Sinomenine Suppress the Vitamin D3 and High Fat Induced Atherosclerosis in Rats via Suppress of Oxidative Stress and Inflammation

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Abstract: Atherosclerosis (AS) is a cardiovascular disease that arise due to dysfunction of lipid deposition and metabolism. AS is causes the mortality and morbidity worldwide. Sinomenine isolated from the Sinomenium acutum is used extensively against the various cardiac diseases in China. However, the anti-atherosclerosis effect of sinomenine still not explore. In this study, we explore the cardioprotective and anti-atherosclerosis effect of sinomenine against Vitamin D3 and High fat induced atherosclerosis in rats. Sprague Dawley (SD) rats were used in this study. The rats were received the vitamin D (60000) and High fat diet to induce the atherosclerosis and divided into groups and received the oral administration of sinomenine (2.5, 5 and 10 mg/kg) and simvastatin (5 mg/kg). Body weight, organ weight and biochemical parameters were estimated. The mRNA expression of MyD88, TLR4, NF-κB and IκB were estimated. Sinomenine treated rats significantly (p<0.001) suppressed the body weight and modulated the organ weight (hepatic, renal and heart). Sinomenine significantly (p<0.001) decreased the level of triacylglycerols (TG), low density lipoprotein cholesterol (LDL-c), total cholesterol (TC), very low-density lipoprotein cholesterol (VLDL-c) and augmented the level of high-density lipoprotein cholesterol (HDL-c). Sinomenine treatment also reduced the level of atherogenic index (TC/HDL-c and LDL-c/HDL-c). Sinomenine treatment decrease the ratio of HMG CoA/Mevalonate and level of collagen and total protein. Sinomenine significantly (p<0.001) altered the level of heart parameters, antioxidant parameters and inflammatory cytokines. Sinomenine significantly (p<0.001) reduced the expression of MyD88, TLR4, NF-κB and IκB. Taken together, sinomenine exhibited the protective effect against the atherosclerosis via alteration of TLR4/NF-κB signaling pathway.

Key words: atherosclerosis, sinomenine, heart parameters, cytokines, TLR4/NF-κB signaling pathway

Abbreviations: AS; Atherosclerosis, SD; Sprague Dawley, TG; Triacylglycerol, LDL-c; Low density lipoprotein cholesterol, TC; Total cholesterol, VLDL-c; Very low density lipoprotein cholesterol, HDL-c; High density lipoprotein cholesterol, CVD; Cardiovascular disease, MDM; Monocyte derived macrophages, SMC; Smooth muscle cells, EC; Endothelial cells, NO; Nitric oxide, AngII; Angiotensin II, eNOS; Endothelial nitric oxide synthase, NF-κB; Nuclear factor-kB, IL-1β; Interleukins-1β, IL-6; Interleukin-6, TNF-α; Tumor necrosis factor-α, COX-2; Cyclooxygenase-2, ICAM-1; Intracellular adhesion molecule-1, VCAM-1; Vascular cell adhesion molecule-1, SM; Sphingomyelin, DOX; Doxorubicin, GPx; Glutathione peroxidase, MDA; Malonaldehyde, SOD; Superoxide dismutase, CAT; Catalase, CMC; Carboxymethyl cellulose, ALP; Alkaline phosphatase, ALT; Alanine aminotransferase, AST; Aspartate aminotransferase, LDH; Lactate dehydrogenase, CK-MB; Creatine kinase-MB, TXB; Thromboxane B₂, cTnI; Troponin I, FFA; Free fatty acids, CPK; Creatine phosphokinase, IKK; Inhibitor of nuclear factor kappa-kinase

