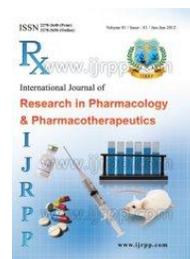




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Research article

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### Effect of serratiopeptidase on Triton X-100 induced hyperlipidemic and atherosclerotic rats

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#### ABSTRACT

**Aim:** To explore the therapeutic usefulness of the proteolytic enzyme Serratiopeptidase in the treatment of Hyperlipidemia and Atherosclerosis.

**Materials & Methods:** We observed the anti-atherosclerotic and anti-hyperlipidemic activity of Serratiopeptidase in atherogenic diet (AD) and Triton X-100 induced male albino wistar rats (150-200g). Animals were separated randomly into 5 groups with 6 rats in each group. Disease control, test groups and standard groups were administered with Triton X-100 (100mg/kg, i.p.) and fed with that of atherogenic diet for 21 days where as normal control rats were fed with normal diet. Test and standard groups were administered with Serratiopeptidase (10 mg/kg, p.o. low dose) and Fenofibrate (50mg/kg, p.o. high dose) subsequently for 21 days. Studies were undertaken to evaluate the effect of serratiopeptidase on physical and serum biochemical parameters by comparing the treated group with disease and normal control for every 7 days. Statistical analysis was carried out using two way ANOVA followed by Bonferroni's multiple comparison test.

**Results:** Serratiopeptidase had shown a significant ( $p < 0.05$ ) reduction in the body weights and lipid levels such as LDL, VLDL, TGL, TC, Atherogenic index and shown a significant ( $p < 0.05$ ) increase in HDL-C. Significant ( $p < 0.05$ ) improvement was also observed in atherogenic index. The results were compared with the standard. Histopathology of coronary arteries indicated the significant reduction in the lesion size in test and standard groups when compared with disease control.

**Conclusion:** Thus, it can be indicated that serratiopeptidase has a significant control in hyperlipidemic and atherosclerotic rats. The study can be further processed clinically as there is a need of a good anti-atherosclerotic & anti-hyperlipidemic drug without or less side effects.

**Keywords:** Serratiopeptidase, Protease, Metalloproteinase, Hyperlipidemia, Atherosclerosis, Fibrinolysis, Fenofibrate, Peroxisome proliferator-activated receptor..

#### INTRODUCTION

Atherosclerosis is a thrombotic and inflammatory disease of the arterial wall that is characterized by the

increased levels of lipoproteins in blood [1]. It develops gradually by lipid accumulation, lipid

oxidation and their modification which increase chronic inflammation in the susceptible walls of all major arteries. Fatty streaks which are developed initially evolve into fibrous plaques, where some of them develop into vulnerable forms to rupture, causing stenosis or thrombosis [2].

Hyperlipidemia is one of the important factors associated with atherosclerosis. It is characterized by excess levels of fatty substances i.e., lipids. The lipids that contribute to hyperlipidemia are cholesterol and triglycerides. They enter into the blood and attached to the proteins and remain dissolved while in circulation. Thus, it can also be called as hyper lipoproteinemia. It can increase the risk of developing coronary artery disease by making the arteries thickened or hardened in the heart muscle which results in chest pain and heart attack [3].

There are many drugs available to treat hyperlipidemia and atherosclerosis. They are statins, bile acid sequestrants, cholestyramine, colestipol fibrates and nicotinic acid derivatives [4]. One of the most effective and widely used drugs for the treatment of hyperlipidemia and atherosclerosis are fibrates. They act mostly in liver where they inhibit lipoprotein synthesis by stimulating the activity of PPAR- $\alpha$  (Peroxisome proliferator-activated receptor) which controls the transcription of regulatory genes of fatty acids and cholesterol metabolism.

Serratiopeptidase is a proteolytic enzyme [5]. It is an extracellular metalloproteinase which belongs to Protease class of enzymes. As the drug has wide range of actions in human body Serratiopeptidase has been called as the “**miracle Enzyme**” or “**super enzyme**”. The enzyme was derived from non-pathogenic Entero bacteria *Serratia E15* microorganisms which are present in the silk worm **Bombyxmori**. It will be produced in the intestines of silkworms to breakdown the cocoon walls. Proteases accelerate peptide bond hydrolysis. The enzyme reacts with substrate and attains tetrahedral transition state and forms Acyl-enzyme intermediate and finally leads to the formation of the product. Serratiopeptidase enzyme does not have any detrimental effects on the host's living cells but it has the ability to dissolve the vital tissue (silkworm protective cocoons) in silkworm intestines. The antigenicity of the enzyme after entering the human body is masked by bounding with  $\alpha$ -2 macroglobulin

in biological fluids i.e., blood in 1:1 ratio. It has an absorption maximum at 275-280 nm.

