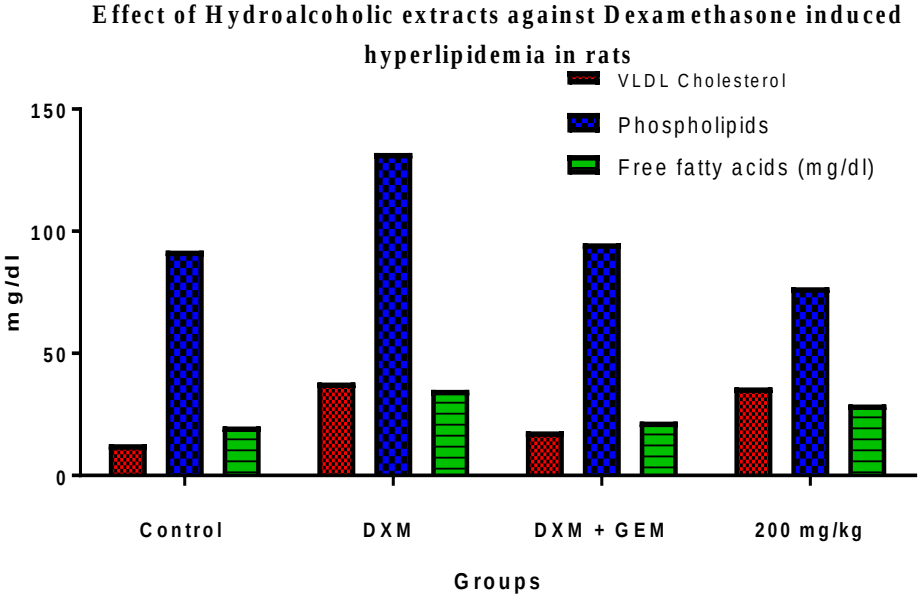

Figure No.11. Dexamethasone induced hyperlipidemia in rats

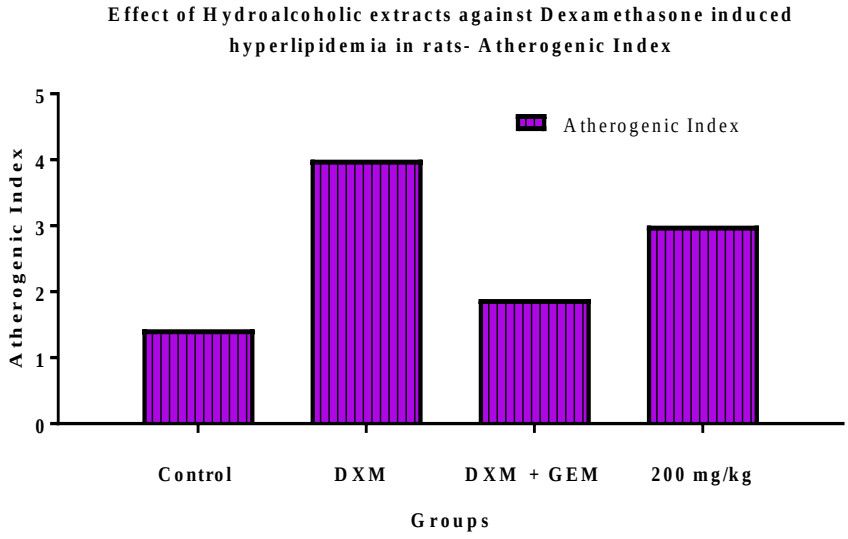

Figure No.12. Atherogenic Index

Invitro antioxidant

Results stated that the attendance of potentially antioxidant substances in Extract, an *in vitro* evaluation of DPPH free radical scavenging at different concentrations was performed. The 50% inhibitory concentration (IC_{50}) and the maximum activity in assay of DPPH free radical scavenging of Extract were equipotent action when compared to BHT as shown in Table. No. 5 & Fig. No.13.

The results clarified that the turnout of potent antioxidant substances in Extract, an *in vitro* evaluation of ABTS free radical scavenging at different concentrations was performed. The 50% inhibitory concentration (IC_{50}) and the maximum activity in assay of DPPH free radical scavenging of Extract were equipotent action when compared to ascorbic acid as shown in Table. No.6 & Fig. No.14.

Table No. 5: *Invitro* antioxidant study by DPPH method

S.No	Concentration	Leaf Extract	Percentage Inhibition (%) of Standard
	50	52.53 ±0.89	35.58±0.37
	100	88.91±0.63	40.20±0.55
	150	91.34±0.32	47.09±0.20
	200	94.39±0.47	40.89±0.73
	250	95.99±0.91	55.41±0.59