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1 Introduction

Cardiovascular disease (CVD) and its consequences are widely regarded as the leading cause of death in the world\(^1\). According to a recent study, more than 80% of CVD-related deaths occur in low- and middle-income nations, such as China\(^2\). Atherosclerosis (AS) is the leading cause of cardio-cerebrovascular disease, such as stroke, and it has the highest mortality rate in the world\(^3\). AS is a complicated condition that is frequently associated with arteriosclerosis and atheromatosis. AS is a proliferative response, fibro-fatty, and excessive formation of inflammatory reaction to damage the arterial wall, which is engaged in several types of cells, including monocyte derived macrophages (MDM), smooth muscle cells (SMC), and endothelial cells (EC), at the cellular level\(^4\). Various researchers assume that AS is triggered by basic endothelial dysfunction, such as that shown in hypertension and hyperlipidemia, which results in an imbalance of nitric oxide (NO) and angiotensin II (Ang II) in the arterial wall. This cascade initiates changes in the expression of adhesion molecules on the endothelium’s surface, proliferation, muscle phenotypic changes, monocyte invasion of the artery wall, and foam cell production\(^5\). A previous report showed that endothelial nitric oxide synthase (eNOS) a critical enzyme observed in the EC and is derived from NO and suppresses the oxidation of LDL-c, smooth muscle cell proliferation, platelet aggregation and adhesion, boosts arterial vasodilation and inhibits monocyte adhesion to endothelium\(^6\). AS is a multi-factorial inflammatory disease, categorized by the occurrence of lesions due to lipid accumulation in the arterial wall\(^7, 8\). A lot of factors, such as environmental, genetic and metabolic, are involved in the evolution and formation of atherosclerotic plaque. Hypercholesterolemia is the major risk factor for AS in humans. Hypercholesterolemia, increased the level of LDL-c and TC and other significant contributors to AS such as insulin resistance, inflammatory and oxidative stress\(^9, 10\). Oxidative stress and lipoprotein oxidation play a significant role in the pathogenesis of atherosclerosis. The pathogenic processes and risk factors for AS have been the focus of research for the past three decades. Despite this study, medicines to prevent or, more critically, reverse the catastrophic effects of AS are still a long way off\(^11, 12\).

Previous research suggests that inflammatory reactions and oxidative stress enhance the AS. The Pro-inflammatory transcription factor, nuclear factor-κB (NF-κB), is a well-known inflammatory reaction mediator and induced by oxidative stress\(^6, 8, 13\). The NF-κB boosts the transcription of inflammatory cytokines such as interleukins (IL-1β and IL-6), tumor necrosis factor-α (TNF-α), eNOS and cyclooxygenase-2 (COX-2). The level of inflammatory mediators includes intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), boosted by the activation of NF-κB in the aorta during the AS\(^14, 15\). During the AS disease, the level of inflammatory molecules is increased and they lead to the expansion of a chronic inflammation state\(^16, 17\).

Increased serum cholesterol has been linked to foods high in cholesterol and saturated fat. According to reports, lipid-rich diets typically accelerate or augment the prevalence of atherosclerotic lesions in AS murine models\(^17\). As a result, consuming a high-fat diet to develop atherosclerosis is a beneficial tool for learning more about the disease and its treatment effects\(^18\). Traditional Chinese medicine significantly contributes to the health care of the population in China. Recently, traditional Chinese medicine is getting more popularity for treating various diseases\(^20, 21\). Plants and plant-derived phyto-constituents have traditionally been a valuable source of medicine for a variety of diseases, and their pharmacological potential is attracting more attention\(^22\).

Sinomenine (SM) (7,8-didehydro-4-hydroxy-3,7-dime-thoxy-17-methylmorphinane-6-one) isolated from the Si-nomenium acutum (Chinese medicinal plant) and treat to various diseases especially rheumatic disease over the 2000 years\(^23\). SM having pharmacological significant against various types of diseases. SM already proved chronic glomerulonephritis, allograft rejection, autoimmune nephritis and mesangial proliferative nephritis\(^24\). Previous research suggest that SM suppress the synovial fibroblast proliferation and lymphocyte as well as macrophage infiltration and reduces the production of inflammatory cytokines\(^24, 26\). SM exhibited the protective effect against podocyte damage. SM also suppressed the free radical production against doxorubicin (DOX) induced nephritic syndrome\(^25\). SM already confirm own anti-inflammatory and antioxidant effects. In this study, we examine the cardio-protective effect of sinomenine against vitamin D and HFD induced atherosclerosis.

