ORIGINAL ARTICLE

Antihyperlipidemic effect of *Adonis vernalis*

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ABSTRACT

Objective: To study the effect of *Adonis vernalis* on triton-induced hyperlipidemic rabbits. Hyperlipidemia has become a major risk factor for a large variety of diseases including coronary artery disease and thus increasing mortality ratio globally. *A. vernalis* (AV) commonly called as Pheasant's eye is a Homoeopathic remedy and considered as cardiotonic due to presence of cardiac gylcosides with strong diuretic action.
Study Design & Methods: The present study investigates the hyperlipidemic effects of *Adonis vernalis* in chemically (triton; 400 mg/kg intraperitoneally) induced hyperlipidemic rabbits. Its effects on lipid profile, glucose, total protein, serum uric acid and liver specific enzyme alanine aminotransferase (ALT) were determined by administering alcoholic extract of *A. vernalis* (5 mg/kg) orally and compared against respective control groups.
Results: AV (5 mg/kg) had shown a significant decrease in serum cholesterol and triglycerides when compared with control, triton-induced hyperlipidemic control (TIC) and triton-induced positive control (TIPC) ($p<0.05$). It slightly increased HDL, clear decrease in LDL and total protein while no effect was found on ALT, uric acid and glucose.
Conclusions: According to the experimental findings the alcoholic extract of *A. vernalis* found to be a potent antihyperlipidemic agent and also showed hepatoprotective property.

Key words: Hyperlipidemia, Coronary artery disease, Cardiotonic, Cardiac glycoside, Diuretic, Antihyperlipidemic, Hepatoprotective.

INTRODUCTION

Irregular metabolic processes results in the severities and abnormalities in biochemical homeostasis.[1] These changes worsen the normal physiological functioning of the body and become the cause of debilitating health outcomes like hyperlipidemia, diabetes, coronary heart diseases etc.[2]

Hyperlipidemia, heterogeneous metabolic disorder, is a silently progressing risk towards life as it not only associated with the integrated mechanisms of diabetes but also with the cardiovascular disorders with overproduction of oxidative cellular stress.[3-5] Due to the broad consequences and complications of hyperlipidemia, researches establish it as one of the significant contributor of death all over the world.[6-7]

There are well established scientific findings which relate hyperlipidemia with an accumulation of visceral adipose tissue,[8] may be due to increase serum total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-c),[9] very low density lipoprotein (VLDL) and decrease serum levels of high density lipoprotein cholesterol (HDL-c). In addition of genetic causes,[10] obesity,[11] stress, sedentary life style habits,[12-13] consumption of alcohols and nicotinic products are reported as secondary causes of hyperlipidemia and associated metabolic disorders.[14-15] In spite of many advancement in the pharmaceutical preparation, there are studies available which support the use of herbal and homeopathic methods for management of diseases such as hyperlipidemia, dyslipidemia associated cardiovascular disorders and others life threatening ailments due to their safest and affordable properties with significant effectiveness.[16]

Adonis vernalis commonly called as Pheasant's eye belongs to the family *Ranunculaceae* is a well known homoeopathic remedy containing a specific cardiac glycoside, Adonidin, which has similar action like digitalin obtained from Foxgloves. Due to presence of cardiac glycoside and diuretic effect *A.vernalis* is used for the treatment of congestive heart failure.[17] At yet, no past literature reports the effect of this medicine on lipid profile. Therefore a preliminary study was undertaken to determine the effect of alcoholic extract of *A. vernalis* on serum levels of lipid profile (TC, TG, LDL-c, HDL-c), total protein, glucose and on liver-

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specific enzyme alanine amino transferase (ALT) in chemically (triton) induced hyperlipidemic animal model. In future further investigations will be done with different magnitude of doses to extend the current theme. This will help to identify the broad therapeutic effectiveness of *A. vernalis.*

MATERIALS AND METHODS

Adonis vernalis

Alcoholic (70%) mother tincture of *A.vernalis* (Schwabe, Germany) was purchased from authentic homeopathic dealer Saddar, Karachi.

Animals

Healthy albnio rabbits of both sexes weighing from 1-1.7 kg were purchased from local supplier of Jinnah University for Women. The animals were provided standard diet and water *ad libitum.* During the overall period, a hygienic condition with no physical stress was provided to study subjects.

Simvastatin

Simvastatin with brand name limitrol purchased from PharmEvo (Pvt.) Ltd, Pakistan and used as positive control at a dose of 20 mg/kg.