The enzyme has wide range of actions. In combination with gatifloxacin serratiopeptidase had a very good antibiotic activity [6]. It possesses good anti-ulcerogenic activity when compared to the NSAIDs [7]. The drug also has significant anti-inflammatory and analgesic effect [8]. It is widely used as an anti-coagulant. In combination with antibiotics it improves immunological response and decreases the bacterial infection. In combination with nattokinase it was used in treating Alzheimers [9]. It can also show improvement in treating the acne vulgaris [10]. Along with these it can be used in implant related infections [11].

As the conventional drugs which are used to treat serratiopeptidase have adverse effects like Rhabdomyolysis, Myositis, Arrhythmias, Gall stones etc there is a need of an effective anti-hyperlipidemic and anti-atherosclerotic drugs with lesser or no side effects. As serratiopeptidase is the drug with fewer side effects we attempted the study [6].

## MATERIALS AND METHODS

### Test materials

Serratiopeptidase drug was purchased from MARS Therapeutics & Chemicals limited, Hyderabad, India. The standard drug Feno fibrate was purchased from USV limited, Himachal Pradesh, India. Assay kits for serum Total Cholesterol (TC), Triglycerides (TGL), High density lipoproteins were purchased from Erba Mannheim, Transasia Bio-Medicals limited, Himachal Pradesh, India. All other chemicals were of analytical grade.

### Animals

The experiment was conducted using [12] male Albino Wistar rats (150-200g), at about 6-8 weeks of age. All the animals were procured from Sanzyme Ltd, Hyderabad, India. The animals were housed in poly acrylic cages (38cm x 23cm x 10cm) which have the capacity to carry six animals per cage. They were exposed to ambient temperature of 26-28°C with 12-h-light/12-h-dark cycle. The rats have free access to standard chow diet and provided with water ad libitum. The maintenance and handling of animals were performed to the rules and regulations of Control and Supervision of Experiments on Animals

(CPCSEA), New Delhi. The research protocols were approved by the Institutional Animal Ethics Committee.

Fibers	1g
Cholesterol	1g
Total weight	100g

### Drug treatment protocol

#### Serratiopeptidase

The LD50 value of serratiopeptidase (test) was 2500mg/kg, p.o.in rats. The effective doses of serratiopeptidase were fixed to be 10 mg/kg, b.w. and 50 mg/kg, b.w. orally. The solubility of serratiopeptidase was found to be in water and was insoluble in ether and alcohol.

#### Fenofibrate

Fenofibrate was considered to be as reference standard [13]. LD50 of fenofibrate (standard) was found to be >1242 mg/kg, p.o.the effective dose of fenofibrate was considered to be 65mg/kg, b.w, orally. The solubility of serratiopeptidase was found to be in 2:3 solutions of DMF and PBS (phosphate buffer solution) of pH-7.2.

#### Induction of disease

Single dose of Triton X 100 (100mg/kg, b.w.)[13] was administered intraperitoneally to the groups II, III, IV, V. Normal controls were injected with saline alone. Triton X-100 was dissolved in freshly prepared, 0.9% sodium chloride immediately before administration to the rats and subsequently fed with atherogenic diet. When Triton X-100 reaches critical micelle concentration (CMC), in the range of 0.19 to 0.20Mm, [14] the cells undergo irreversible permeabilization of its membrane and structural collapse which results in progression of the disease. Physical parameters were estimated at 1, 7, 14, 21 days. Biochemical parameters were also estimated in the same intervals by collecting the blood samples from retro orbital plexus under mild ether anesthesia. Then the rats were sacrificed on 22<sup>nd</sup> day, the heart was dissected out and proximal aortas were isolated for histopathological studies.

#### Composition of atherogenic diet-[15]

Protein (milk powder)	10g
Carbohydrates (wheat flour)	61g
Sugar	5g
Fat (Butter)	16g
Salts	4g
Vitamins	2g

### Grouping and Treatment Protocol

30 rats were selected randomly and divided into 5 groups with 6 animals in each group.

The animals were divided into 6 groups (Six rats/group)

**Group-1:** Normal control with normal diet

**Group-2:** Disease control- Triton X-100mg/kg b.w. + animals fed on atherogenic diet

**Group-3:** Test 1- Low dose - Triton X-100mg/kg b.w. + atherogenic diet + Serratiopeptidase (10mg/kg)

**Group-4:** Test 2- High dose -Triton X-100mg/kg b.w. + atherogenic diet + Serratiopeptidase (50mg/kg)

**Group-5:** Std- Triton X-100mg/kg b.w. + atherogenic diet + Fenofibrate (65mg/kg)

The animals were fed for a period of 21 days [13] with atherogenic diet and administered with single dose of Triton X-100 with a dose of 100 mg/kg i.p.

