



EVALUATION OF ANTIHYPERLIPIDEMIC ACTIVITY OF ETHANOLIC EXTRACT OF *WITHANIA SOMNIFERA* IN TRITON X-100 INDUCED HYPERLIPIDEMIC RATS

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ABSTRACT

Hyperlipidemia is the greatest risk factor of coronary heart disease. The present study was designed to investigate the antihyperlipidemic activity of *Withania somnifera* in Triton X-100 induced hyperlipidemic rats. *Withania somnifera* extract was administered at different dose of 200mg/kg and 400mg/kg (p.o) daily for 7 days to hyperlipidemic rats. Atorvastatin is used as reference standard. The statistical analysis was carried out using one-way ANOVA followed by Dunnet's multiple comparison test. *Withania somnifera* showed a significant decrease in the levels of serum cholesterol, triglycerides, LDL, VLDL and significant increase in the level of serum HDL at the dose of 400mg/kg (p.o) against Triton induced hyperlipidemic rats. Therefore, it effectively suppressed the Triton induced hyperlipidemia in rats, suggesting the potential protective role in Coronary heart disease.

KEY WORDS

Withania somnifera, Hyperlipidemia, Triton X-100.

INTRODUCTION

Hyperlipidemia is defined by abnormally elevated levels of one or more lipids such as cholesterol or triglycerides in the bloodstream. It also involves elevated levels of lipoproteins especially LDL-cholesterol and this is the most common forms of dyslipidemia which comprises a triad of decreased levels of high density lipoprotein (HDL), increased levels of low density lipoprotein (LDL), and elevated levels of triglycerides (Musunuru et al, 2010).

Hyperlipidemia is a disorder of lipoprotein metabolism manifested as hypercholesterolemia, hypertriglyceridemia, or a combination, with elevated plasma apolipoprotein B. Hyperlipidemic is a risk factor for gall stone, pancreatitis and xanthomas, whereas hyperlipidemic is a risk factor for coronary artery disease (CAD), myocardial infarction (MI), hypertension and cerebrovascular accidents. CAD could be

considered as the most common cause of death globally, including India, by 2020 (Yusuf S et al, 2001). Hyperlipidemia is the result of complex interactions between environmental and genetic factors (Haffner et al, 1999). Hyperlipidemia is the main cause of congestive heart diseases in adulthood. It is also the main cause of atherosclerosis which is the pathophysiological cause of vascular diseases such as angina pectoris, myocardial infarction, and stroke (Klag et al, 1993).

Withania somnifera, also known as *ashwagandha*, Indian ginseng, and winter cherry, has been an important herb in the Ayurvedic and indigenous medical systems for over 3000 years.

Ashwaganda is also traditionally used as an adaptogen for patients with nervous exhaustion, insomnia, and debility due to stress, and as an immune stimulant in patients with low white blood cell counts.

MATERIALS AND METHODS

Plant material

Withania somnifera root were collected from local area supplier of Delhi. Sample of plant material was sent to Dr. K Madava Chetty, Assistant Professor Dept. of Botany, Sri Venkateshwara University, for identification and taxonomic authentication.

Chemicals

Triton X-100 was obtained from Best Nauticals, Ballimaran, Chandni Chowk, Delhi 110006, India. Atorvastatin was obtained from Baldev Pharmaceuticals Ltd. Ghaziabad, U.P. All other chemicals were of analytical grade and obtained locally.

Experimental Animals

Wistar albino adult male rats weighing 180-200g were obtained from the animal house National Institute of Biologicals, A-32, Sector-62, Noida- 201307, U.P, India. The animal were grouped and housed in polypropylene cages (38x 23x 10cm) with not more than six animals per cage and maintained under standard laboratory conditions. They were allowed free access to standard dry pellet diet and water ad libitum. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) constituted under CPCSEA.