2 Methods

2.1 Chemical

Sinomenine (98\%) was purchased from the Sigma Aldrich (St. Louis, USA). All the chemical and reagent used in this study was analytical grade.

2.2 Animals

SD (aged: 8-10 weeks; sex: male; weight: 200 ± 25 g) were procured from the Experimental animal center. The rats were housed in the standard condition of an animal house. The rats were housed at 22°C and 70% relative humidity. The rats were also maintained on a light/dark cycle (12 h) and received the basic diet and HFD (Table 1). All animal protocols followed animal welfare guidelines, and all experimental methods followed the Institute’s Guide for the Care and Use of Laboratory Animals and relevant ethical re-
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2.3 Preparation of the drugs

The drugs are freshly prepared and intragastrically administered to the animal as a suspension. The suspension was prepared with an appropriate quantity of sinomenine dissolved in a 1% carboxymethyl cellulose (CMC) solution.

2.4 Experimental procedure

The rats were acclimatized for one week before adopting the laboratory conditions. The rats were divided into the following groups as follows:

- Group I: Normal control (received oral administration of 1% CMC),
- Group II: AS control group (received oral administration of 1% CMC),
- Group III: AS + Sinomenine (2.5 mg/kg),
- Group IV: AS + Sinomenine (5 mg/kg),
- Group V: AS + Sinomenine (10 mg/kg) and
- Group VI: AS + Simvastatin (5 mg/kg), respectively.

In this experimental protocol, all groups contain 12 rats. A high fat diet and vitamin D3 were used for the induction of AS. AS group rats received a high fat diet along with vitamin D3 (600000 IU/kg). The rats received the diet for 17 weeks, except control group rats (received a normal diet). After 7 weeks of high fat treatment, rats received the oral administration of sinomenine and simvastatin till end of the experimental protocol. The tested drug was decided via titrated according to the animal weight. All the drugs are prepared immediately before the intragastrically administration.

2.5 Sample collection

At end of the experimental protocol, the rats were fasted overnight and anesthetized using diethyl ether to collect the blood. The blood sample was collected from the retro orbital plexus. The collected blood was centrifuged for 15 min at 5000 rpm at 4°C to separate the serum.

2.6 Biochemical parameters

Enzymatic kit was used for the estimation the level of serum TG, LDL-c, TC and HDL-c using Hitachi 7150 Autoanalyzer (Hitachi High Technology, Tokyo, Japan). All the lipid parameters were estimated using the enzymatic kits (Zhongsheng Beikong Biotechnology and Science Inc., Beijing, China) following the manufacture protocol. The level of LDL was estimated using the following formula.

\[
\text{LDL-c} = \text{TC} - (\text{TG}/5 + \text{HDL-c})
\]

Calcium level was estimated in the tissue and serum.

Table 1 List of High fat diet composition.