### Biochemical analysis

#### Collection of blood

Blood was collected from all the groups in regular intervals of 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup>, and 21<sup>st</sup> through retro orbital sinus puncture, under mild ether anesthesia. For serum separation the blood samples were subjected to centrifugation at 4000rpm for 10minutes at 37<sup>o</sup>c temperature. All the separated serum samples were stored at -20<sup>o</sup>c temperature until analysis. The serum was separated by micropipettes and it was used for various biochemical estimations. These estimations were carried out for 4 times in regular time intervals. At the end of the experiment, the animals were sacrificed by using CO2 inhalation chamber and the heart slices were excised out for histopathological evaluation.

#### Serum analysis

Atherosclerosis was assessed by quantifying the serum levels of Total cholesterol (TC), High density lipoproteins (HDL-C), Triglycerides (TGL), Low density lipoproteins (LDL-C), Very low density

lipoproteins (VLDL-C) were calculated as per the standard methods.

**Calculation of LDL Cholesterol (mg/dl):[16]**

$$\text{LDL cholesterol} = \text{Total cholesterol} - (\text{triglycerides}/5) - \text{HDL cholesterol}$$

**Calculation of VLDL Cholesterol (mg/dl): [17]**

$$\text{VLDL cholesterol} = \text{Triglycerides}/5$$

**Calculation of Atherogenic Index (AI)**

$$\text{AI} = \frac{\text{Total serum cholesterol}}{\text{Total serum HDL} - \text{Cholesterol}}$$

**Physical parameters**

**Body weight**

Exposure of rats to atherogenic diet and Triton X-100 induction led to increased weight gain due to the acceleration of hepatic cholesterol synthesis and absorption of dietary lipids by Triton X-100. Body weights of rats were measured in animal weighing balance at 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> days.

**Histopathological studies**

Rats were sacrificed and dissected. The heart was separated out from each rat and kept in 10% buffered formalin. Then the proximal aortas were isolated, made into slices and segmented [12]. The heart slices were fixed in 10% buffered formalin and were processed for embedding in paraffin. Sections (5-6

µm) were cut and stained with haematoxylin and eosin and examined for histopathological changes under 60X using photo light microscope.

**Statistical analysis**

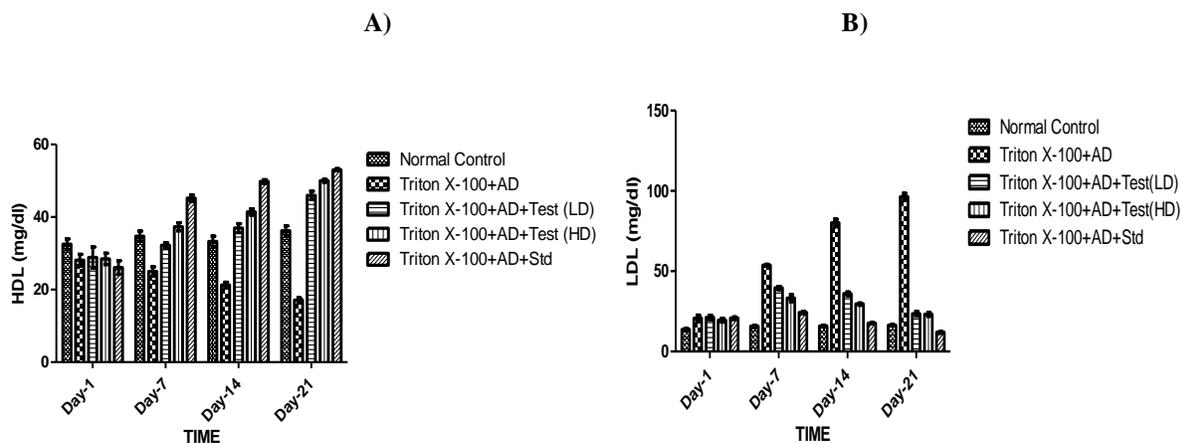
Statistical analysis of all obtained results was performed by two way ANOVA using graph pad prism software version 5.0 followed by Bonferroni's multiple comparison test. All the results were expressed as mean±SEM. A probability of p<0.05 was considered as significant.