METHODS

Preparation of extract

The Root of plant *Withania somnifera* were collected and were authenticated. The root of the plant were collected and subjected to shade drying. The size were reduced and made to coarse powder and then further passed through the appropriate sieve no. to obtain uniform particle size. The powdered root part extracted with ethanol and water by soxhlet apparatus. The root extracts were filtered and collected and concentrated by using Rotatory Flash Evaporator. The extract were used for the further experimental models.

Qualitative chemical tests

Ethanol extract of the plant was subjected to chemical tests for the identification of their active constituents.

Acute toxicity study

Acute oral toxicity was performed by following OECD-423 guidelines (acute toxic class method). The ethanol extract of plant *Withania somnifera* was found to be safe up to 2000 mg/kg body wt. by oral route. After 24hr animals were found well tolerated. There was no mortality and no signs of toxicity. So two dose levels i.e. 200mg/kg and 400mg/kg body weight were selected for the present study.

Experimental Models for Evaluation of Anti Hyperlipidemic Activity

The antihyperlipidemic activity was performed by experimental model, triton x 100 induced hyperlipidemia.

Induction of Hyperlipidemia

Hyperlipidemia was induced in Male Wistar albino rats by single intraperitoneal injection of freshly prepared solution of Triton-X-100 (100 mg/kg) in physiological saline solution after overnight fasting for 18 h. The animals were divided into five groups of six rats each. The first group was given standard pellet diet, water. The second group was given a single dose of triton x-100 administered at a dose of 100mg/kg, i.p. Third group was administered with the standard 10 mg/kg, p.o. for 7 days. To the fourth and fifth group were administered with 200mg/kg and 400mg/kg respectively of ethanolic extract of *Withania somnifera* p.o., daily for 7 days, after inducing hyperlipidemia.

Collection of blood

On the 8th day, blood was collected by retro orbital sinus puncture, under mild di ethyl ether anaesthesia. The collected samples were centrifuged for 15 minutes. Then serum samples were collected and used for various biochemical experiments. The animals were then sacrificed, and the liver and heart collected.

Biochemical analysis

The serum and liver extract were assayed for total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) using standard protocol methods.

Histopathological studies

At the end of the study period, animals from experimental group were sacrificed liver and heart were collected. The transverse section of liver and heart were prepared using the usual techniques for preparation of permanent slides and these sections were observed for histopathological changes in liver and heart cells. Histopathological analysis of liver and heart were examined under light microscope.

Statistical analysis

The results were expressed as mean $\pm$ S.E.M. Statistical analysis was carried out by using ANOVA followed by Dunnett's multiple comparison tests using Graph pad PRISM software version 5. P values <0.05 were considered as statistically significant.

RESULTS

Effect of *Withania somnifera* on total Cholesterol levels.

In the normal rats the total cholesterol levels were to be found be 63.77 ± 1.476 . Treatment with Triton-X-100 caused a significant rise in the levels of cholesterol ($174. \pm 3.040$). Administration of various doses of the plant extract after the treatment with Triton-X-100 resulted in the lowering of Cholesterol levels in a dose dependent manner. The total cholesterol levels of groups treated with 200 and 400 mg/kg were 147.5 ± 1.28 , 91.23 ± 1.116 respectively. The reduction in cholesterol level produced by 400mg/kg extract was significant at ($p < 0.05$).

Effect of *Withania somnifera* on Triglyceride levels.

Induction of hyperlipidemia resulted in significantly raised triglyceride levels (111.6 ± 2.48) compared to the normal (74.77 ± 2.07). Administration of various doses of the plant extract was able to produce a dose dependent decrease in the triglyceride levels. The respective triglyceride values for rats treated with 200 and 400 mg/kg of extract were 95.9 ± 3.57 and 85 ± 1.461 .

Effect of *Withania somnifera* on serum LDL levels.

The LDL levels in normal rats were found to be 61.43 ± 1.647 . Administration of Triton-X-100 resulted in a rise in LDL levels (97.23 ± 1.28). In Atorvastatin group the LDL was reduced to 64.14 ± 3.43 , whereas groups treated with 200 and 400 mg/kg of extract showed a dose dependant decrease in the LDL levels (88.13 ± 1.47 , 74.77 ± 1.65 respectively)

Effect of *Withania somnifera* on serum VLDL levels.