| S. NO | Ingredient                          | Standard diet            | High fat diet            |
|-------|------------------------------------|--------------------------|--------------------------|
| 1     | Corn Starch                        | 495.7 g (1983 kcal)      | 72.8 g (291 kcal)        |
| 2     | Casein (30 mesh)                   | 140 g (560 kcal)         | 200 g (800 kcal)         |
| 3     | Maltodextrin 10                    | 125 g (500 kcal)         | 100 g (400 kcal)         |
| 4     | L-Cystine                          | 1.8 g (7.2 kcal)         | 3 g (12 kcal)            |
| 5     | Soybean Oil                        | 40 g (360 kcal)          | 25 g (225 kcal)          |
| 6     | Sucrose                            | 100 g (400 kcal)         | 172.8 g (691 kcal)       |
| 7     | t-Butylhydroquinone                | 0.008 g (0 kcal)         | 0 g (0 kcal)             |
| 8     | Cellulose, BW200                   | 50 g (0 kcal)            | 50 g (0 kcal)            |
| 9     | Mineral Mix S10022M                | 35 g (0 kcal)            | 0 g (0 kcal)             |
| 10    | Vitamin Mix V10037                 | 10 g (0 kcal)            | 0 g (0 kcal)             |
| 11    | DiCalcium Phosphate                | 0 g (0 kcal)             | 13 g (0 kcal)            |
| 12    | Calcium Carbonate                  | 0 g (0 kcal)             | 5.5 g (0 kcal)           |
| 13    | FD&C Red Dye#40                   | 0 g (0 kcal)             | 0.05 g (0 kcal)          |
| 14    | Mineral Mix S10026                 | 0 g (0 kcal)             | 10 g (0 kcal)            |
| 15    | Choline Bitartate                  | 2.5 g (0 kcal)           | 2 g (0 kcal)             |
| 16    | Vitamin Mix V10001                 | 0 g (0 kcal)             | 10 g (40 kcal)           |
| 17    | Cholesterol                        | 0 g (0 kcal)             | 18 g (0 kcal)            |
| 18    | Lard                               | 177.5 g (1598 kcal)      | 177.5 g (1598 kcal)      |
| 19    | H2O                                | 0 g (0 kcal)             | 16.5 g (0 kcal)          |
| 20    | Total                              | 1000 g (3850 kcal)       | 876.15 g (4057 kcal)     |
using commercial kits following the manufacture protocol (Nanjing Jiancheng Biotechnique Institute, Nanjing, China). For the estimation of calcium level in the aortic tissue, the tissue was homogenized in saline and centrifuged for 30 min at 3000 rpm. After centrifuge, separate the supernatant and estimate the protein concentration using a spectrophotometer. The level of NOx(NO2 + NO3) was estimated using the fluorometric assay kit.

Hepatic parameters such as alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated using commercial kits (Nanjing Jiancheng Biotechnique Institute, Nanjing, China) following the manufacturer’s instructions.

The heart parameters such as lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB), thrombaxone B2 (TXB2), Troponin I (cTnI) were estimated using the commercially available kits (Nanjing Jiancheng Biotechnique Institute, Nanjing, China) following the manufacture instructions.

Antioxidant parameters such as malonaldehyde (MDA), glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) were estimated using the previous reported method with minor modification5,7,28,29.

The level of total protein, collagen and HMG-CoA/mealonate ratio was estimated using the previous reported method with minor modification5,7,28,29.

The antioxidant parameters include glutathione peroxidase (GPx), malonaldehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) were determined as per the previous method with minor modification5,7,28,29.

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2.7 mRNA expression

A series of involvements were utilised to isolate total RNA using real-time PCR TRizol reagent. The RNA was measured with a NanoDrop spectrophotometer, and the specified amount of cDNA was generated using the iScript cDNA synthesis kits manufacturing technique. The primer sequences are provided in Table 2. As an internal standard, GAPDH was used.

Table 2 list of primers.

| S.no | Genes | Forward | Primers | Reverse |
|------|-------|---------|---------|---------|
| 1    | TLR4  | GTCTCCTCCACATCCCTCCCT | CTCCCAGAAACCAACCGATG |
| 2    | MyD88 | GTCTCCTCCACATCCCTCCCT | CAGTGGCCGGATCTCCAAAGT |
| 3    | NF-κB | TGGGAAGTGTAGGTCCTCTCAAC | TCTCTGTACCCGGCGATTAGC |
| 4    | NLRP3 | GCTCCTTGGCTGGTCTCAGCGC | TTGTGCTTCCAGATGGCGTCAG |
| 5    | GAPDH | AAAATCAAGTGGGGGCGATG | GATGACCCCTTTTGTCCTCCC |

2.8 Statistical analysis

The statistical analysis was performed using Graph Pad Prism version 7 (St. Louis, USA). All of the data was given as a mean with standard deviation. For repeated comparisons of therapy between the groups (disease versus tested), ANOVA was employed, followed by Dunnett’s test. It was determined that p < 0.05 was statistically significant.