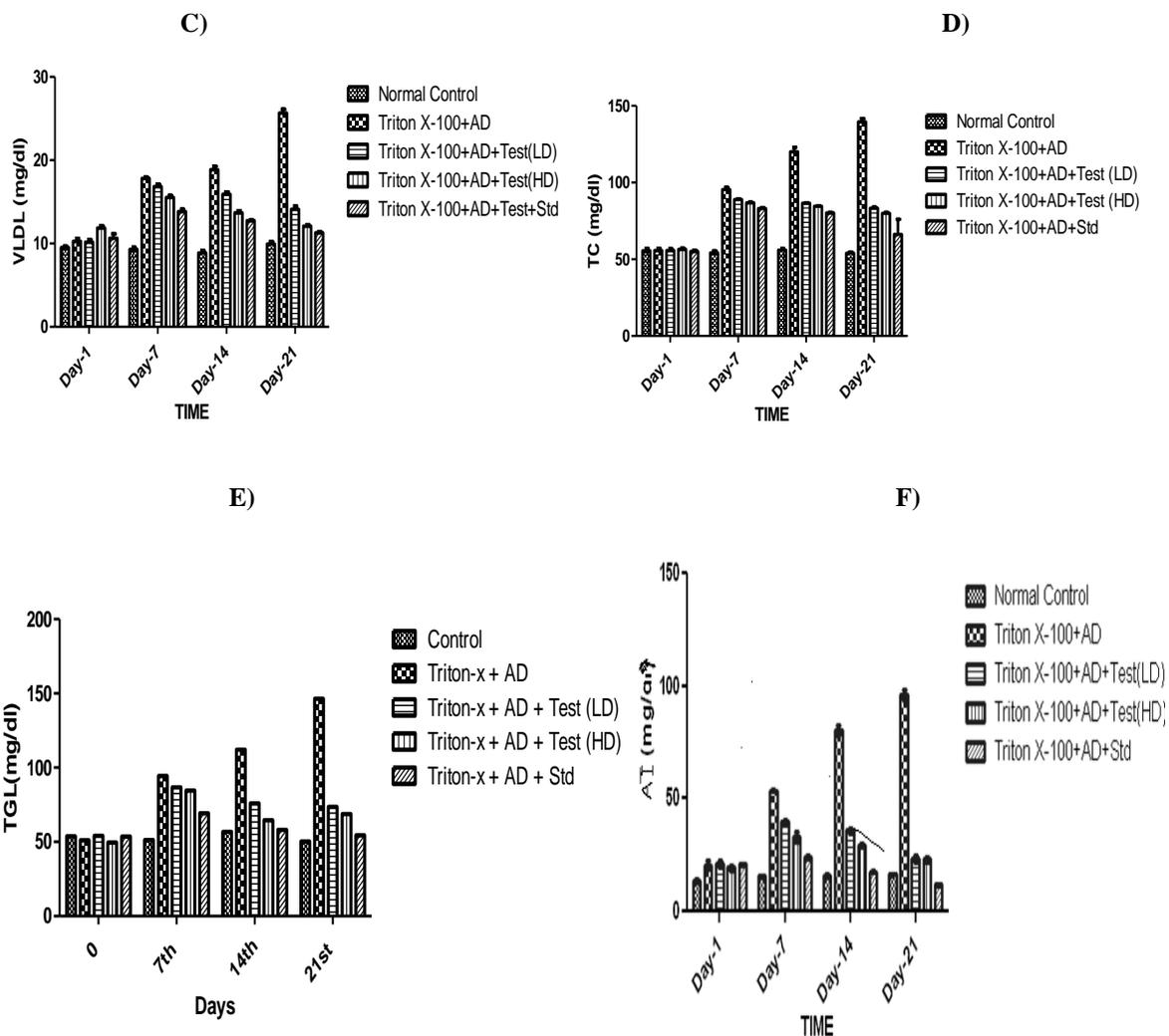
**RESULTS**

**Serum analysis**

**Effect of serratiopeptidase on Serum Lipid profile**

Disease control rats produced significant (p<0.05)increase in serum cholesterol (TC), Triglycerides (TGL), Low density lipoproteins (LDL-C), Very low density lipoproteins (VLDL-C), Atherogenic Index levels (AI) and significant (p<0.05)decrease in High density lipoproteins (HDL-C). Treatment with serratiopeptidase (test) at doses of 10 mg/kg and 50 mg/kg p.o. showed significant (p < 0.05) decrease in TC, LDL-C, VLDL-C, TGL, AI and significant (p < 0.05) increase in HDL-C. Treatment with standard drug fenofibrate also prevented the elevation of TC, LDL-C, VLDL-C, TGL, AI significantly (p < 0.05) and increased the HDL-C levels.