The VLDL levels in normal rats were found to be 15.83 ± 0.41 . Administration of Triton-X-100 resulted in a rise in VLDL levels (21.43 ± 0.49). In Atorvastatin group the VLDL was reduced to 14.67 ± 0.49 , whereas groups treated with 200 and 400 mg/kg of extract showed a dose dependant decrease in the VLDL levels (20.13 ± 0.37 , 18.19 ± 0.29 , 16.20 ± 0.29 respectively)

Effect of *Withania somnifera* on serum HDL levels.

The HDL levels in normal rats were found to be 47.75 ± 1.52 . Administration of Triton-X-100 resulted in a fall in HDL levels (24 ± 1.09). In Atorvastatin group the LDL was elevated to 40 ± 1.31 , whereas groups treated with 200 and 400 mg/kg of extract showed a dose dependant increase in the HDL levels (32.47 ± 1.72 , 27.23 ± 1.49 respectively).

Table 1: Effect of ethanolic extract of *Withania somnifera* (EEWS) on serum lipids

Treatments groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Normal control	63.77 ± 1.476	74.77 ± 2.07	47.75 ± 1.52	61.43 ± 1.647	15.83 ± 0.41
Triton X- 100	174.6 ± 3.040	111.6 ± 2.48	24 ± 1.09	97.23 ± 1.28	21.43 ± 0.49
Atorvastatin (10mg/kg)	72.90 ± 1.673	79.87 ± 1.87	40 ± 1.31	64.14 ± 3.43	14.67 ± 0.79
EEWS (200mg/kg)	$147.5 \pm 1.28^{**}$	$95.9 \pm 3.57^{**}$	27.23 ± 1.49	88.13 ± 1.47	$18.19 \pm 0.29^{**}$
EEWS (400g/kg)	$91.23 \pm 1.116^{**}$	$85 \pm 1.461^{**}$	32.47 ± 1.72	$74.77 \pm 1.65^{*}$	$16.20 \pm 0.39^{**}$

All values are expressed as MEAN $\pm$ SEM; (n=6), *: $p < 0.05$ compared to triton treated group, **: $p < 0.01$ compared to triton treated group. Test result of serum lipids in mg/dl.