Triton (*X-100* Art 12298)

Non-ionic detergent triton X-100 (Iso octyl polyoxy ethylene phenol, formaldehyde polymer) was obtained from the **MERCK** Chemicals Pakistan and experimentally used as hyperlipidemia inducing agent. Freshly prepared doses of Triton intraperitoneally in a dose of 400 mg/kg were used in this study.[18]

Preparation of alcoholic extract of *A. vernalis*

The Alcoholic mother tincture of *A. vernalis* (70%) was subjected to evaporate by using rotary vacuum evaporator to obtain brown residue that was reffered as alcoholic extract of *A. vernali*.[19]

Dimethylsulphooxide (DMSO)

Analytical reagent grade DMSO was purchased from Fisher Scientific (UK) and its 0.05% concentration in distilled water was used as vehicle for injecting triton (i.p) and administrating the doses of alcoholic extract of *A. vernalis* in experimental test rabbits.[20]

Experimental Procedures

An overnight fasted twenty four experimental rabbits were divided into four groups *viz.*, control, triton-induced hyperlipidemic control (TIC), triton-induced hyperlipidemic positive control (TIPC) and test group. Each group individually contained six (06) rabbits. The control was treated orally with 1ml of distilled water. TIC was treated with 400 mg/kg (intraperitoneally) and 1 ml distilled water orally while TIPC was treated with simvastatin (20 mg/kg) after i.p. triton treatment. Test rabbits were treated orally with alcoholic extract of *A. vernalis* (5 mg/kg) after the i.p. dose of triton. After 18 hours of respective treatments, animals were sacrificed and blood was collected from each group to separate serum to analyze biochemical parameters by spectro UV- Visible Auto, PC Scanning Spectro-photometer, Labomed, Inc.

Biochemical Analysis

Lipid profile includes serum total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-c), serum glucose, total protein (TP), liver specific enzyme Alanine aminotransferase (ALT) and serum uric acid (UA) were determined by the commercially available Kits (Randox). LDL-c was calculated by formula given in reagent kit (Randox, UK) as:

LDL-c = Total cholesterol – Triglycerides/5 – HDL-cholesterol

Statistical Analysis:

The results are presented as mean ± SD. The data were analyzed by one way ANOVA followed by LSD (least significant difference) test (SPSS, version 17.0). The differences were found significant when $p<0.05$.

RESULTS

Effect of alcoholic extract of *Adonis vernalis* on Lipid Profile:

The alcoholic extract of *Adonis vernalis* at 5 mg/kg had shown significant decrease ($p<0.05$) in serum TC level up to 182.5 mg/dL when compared with TIC that showed TC 205.2 mg/dL in rabbits treated only with triton (400 mg/kg) intraperitoneally. Oral administration of same dose of *A. vernalis* also induced decrease in serum TG level significantly up to 170.1 mg/dL when compared with control and TIC having values 191.46 and 212.63 mg/dL ($p<0.05$). *A. vernalis* at same dose decreased serum LDL-c level in test group (39.37 mg/dL) as compared to control, TIC, TIPC but it was not statistically significant. The LDL-c levels found in control, TIC and TIPC were 54.28, 66.10 and 64.78 mg/dL respectively. Similarly, serum HDL-c level (108.9 mg/dL) was slightly increased in test group but not found statistically significant when compared with control, TIC and TIPC. The HDL-c levels found in control, TIC and TIPC were 98.7, 96.6 and 88.14 mg/dL respectively (Figure I).

Effect of alcoholic extract of *Adonis vernalis* on liver specific enzyme:

The serum ALT levels was found normal ranging from 1.93 to 5.59 U/L in all groups including control, TIC, TIPC and test groups (Table I).