**Fig-1:** Effect of serratiopeptidase on changes in serum biochemical parameters of rats with AD and Triton X-100. A) High Density Lipoproteins B) Low Density Lipoproteins C) Very Low Density Lipoproteins D) Total Cholesterol E) Triglycerides F) Atherogenic index

Values are at mean ± SEM, error bar indicating the standard deviation, n = 6 animals. Treated group \* p ≤ 0.05 vsatherogenic control. \*\* p ≤ 0.05. Normal control. \*\* p ≤ 0.05 vsatherogeniccontrol \* p ≤ 0.05.

**Table 1:** Effect of serratiopeptidase on lipid parameters in rats fed with atherogenic diet and Triton X-100 induced rats for 21 days

GROUPS	TC	LDL-C	VLDL-C	HDL-C	TGL	AI
<b>Normal</b>						
Day-0	32.511±1.46	13.660±0.72	9.446±0.12	32.511±1.46	45.615±0.79	1.54±0.03
Day-7	34.726±1.42	15.705±0.46	9.238±0.17	34.726±1.42	46.267±1.17	1.59±0.23
Day-14	33.250±1.48	15.821±0.43	8.905±0.18	33.250±1.48	45.978±1.14	1.5±0.10
Day-21	36.243±1.33	16.373±0.36	9.858±0.19	36.243±1.33	48.303±0.75	1.26±0.12

**Disease control**

Day-0						
Day-7	28.126±1.58	20.630±1.89	10.365±0.26	28.126±1.58	46.785±0.72	1.62±0.13
Day-14	25.016±1.23	53.733±0.48	17.808±0.13	25.016±1.23	88.050±0.63	4.03±0.28
Day-21	21.255±0.79	80.026±2.27	18.968±0.26	21.255±0.79	106.733±3.8	4.27±0.29
	17.128±0.57	96.311±2.14	25.828±0.30	17.128±0.57	143.182±2.9	4.82±0.70

**Test-1**

Day-0	28.885±2.85*	21.141±1.25*	10.230±0.22	28.885±2.85*	47.58±1.10*	1.65±0.16*
Day-7	32.213±0.72*	39.51±0.87*	16.940±0.19*	32.213±0.72*	81.98±0.95*	3.88±0.09*
Day-14	36.960±1.21*	35.935±1.06	16.155±0.24*	36.960±1.21*	75.95±1.02	2.13±0.46*
Day-21	45.988±1.13*	23.49±1.36*	14.151±0.26*	45.988±1.13*	64.05±1.18*	1.74±0.09*

**Test-2**

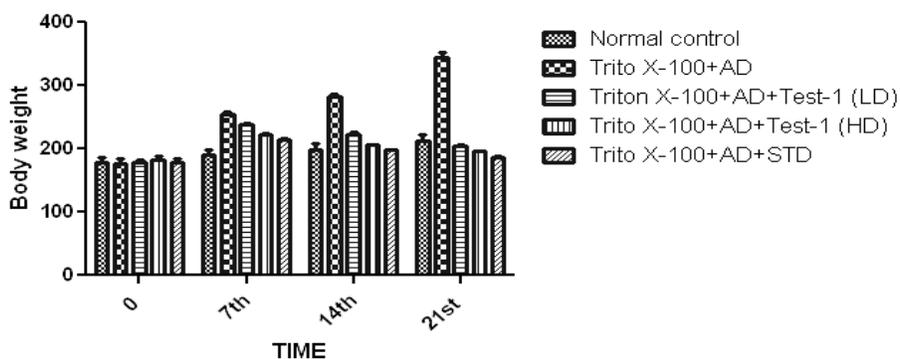
Day-0	28.463±1.53*	19.481±1.05*	12.062±0.25*	28.463±1.53*	48.10±0.88*	1.69±0.06*
Day-7	37.308±1.09*	33.23±2.08*	15.666±0.15*	37.308±1.09*	71.23±2.20*	2.94±0.04*
Day-14	41.435±0.83*	29.50±0.62*	13.781±0.17*	41.435±0.83*	61.67±1.57*	2.11±0.05*
Day-21	50.006±0.35*	23.11±1.03*	12.045±0.10*	50.006±0.35*	55.99±0.68*	1.53±0.05*

**Standard**

Day-0	26.091±1.87*	20.860±0.61*	10.888±0.38	26.091±1.87*	48.36±1.50*	1.72±0.01*
Day-7	45.225±0.91*	24.22±0.71*	13.943±0.27*	45.225±0.91*	65.57±1.82*	2.13±0.05*
Day-14	49.805±0.48*	17.57±0.31*	12.748±0.08*	49.805±0.48*	59.04±1.59*	1.78±0.04*
Day-21	53.031±0.27*	11.93±0.39*	11.340±0.10*	53.031±0.27*	52.752±2.0*	1.50±0.06*