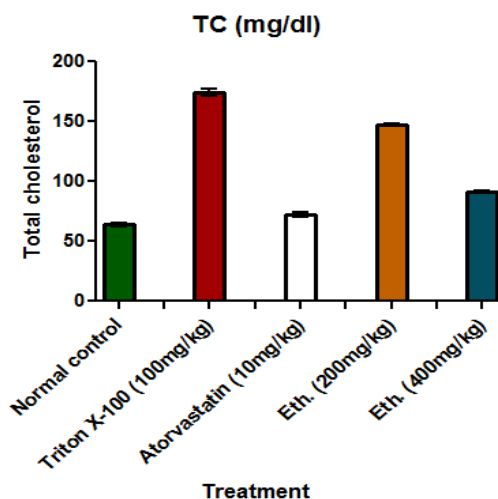


Figure No. 1: Effect of different quantity of different doses on Conc. of TC

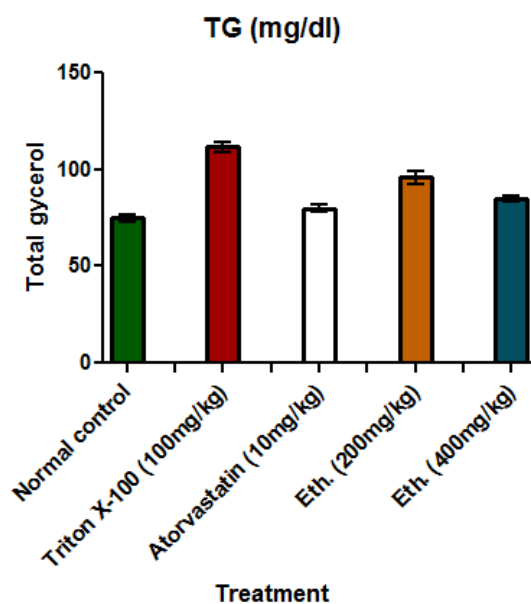


Figure No. 2: Effect of different quantity of different doses on Conc. of TG

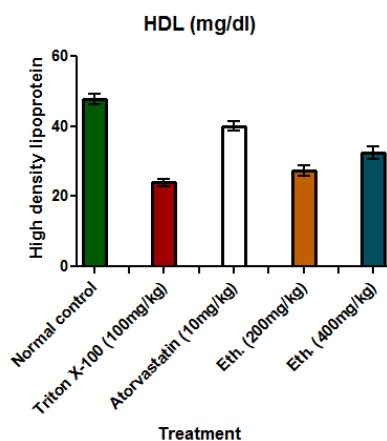


Figure No. 3: Effect of different quantity of different doses on Conc. of HDL

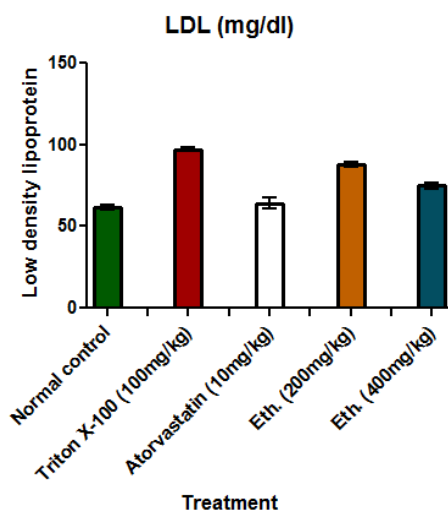


Figure No. 4: Effect of different quantity of different doses on Conc. of LDL

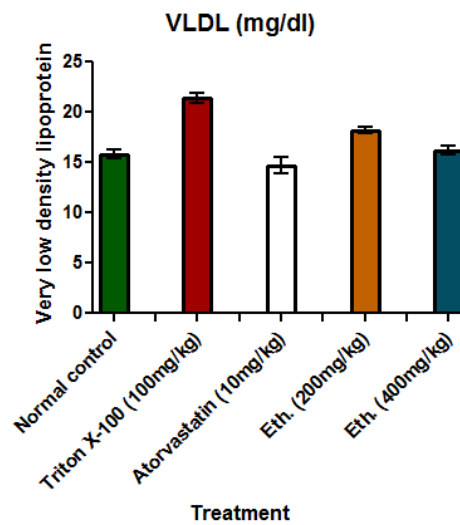


Figure No. 5: Effect of different quantity of different doses on Conc. of VLDL

HISTOPATHOLOGY OF RAT HEART

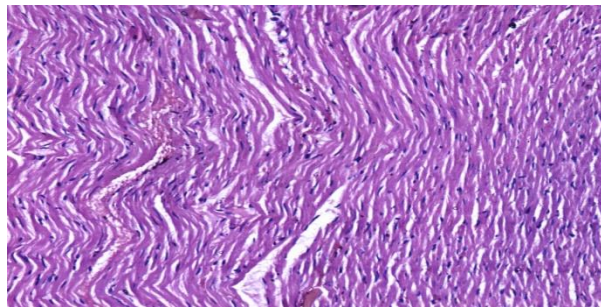


Figure No. 1: Rat heart of normal control group showing normal cardiac myocytes. (H&E X 200)

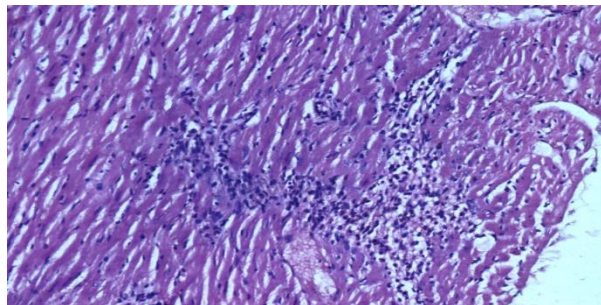


Figure No. 2: Heart of rat treated with Triton x-100 shows focal myocyte necrosis with inflammatory infiltrate and focal edema leading to mild separation of cardiac myocytes.