3 Results

3.1 Body and organ weight

The effect of the tested and standard drugs on body and organ weight is shown in Fig. 1. The regular pattern of body weight gain was seen in normal rats. Due to continuous received the HFD diet, AS rats showed the increased body weight. When compared to AS group rats, Sinomenine treated rats had a lower body weight. The body weight of simvastatin rats was reduced (Fig. 1a).

Figure 2b showed the increased liver weight and Figs. 2c and 2d demonstrated the reduced heart and kidney weight in AS rats. Simomenine and simvastatin treated rats demonstrated the reduction in the liver weight and enhancement in the heart and kidney weight.

3.2 Lipid profile

During the AS disease, altered the lipid parameters and similar result was observed in the AS group. AS group showed the enhancement in the level of total cholesterol (Fig. 2a), triacylglycerol (Fig. 2b), LDL-c (Fig. 2c), VLDL-c (Fig. 2d) and reduction in the level of HDL-c (Fig. 2e). Figure 2f exhibited significantly (p<0.001) increased level of FFA in the AS group. Sinomenine treated rats presented the decreased level of total cholesterol (Fig. 2a), triacylglycerol (Fig. 2b), LDL-c (Fig. 2c), VLDL-c (Fig. 2d) and reduction in the level of HDL-c (Fig. 2e) and upregulation in the level of HDL-c (Fig. 2e). Sinomenine treated rats also reduced the level of FFA.

Figure 3 showed the upregulation in the ratio of TC/HDL-c (Fig. 3a) and LDL-c/HDL-c (Fig. 3b) in the AS group. Sinomenine treated rats showed the reduction in the ratio of TC/HDL-c and LDL-c/HDL-c.
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Fig. 1  Effect of sinomenine on the body and organ weights of vitamin D and high fat induced atherosclerosis in rats. a: body weight, b: liver weight, c: heart weight and d: kidney weight. Data are presented as mean ± SEM, compared to atherosclerosis group. Where *p<0.05, **p<0.01 and ***p<0.001.

Fig. 2  Effect of sinomenine on the lipid parameters of vitamin D and high fat induced atherosclerosis in rats. a: total cholesterol, b: triacylglycerol, c: low density lipoprotein, d: high density lipoprotein, e: very low density lipoprotein and f: free fatty acid. Data are presented as mean ± SEM, compared to atherosclerosis group. Where *p<0.05, **p<0.01 and ***p<0.001.

Fig. 3  Effect of sinomenine on the atherogenic index of vitamin D and high fat induced atherosclerosis in rats. a: TC/HDL-c and b: LDL-c/HDL-c. Data are presented as mean ± SEM, compared to atherosclerosis group. Where *p<0.05, **p<0.01 and ***p<0.001.

3.3 Collagen, total protein and HMG CoA/Mevalonate ratio
AS group rats showed the increased ratio of HMG CoA/Mevalonate (Fig. 4a), collagen (Fig. 4b) and total protein (Fig. 4c) as compared to normal group. Sinomenine treated rats showed the significantly (p<0.001) decreased the ratio.
of HMG/CoA/Mevalonate, collagen and total protein.

3.4 LDH, CK-MB and cTnI

AS group rats demonstrated the increased level of LDH (Fig. 5a), CK-MB (Fig. 5b) and cTnI (Fig. 5c) and sinomenine treated rats significantly ($p<0.001$) showed the reduction in the level of LDH, CK-MB and cTnI.