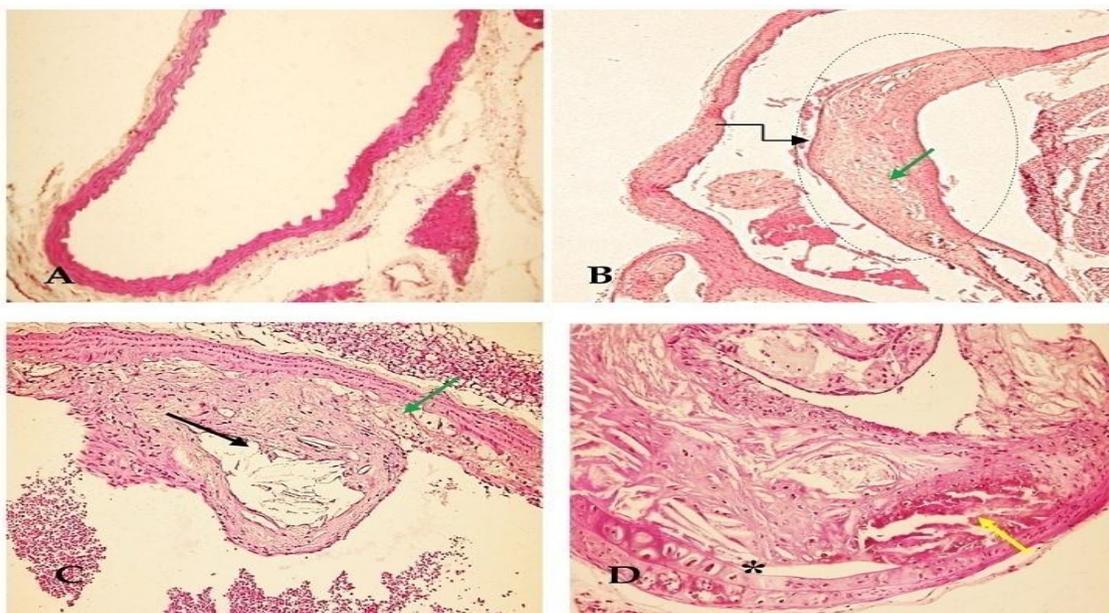
**II) Effect on physical parameters**

Effect on Body weights



GROUPS	Day-0	Day-7	Day-14	Day-21
Normal	177.500±8.34	190.500±8.66	199.16±8.604*	213.33±9.08
Disease control	176.667±6.67	254.883±2.587	282.50±4.425*	343.333±8.33
Test-1	179.167±3.05*	238.167±2.272*	223.00±2.436*	204.83±1.86*
Test-2	182.333±6.25*	222.500±1.118*	105.833±1.447	196.33±0.71*
Standard	178.167±6.90*	215.00±1.238*	198.33±0.919*	187.17±1.62*

**Effect of Serratiopeptidase treatment on histopathological changes of rat myocardium**



**Representative cross-sections of rat aortas.**

**A) Standard drug treated**

No atherosclerotic lesions were found in standard drug (fenofibrate) treated group.

**B) Serratiopeptidase drug high dose treated**

Atherosclerotic plaque (outlined) characterized by a thin fibrous tissue cap (elbow black arrow), particularly superficial accumulation of foam cells (green arrow) without a necrotic core and encapsulated by collagen rich fibrous tissue;

**C) Serratiopeptidase drug low dose treated**

accelerated atherosclerosis and deposition of cholesterol crystals (black arrow) in the endothelium of the aorta wall

**D) Disease control group**

Advanced lesions were developed. All the characters of atherosclerotic plaque can be seen. Like xanthoma

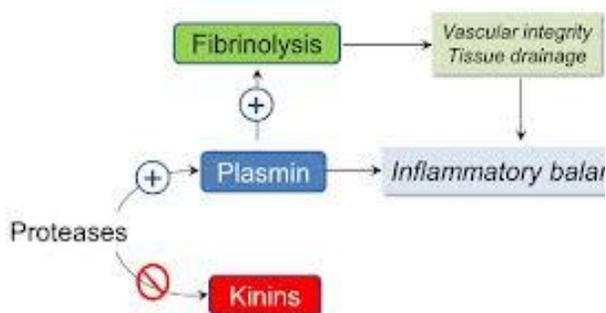
formation, cartilage tissue (asterix) and calcified nodules (yellow arrow) with an underlying fibrocalcific plaque with minimal or absence of necrosis occur (H&E staining, microscopic magnification applied x 100).