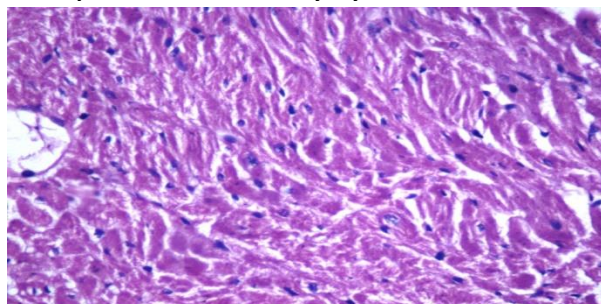


Figure No. 3: Heart of rat treated with low dose extract shows mild increase in thickness of myocardial wall. (H&EX 40)

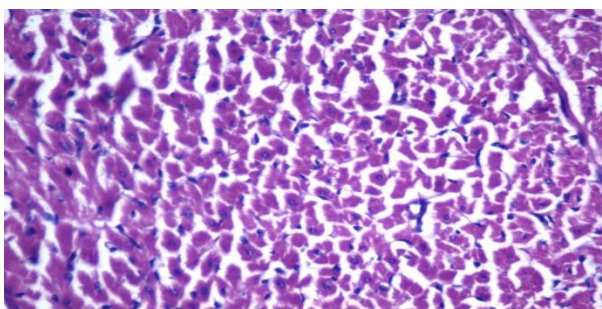


Figure No. 4: Heart of rat treated with high dose extract shows significant myocardial edema with separation of cardiac myocytes. (H&E X 400)

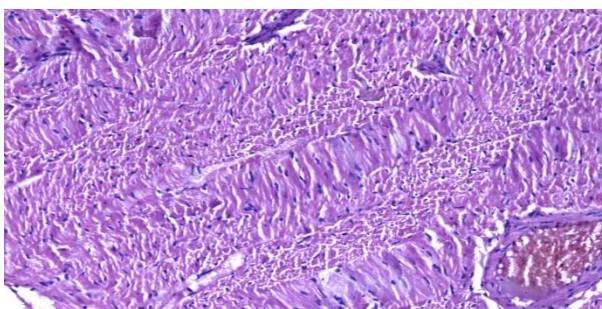


Figure No. 5: Heart of rat treated with Atorvastatin shows normalization of myocardium. (H&E X 200)

HISTOPATHOLOGY OF RAT LIVER

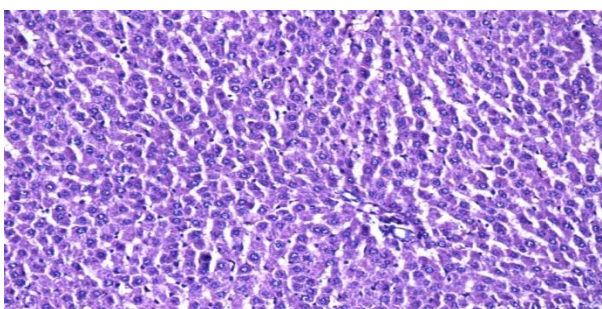


Figure No. 1: Rat liver of normal control group showing normal liver architecture with normal hepatocytes and portal tract. (H&E X 200)