Standard: Butylated hydroxyl toluene

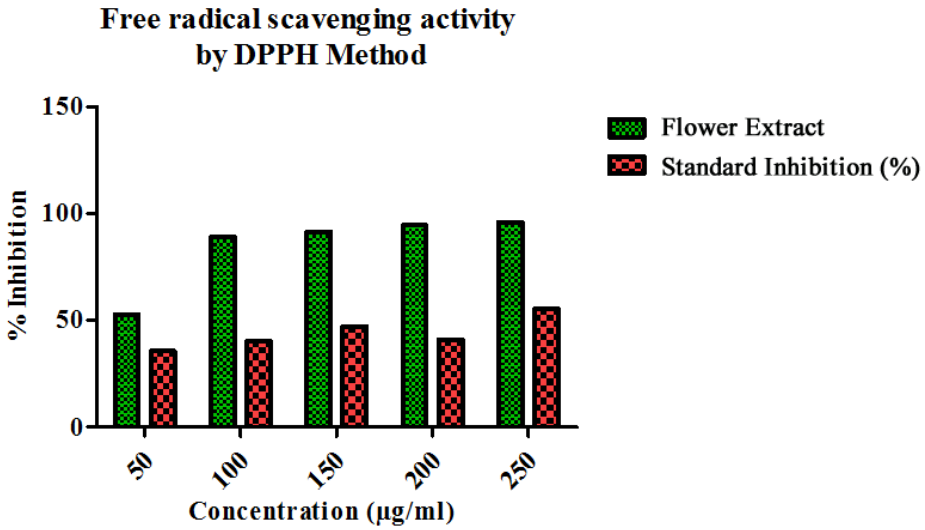

Figure No.13. Antioxidant study by DPPH method

Table No. 6: Free radical scavenging activity by ABTS Method

S.No	Concentration	Flower Extract	Percentage Inhibition (%) of Standard
1.	200	0.64±0.56	0.37±0.05
2.	300	0.69±0.06	0.53±0.06
3.	400	0.76±0.95	0.62±0.09
4.	500	0.74±0.53	0.84±0.54
5.	600	1.14±0.28	0.96±0.08

Standard: Ascorbic acid

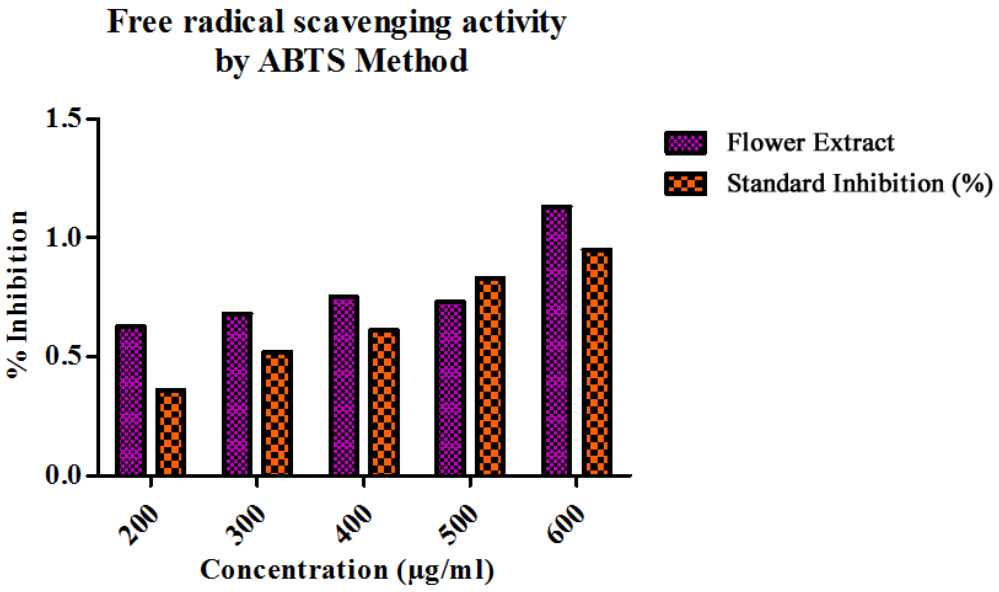

Figure No.14: Antioxidant study by ABTS$^+$ method

CONCLUSION

The current research focuses on Antihyperlipidemic activity in dexamethasone-induced hyperlipidemia in male Wistar rats, with the extracts of *Achillea millefolium.* The antihyperlipidemic potential of the *Achillea millefolium* has been planned to be assessed for its activity against controlling the lipid profile of the animal selected models. Thus the results of the present investigation clearly indicated that the selected medicinal plants possess good antihyerlipidemic activity in dexamethasone induced hyperlipidemic rats and led to the possessing of antihyperlipidemic and selected species activities. The results found are encouraging for further studies on the selected plants and to identify the bioactive compounds.

Oxidative balance in the body was regulated by endogenous and exogenous mechanisms, in which surplus of free radicals connected to many diseases. By regulation of the excess of oxidative molecules includes especially exogenous intake of antioxidants, which are largely found in natural sources. The chemical composition of these plants has shown that same classes of polyphenols may present which are exert such function such as flavonoids. The capacity of Extract of DPPH free radicals scavenging was intermediary among standard antioxidants just about higher than that of BHT.

The significance of novel products in the action and avoidance of dyslipidemias becomes necessary to reduce the mortality and morbidity due to cardiovascular complications. In addition, the search for less toxic drugs has augmented the interest of the scientific community for natural products. The Extract showed to able to manage hyperlipidemia induced by high-fructose diet, reducing serum levels of total cholesterol and triglycerides, without signs may be indicated change in hepatic and renal function, suggesting that Extract is safe in the evaluated conditions. The hypolipidemic activity of natural products can be correlated to the presence of flavonoids due to their properties of inhibiting cholesterol biosynthesis and absorption and modifying the activity of lipogenic and lipolytic enzymes, leading to reduced lipid metabolism, as observed in hyperlipidemic rats treated with Extract which showed important reduction in the levels of total cholesterol and triglycerides. Other molecules clever to decrease the serum level of cholesterol are saponins, also present in Extract. It is very interesting that Extract was able to reduce both serum level of cholesterol and total triglycerides.

In conclusion, our results showed that Flowers of *Achillea millefolium* reduce oxidative stress by

free radical scavenging and protect adjacent to lipid peroxidation and also able to manage hyperlipidemia by decreasing serum level of cholesterol and triglycerides, similarly to standard drugs.

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