**DISCUSSION**

Hyperlipidemia has been documented as one of the major causative factor for atherosclerosis, resulting in coronary heart diseases (CHD). Among all the lipids LDL-C plays a major role in the atherosclerotic plaque production, by the formation of ox-LDL free radicals. Hence the major target in reducing the fatty plaque is to reduce the levels of LDL-C in the blood supply to the organs which have risk of CHD. Along with LDL-C the other lipid parameters like TGL, VLDL-C, atherogenic index, body weight should also be considered.

Triton X-100 is a non-ionic surfactant which accelerates the hepatic cholesterol synthesis and

increases the intestinal lipid absorption by the process of emulsification. It has been proved that non-ionic detergent like triton X-100 results in a progressive disturbance on membrane permeability of the cells [18]. Triton X-100 cause lysis of cells, permeabilize the living cell membrane for transfection. On prolonged exposure to Triton X-100 the cells die because of the polar head groups of surfactant disrupts hydrogen bonding present within the cells lipid bilayer which results in destruction of the compactness and integrity of the lipid membrane [18]. Hence this mechanism can be extended to the endothelial cells of blood vessel. The proteolytic activity of serratiopeptidase may be expected to have plaque dissolving capacity [2] as atherosclerotic plaque is made up of proteins.



The present study was aimed to evaluate the anti-hyperlipidemic and anti-atherosclerotic effect of the drug serratiopeptidase in atherosclerotic rats and compare its response with that of standard fenofibrate. In that concern triton X-100 was administered intraperitoneally to the rats. Triton X-100 increases osmotic pressure in the RBC, hemolysis consequently to the death of animals. Hence its dose was monitored. On administration of serratiopeptidase in atherosclerotic rats, it had shown beneficial effect by maintaining the abnormal biochemical parameters which are altered in atherosclerosis to the normal state and reversed the histopathological changes observed in tissue sections of infarcted heart. Serratiopeptidase was a proteolytic enzyme which was assumed to have plaque dissolving capacity in atherosclerotic rats and by the present study, it was proved that serratiopeptidase can be used as an anti-atherosclerotic drug. As several anti-atherosclerotic drugs show many adverse effects, there was a need of an effective anti-atherosclerotic drug with no/less side effects. Serratiopeptidase was one such drug, which

possess only one side effect i.e., hemorrhage. Hence serratiopeptidase can also be used as an anti-coagulant because it has a side effect of hemorrhage which can be useful. The anti-atherosclerotic effect of serratiopeptidase was compared with fenofibrate and found that serratiopeptidase possess almost near activity to that of fenofibrate.

Administering triton X-100 I.P 100mg/kg along with the atherogenic diet progressively increased the levels of lipids in the blood like cholesterol, LDL-C, TGL, VLDL-C, atherogenic index, body weight and caused decrease in HDL-C and atherogenic index. Our findings demonstrate that treatment with serratiopeptidase led to the reduction in body weight in triton X and diet induced obese rats. Body weights of untreated rats were significantly higher than those in normal rats. A decrease in body weight ( $p < 0.05$ ) was observed in hyperlipidemic atherosclerotic rats after the treatment with serratiopeptidase for 21 days and the effect was compared with the fenofibrate (STD). There was a significant ( $p < 0.05$ ) control in the levels of serum lipids and increase ( $p < 0.05$ ) in HDL-C levels in standard and serratiopeptidase treated hyperlipidemic rats. The serum lipids were significantly higher in the untreated hyperlipidemic atherosclerotic rats compared to those in the normal rats, while HDL-C levels were significantly decreased in untreated hyperlipidemic rats compared to normal rats. The atherogenic index which is the measure of the extent of atherosclerotic lesions based on the serum lipids. This was estimated in all groups. This index reports the extent of lipid deposition as plaque or fatty streaks in coronaries, aorta, liver and kidney. Treatment with standard fenofibrate caused reduction in AI significantly ( $p < 0.05$ ) and the effect of serratiopeptidase was almost near to the standard response. We can also observe the protection of endothelium in treated groups compared to the disease induced groups by histopathological reports. Moreover, the protein called Abresham which was originated from silk worm cocoon was proved to have cardio protective action isoprenaline induced myocardial infarction in rats. The enzyme serratiopeptidase was also expected to have cardio protective effect because Abresham and serratiopeptidase are similar in origin. But further study should be conducted to prove this [19].