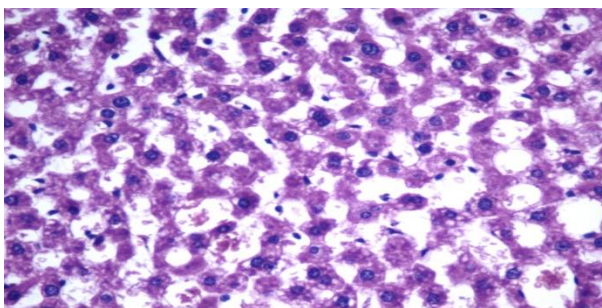


Figure No. 2: Rat liver treated with TritonX-100 shows fatty infiltration and granular degeneration with edema. (H&E X 400)

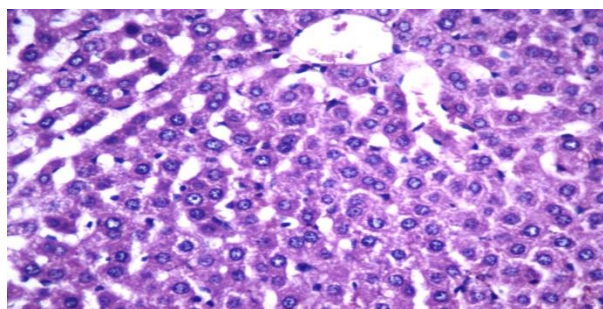


Figure No. 3: Rat liver treated with low dose extract shows mild fatty infiltration and granular degeneration. (H&E X400).

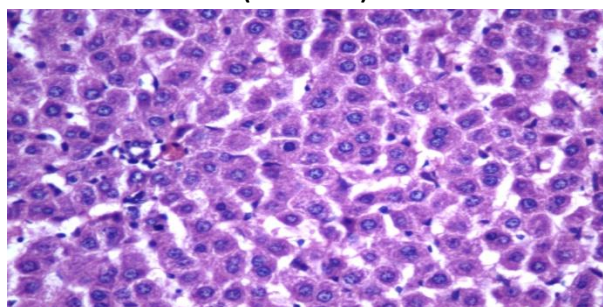


Figure No. 4: Rat liver treated with high dose extract shows mild granular degeneration and edema. (H&E X400)

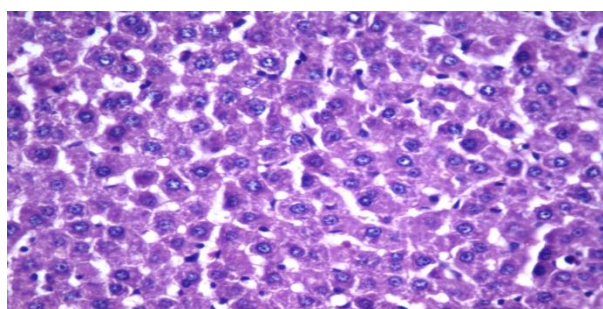


Figure No. 5: Rat liver treated with atorvastatin shows negligible fatty infiltration and granular degeneration. (H&E X400)

DISCUSSION

Atherosclerosis is a serious complication produced by hyperlipidemia. It eventually causes coronary heart disease (CHD) and in modern times the number of hyperlipidemic patients has been continuously increasing. Life style changes, especially high fat diet is the predominant factor resulting in hyperlipidemia. Hyperlipidemia constitutes etiopathological factors for atherosclerosis. Most recent findings indicate a multi-faced cause to the problem of cardiovascular disease, including excessive intake of saturated fats, carbohydrate, and metabolic dysfunction.

The plant has been chosen for this study as it was easily available and the antihyperlipidemic activity has not been reported earlier.

The major biochemical constituents of *ashwaganda* root are steroidal alkaloids and steroidal lactones in a class of constituents called withanolides.¹ At present, 12 alkaloids, 35 withanolides, and several sitoindosides from this plant have been isolated are known to have anti-hyperlipidemic properties.

The major chemical constituent present in this plant is two main withanolides, withaferin A and withanolide D which are responsible for antihyperlipidemic activity.