3.5 TXB$_2$, ET-1 and NO

AS group rats showed the increased level of TXB$_2$ (Fig. 6a), ET-1 (Fig. 6b) and reduced level of NO (Fig. 6c). Sinomenine treated rats significantly ($p<0.001$) suppressed the level of TXB$_2$ (Fig. 6a), ET-1 (Fig. 6b) and increased level of NO (Fig. 6c).

3.6 Calcium

Figures 7a and 7b demonstrated the altered level of calcium in the serum and aortic. Figure 7a showed the increased aortic calcium level and Fig. 7b showed the reduced serum calcium level and sinomenine treated rats.
showed the reduced aortic calcium level and increased the serum calcium level.

3.7 Aorta media and platelet aggregation
AS group rats showed the increased thickness of aortic media (Fig. 8a) and reduction of maximum platelet aggregation (Fig. 8b) and sinomenine significantly (p<0.001) reduced the thickness of aortic media and increased the maximum platelet aggregation.

3.8 Hepatic parameters
Figure 9 showed the effect of the sinomenine on the
level of hepatic parameters. AS group rats demonstrated the increased level of ALT (Fig. 9a), AST (Fig. 9b) and ALP (Fig. 9c) and sinomenine treatment significantly \(p < 0.001\) suppressed the level of hepatic parameters.

3.9 Antioxidant parameters

Figure 10 demonstrated the level of antioxidant parameters of different group of rats. AS group rats demonstrated the decreased level of SOD (Fig. 10a), GPx (Fig. 10b), CAT (Fig. 10c) and improved the level of MDA (Fig. 10d) and sinomenine significantly \(p < 0.001\) altered the level of antioxidant parameters.

3.10 Pro-inflammatory cytokines

AS group rats demonstrated the enhanced level of IL-1β (Fig. 11a), TNF-α (Fig. 11b) and IL-6 (Fig. 11c) and sinomenine treatment significantly \(p < 0.001\) suppressed the level of inflammatory cytokines.

3.11 mRNA expression

mRNA expression of TRIL4 (Fig. 12a), MyD88 (Fig. 12b), NF-κB (Fig. 12c) and NLRP3 (Fig. 12d) boosted in the AS group and sinomenine and simvastatin treatment significantly \(p < 0.001\) reduced the mRNA expression.
4 Discussion

Lipid metabolism is widely known for maintaining fat breakdown and synthesis of fat in the biological tissue\(^\text{[17]}\). Cholesterol is an essential component of biological membranes and a key precursor for the production of a variety of chemicals, including vitamin D, bile acids, and steroidal hormones\(^\text{[17, 18, 30]}\). The higher concentration of cholesterol in the serum enhances the risk for the expansion of coronary heart diseases. In this study, AS group rats exhibited an augmented level of TG, FFA, LDL and TC and reduced the HDL level in the circulation. Dyslipidaemia, which increases the deposition of oxidised LDL in the arterial wall, is one of the key risk factors for coronary artery disorders including atherosclerosis\(^\text{[18, 31]}\). According to several reports, LDL-c oxidation may play a critical part in the progression of the early stages of atherosclerosis, with thrombosis being one of the most lethal clinical consequences of this condition\(^\text{[18, 32]}\). Previous reports suggest that oxidised LDL-c plays a foremost role in the progression and initiation of cardiovascular dysfunction related to atherogenesis or atherosclerosis\(^\text{[18, 33]}\). As a result, minimising or maintaining dyslipidaemia is just as important as reducing or main-

**Fig. 11** Effect of sinomenine on the level of pro-inflammatory cytokines of vitamin D and high fat induced atherosclerosis in rats. a: IL-1β, b: TNF-α and c: IL-6. Data are presented as mean ± SEM, compared to atherosclerosis group. Where \(* p<0.05\), \(** p<0.01\) and \(*** p<0.001\).