## CONCLUSION

The above data suggest that serratiopeptidase possess weight reduction properties with no interference with inflammation. The findings of the study ensure that serratiopeptidase effectively able to reduce the serum lipids like LDL-C, TC, TGL, VLDL-C, AI and

significant increase in HDL-C. It can be concluded that serratiopeptidase has a potential role in suppressing triton X induced hyperlipidemia and atherosclerosis. Further investigation is needed to confirm the anti-atherosclerotic effect of serratiopeptidase clinically.

## REFERENCES

- [1] S.P Prabha, P.N Ansil, A. Nitha, P.J Wills, M.S Latha. Anti-atherogenic activity of methanolic extract of gardenia gummiferalinn.f. on high fat diet induced atherosclerosis in rats . Int J Pharm Sci. 5(2), 2013, 388-93.
- [2] Insull W Jret al. The pathology of atherosclerosis: plaque development and plaque responses to medical treatment. Am J Med. 122, 2009, S3-S14.
- [3] K. Hari Kumar et al. A Review on Hyperlipidemic. IJNTPS. 3, 2013, 159-171.
- [4] Sagar P et.al. Therapeutic approaches to drug targets in hyperlipidemia. Biomedicine. 2, 2012, 137-146.
- [5] Chaudhari SA, Mali AK. Production, characterization & optimization of potent protease (serratiopeptidase) from *Serratiamarscens* E15. Int. Res J Pharm. App Sci. 3(4), 2013, 95-8.
- [6] Jyothi P, Kiran KJ, Radhika M. Antimicrobial studies of selected antibiotics and their combination with enzymes. Int J Pharmacy Pharm Sci. 2 (3), 2010, 43-4.
- [7] Misraulia.K.S, Kaskhedikar.P. Ulcerogenic activity of certain non-steroidal anti-inflammatory drugs and enzymes on gastric mucosa of rats. *Indian J. Field Vet.*6, 2010, 42-4.
- [8] R. Garg, Shafiq.A, Garg.A, Walia.R. Prospective Comparative Study of serratiopeptidase and aceclofenacin Upper and Lower Limb Soft Tissue Trauma Cases. IJPPT.1, 2012, 2277 –3436.
- [9] Fadi NN, Ahmed HH, Booles HF, Sayed AH. Serrapeptase and nattokinase intervention for relieving Alzheimer's disease pathophysiology in rat model. Hum Exp Toxicol Jul. 32(7), 2013, 721-35.
- [10] Ehab MM, Mahdi YM. Serratiopeptidase a Hope in a Rapid and Better Improvement of Inflammatory Acne Vulgaris. Iraqi J Pharm Sci. 21(1), 2012, 78-81.
- [11] Mecikoglu M, Saygi B, Yilidrim Y, Karadag-Saygi E, Ramadan SS, Esemeli T. (2006) The effect of proteolytic enzyme serratiopeptidase in the treatment of experimental implant-related infection. J Bone Joint Surg Am. 88(6), 2006, 1208-14.
- [12] Gerhard Vogel. (2002). Anti-Atherosclerotic Activity, 2<sup>nd</sup> edition (p.1676-77). Newyork: Springer-Verlag Berlin Heidelberg.
- [13] Sudha S.S, Karthic R, R. Naveen, J.Rengaramanujam. Anti hyperlipidemic activity of *Spirulina platensis* in Triton X-100 induced hyperlipidemic rats. Hygeia.J.D.Med. 3(2), 2011, 32-37.
- [14] Chatzizisis YS, et al. Prediction of the localization of high-risk coronary atherosclerotic plaques on the basis of low endothelial shear stress: an intravascular ultrasound and histopathology natural history study. Circulation. 117(8), 2008, 993-1002.
- [15] Dhandapani R. Hypolipidemic activity of *Eclipta prostrata* (L.) L. leaf extract in atherogenic diet induced hyperlipidemic rats. Indian J Exp Biol. 45(7), 2007, 617-9.
- [16] Friedewald, W.T, Levy, R.I, Friedrickson, D.S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin.Chem. 18, 1972, 499-502.
- [17] Davidson M et.al. Measurement of LDL-C after treatment with the CETP inhibitor anacetrapib. Lipid Res. 54, 2013, 467-72.
- [18] Koley D, Bard AJ. Triton X-100 concentration effects on membrane permeability of a single HeLa cell by scanning electrochemical microscopy (SECM). Proc Natl Acad Sci. 107, 2010, 16783-87.
- [19] RK Srivastav, Siddiqui HH, Imam MT, Ahsan F. Evaluation of cardioprotective effect of silk cocoon (Abresham) on isoprenaline-induced myocardial infarction in rats. Avicenna J Phytomed. 3(3), 2013, 216-23.