At the end of the study period, animals from experimental group were sacrificed liver and heart were collected. The transverse section of liver and heart were prepared using the usual techniques for preparation of permanent slides and these sections were observed for histopathological changes in liver and heart cells. Histopathological analysis of liver and heart were examined under light microscope.

Treatment of Triton x-100 induced Hyperlipidemia rats with selected plant extracts and reference standard Atorvastatin (10mg/kg), an HMG CoA inhibitor showed a significant decrease of serum triglycerides, cholesterol, LDL and VLDL and significant increase of serum HDL-C levels.

CONCLUSION

The present study was designed to investigate the antihyperlipidemic activity of *Withania somnifera* extract in Triton X-100 induced hyperlipidemic rats. Administration of triton-X-100 (100mg/kg) to rats caused an elevation of total cholesterol, total triglycerides, VLDL and LDL and reduction in HDL levels. *Withania somnifera* was administered at various doses 200, 400 mg/kg day, (p.o) to Triton induced hyperlipidemic rats. Atorvastatin was used as reference standard. Treatment with plant extract was able to significantly ($p < 0.05$) decrease the levels of TC, TG, VLDL and LDL. Also, the extract was found to cause a significant ($p < 0.05$) increase in the HDL levels. Therefore, it can be concluded that *Withania somnifera* extract is able to effectively suppress Triton induced hyperlipidemia in rats. Therefore, 400mg/kg extract is more effective than 200mg/kg extract.

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REFERENCES

- Haffner, S. M. 1999. Diabetes, hyperlipidemia, and coronary artery disease. *American Journal of cardiology*, 83, 17f-21f.
- Klag, M. J., Mead, L. A., Ford, D. E., Coresh, J. & Levine, D. M. 1993. Serum-cholesterol in young men and risk of subsequent injuries. *Circulation*, 88, 124-124.
- Allain CC, Poon LS and Chan CSG. Enzymatic determination of total serum cholesterol. *Clin Chem*. 1974; 20:470-475.
- Bucolo G and David H. Quantitative determination of serum triglycerides by use of enzymes. *Clin Chem*. 1973; 19:476-482
- Bairaktari E, Elisaf M, Katsaraki A, Tsimihodimos V, Tselepis AD, Siamopoulos KC and Tsolas O. "Homogeneous HDL-cholesterol assay versus ultracentrifugation/dextran sulfate-Mg²⁺ precipitation and dextran sulfate-Mg²⁺ precipitation in healthy population and in hemodialysis patients". *Clinical Biochem*. 1999;32(5):339.
- Friedewald WT, Levy RI and Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972; 18:499-502.
- Vogel G, Vogel WH. Influence of lipid metabolism. In: *Drug Discovery and Evaluation Pharmacological Assay*. Springer-Verly: Berlin; 1997;604-8.
- Aggarwal R., Diwanay S., Patki P., Patwardhan B. Studies on immunomodulatory activity of *Withania somnifera* (Ashwagandha) extracts in experimental immune inflammation. *J Ethnopharmacol*. 1999; 97: 27-35.
- Davis L., Kuttan G. Immunomodulatory activity of *Withania somnifera*. *J Ethnopharmacol*. 2000; 71 (1-2): 193-200.
- Durrington PN. Hyperlipidaemia. Cambridge: Butterworth-Heinemann, Ltd. 1995.
- Harbourne JB. Phytochemical analysis. London: Chapman and Hall company Ltd. 1973.
- Harborne JB. 1984. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*, 2nd.
- Harborne JB, 1984. "Phytochemical methods" (2nd Ed). Chapman and Hall, London and New York. pp288.
- Rasool M., Varalakshmi P. Immunomodulatory role of *Withania somnifera* root powder on experimental induced inflammation: *Vascul Pharmacol*. 2006; 44 (6): 406-410.
- Ziauddin M., Phansalkar N., Patki P., Diwanay S., Patwardhan B. Studies on the immunomodulatory effect of Asgandh. *J Ethnopharmacol*. 1996; 50 (2): 69-76.

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