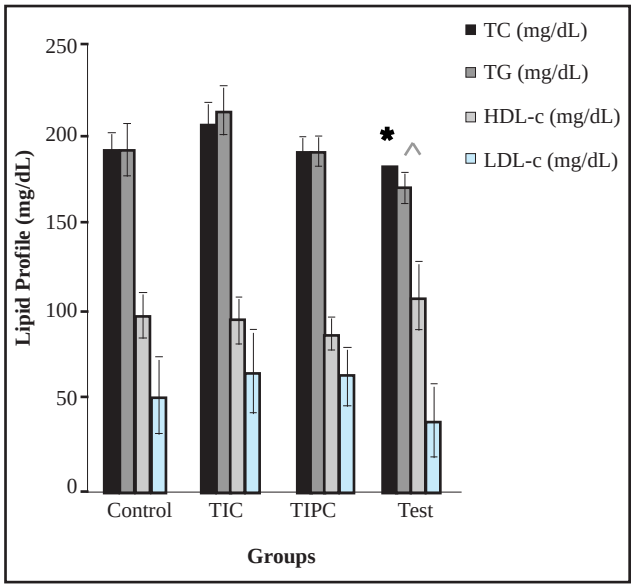

Figure I: Effect of Adonis vernalis on lipid profile (mg/dL). Control and TIC (Triton-induced control) is treated with distilled water (1 mL/kg) while TIPC (Triton induced positive control) treated with limitrol (20 mg/kg) and test with alcoholic extract of Adonis vernalis (5 mg/kg) orally after administration of triton @ 400mg/kg (intraperitoneally) to all groups except control. Each value expressed as mean ± SD (n=6), where *, ^ = $p<0.05$ (statistically significant when compared with control and TIC respectively).

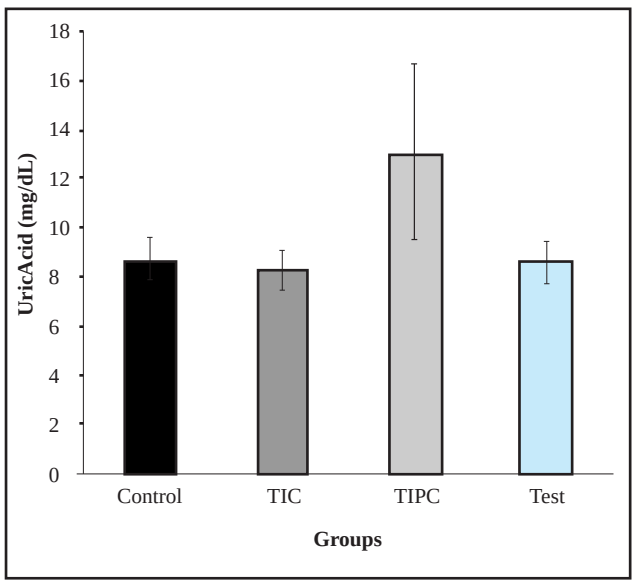

Figure II: Effect of Adonis vernalis on Uric Acid (mg/dL). Control and TIC (Triton induced control) is treated with distilled water (1 mL/kg) while TIPC (Triton induced positive control) treated with limitrol (20 mg/kg) and test with alcoholic extract of Adonis vernalis (5 mg/kg) orally after administration of triton @ 400mg/kg (intraperitoneally) to all groups except control. Each value expressed as mean ± SD (n=6).

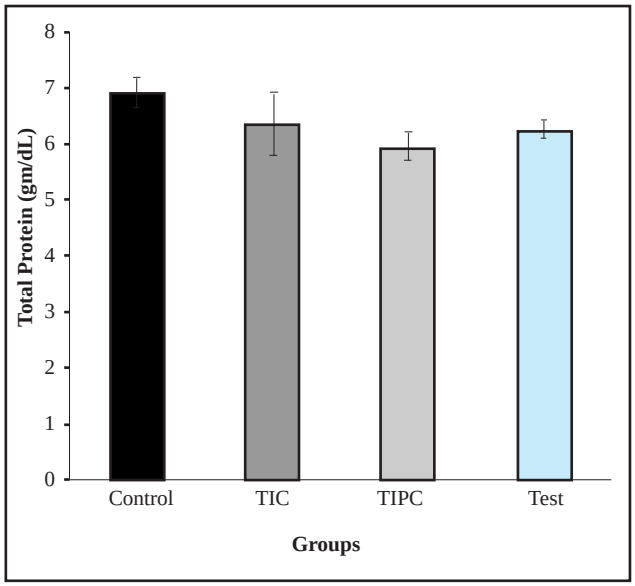

Figure III: Effect of Adonis vernalis on Total Protein (gm/dL). Control and TIC (Triton induced control) is treated with distilled water (1 mL/kg) while TIPC (Triton induced positive control) treated with limitrol (20 mg/kg) and test with alcoholic extract of Adonis vernalis (5 mg/kg) orally after administration of triton @ 400mg/kg (intraperitoneally) to all groups except control. Each value expressed as mean ± SD (n=6).

S. No	Groups	Glucose (mg/dL)	ALT (U/L)
1	Control	94.167±6.799	5.59±2.97
2	TIC	84.833±5.269	3.297±0.334
3	TIPC	94.533±15.638	2.90±0.585
4	Test	91.033±0.924	1.93±0.331

Table I: Effect of Adonis vernalis on Glucose (mg/dL) and Alanine aminotransferase (ALT) (U/L). Control and TIC (Triton induced control) is treated with distilled water (1 ml/kg) while TIPC (Triton induced positive control) treated with limitrol (20mg/kg) and test with alcoholic extract of Adonis vernalis (5 mg/kg) orally after administration of triton @ 400mg/kg (intraperitoneally) to all groups except control. Each value expressed as mean ± SD (n=6).

Effect of alcoholic extract of *Adonis vernalis* on serum glucose, TP and UA:

The alcoholic extract of *Adonis vernalis* @ 5 mg/kg did not produce any significant effect in serum glucose level when compared with control, TIC and TIPC rabbits. Glucose levels found in control, TIC, TIPC and test groups were 94.16, 84.83, 94.53 and 91.03 mg/dL respectively (Table I). A clear decrease was observed in serum TP level of the test group (6.26 gm/dL) administered with alcoholic extract orally at 5mg/kg with respect to control having the same parameter as 6.93gm/dL (Figure III). However, no effect was observed in serum UA level of test group when compared with respective control groups (Figure II).

DISCUSSION

Hyperlipidemia in medical sciences characterized as the foundation of coronary heart diseases because the increase in the cholesterol biogenesis beyond the immediate needs leads to the patho-pysiology of cardiac related vascular diseases.[21-22] The consequences leads to hyperlipidaemia are very vast to understand because the circulatory lipids (cholesterol, triglyceride, free fatty acids and phospholipids) are not in proper circulation due to the inappropriate synthesis or may be the disturbance in biochemical machinery. Prevention of hyperlipidemia is essential due to proper maintenance of lipids concentration in blood because it reduces the risk of myocardial infarction, angina pectoris, stroke and other related cardiac disorders.[23] It is observed that alternate treatment including use of natural products and homoeopathy are nowadays more focused to find out new antihyperlipidemic agents with minimum side effects rather than conventional treatment.[24]

Adonis vernalis (AV) already reported in the treatment of heart disease due to its cardiotonic and diuretic properties.[17] The present study investigates the effect of AV in lowering the serum level of cholesterol, triglycerides, LDL-c and increasing the level of HDL-c which could be the best activity of any drug used in the treatment of hyperlipidemia and related heart diseases. As it was observed that elevated levels of TC, TG, and LDL-c are the major contributors of cardiovascular disease including hypertension.[25-26] In the present study significant decrease was observed in the level of cholesterol may be due to increase rate of secretion of cholesterol into intestinal tract or reabsorption of cholesterol or both by enhancing enterohepatic circulation of cholesterol[25-27] or may be AV decreases the HMG Co A reductase (3-hydroxy-3-methyl-glutaryl Co enzyme A) activity, the rate-limiting enzyme of cholesterol biosynthesis.[28-29] Whereas a significant decrease in TG level was also found may be by enhancing the activity of lipase enzyme that hydrolyzes TG or by increasing excretion of TG via feces.[30] From scientific proofs it was evident that increase in the level of HDL-c is a fine biochemical sign which referred as good cholesterol. In the present study slight increase in the level of HDL-c improves the cholesterol transportation to liver via cholesterol reverse transport pathway.[31] This indicates that AV not only acts as cardiotonic but also has antihyperlipidemic activity reducing the risk of atherosclerosis, heart attack and cardiovascular diseases.

No hepatoxicity was observed due to AV administration in test rabbits as normal serum levels of ALT and TP were found in all groups including test. It is reported that increase in TP and ALT indicates the presence of infection, inflammation and liver disease.[32-33] Therefore, ALT normalizing and TP reducing effects of *A. vernalis* indicate that it has no toxic effect on liver and is involve in decreasing formation of inflammation which could be induced by developing hyperlipidemia with the help of triton X-100 in test rabbits hence AV reduces the risk of coronary atherosclerosis.

CONCLUSION

According to results, the alcoholic extract of *Adonis vernalis* (5 mg/kg) showed significant decrease in TC, TG, and TP hence it can be stated that alcoholic extract of *Adonis vernalis* has anti-hyperlipidemic and hepato-protective effects or may be used for treating hyperlipidemia.

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