**Fig. 12** Effect of sinomenine on the mRNA expression of vitamin D and high fat induced atherosclerosis in rats. a: TRL4, b: MyD88, c: NF-κB and d: NLRP3. Data are presented as mean ± SEM, compared to atherosclerosis group. Where \(* p<0.05\), \(** p<0.01\) and \(*** p<0.001\).
taining oxidative stress. The function of oxidative stress in the progression of atherogenesis is well understood. The oxidative alteration of LDL-c is the primary step in the conversion of LDL-c into atherogenic form\textsuperscript{18, 22, 31}. Some clinical reports suggest a relationship between atherogenic lipoproteins and plasma level of oxidants in patients having cardiovascular dysfunction\textsuperscript{22, 31}. SM treatment significantly suppressed the higher levels of TG, FFA, LDL and TC and boosted the level of HDL. Previous study showed the similar type of result, which give the strength of our findings\textsuperscript{7, 18, 36}. Simvastatin (positive control) treated group showed the reduction in the level of LDL, TG, FFA, TC and upregulation in the level of HDL.

HDL-c is inversely related to total body cholesterol, and a decrease in HDL-c levels in the circulation may promote the progression of atherosclerosis, which can lead to the onset of ischemic heart disease, by reducing cholesterol clearance from the arterial wall\textsuperscript{14, 34}. Excess tissue cholesterol is taken up and processed by HDL-c particles as a result of reserve cholesterol transport before being transported to the liver for further metabolism and removal. The enzyme lecithin cholesterol-O-acyltransferase (LCAT) is essential for cholesterol trans-esterification, HDL maturation, and the transport of cellular cholesterol to HDL. Increased HDL levels, on the other hand, may help to reduce the risk of atherosclerosis\textsuperscript{17–19}. AS control rats showed an increased level of HDL-c and SM treatment significantly reduced the level and suggesting the suppression of atherosclerosis. SM may play a role in increasing the level of HDL, which is linked to the mobilisation of cholesterol from peripheral cells to the liver via LCAT and plays a crucial in the metabolism of lipoprotein\textsuperscript{5, 22, 35}. A previous report suggests a direct correlation between atherosclerosis and LDL-c. Previous reports showed that the reduction in the level of LDL-c targeted to treat atherosclerosis\textsuperscript{17, 18}. In this study, SM significantly reduced the level of LDL-c along with TC and TG.

Epidemiological investigations have revealed that atherosclerosis is a multifaceted disease including oxidative stress, platelet aggregation, inflammation, and hyperlipidaemia\textsuperscript{5, 17, 34}. According to previous studies, lipoproteins and some of their increased combined ratios (LDL-c/HDL-c and TC/HDL-c) and show a stronger statistical link with the severity and prevalence of coronary artery disease with lipid levels\textsuperscript{18, 36}. hsCRP is a significant sensitive marker of inflammation. SOD and MDA, both are the significant markers of oxidative stress\textsuperscript{17, 18}. The level of these molecules is associated with the severity of coronary atherosclerosis\textsuperscript{27, 37}. AS group rats displayed an augmented level of MDA and reduced level of SOD, along with suppression of the ratio of TC/HDL-c and LDL-c/HDL-c. Additionally, the SM treatment suppressed the levels of LDL, TC, VLDL, TG and increased the level of HDL. SM treatment also reduced the level of hs-CRP and maximum platelet aggre-
gation rate. Taken together, SM treatment suppressed the all parameters and suggesting the anti-oxidant, anti-hyperlipidaemia, anti-platelet aggregation and anti-inflammatory effects and may enhance hyperlipidaemia in rats via preventing inflammation, lipid peroxidation, thrombosis and regulating lipid levels under atherosclerotic conditions.

Atherosclerosis is a degenerative condition that underlying a wide range of cerebrovascular and cardiovascular disorders\textsuperscript{40, 42}. More than 80% of cases of acute myocardial infarction are caused by coronary atherosclerosis (AS) with superimposed luminal thrombus (AMI). The detection of myocardial enzymes such as LDH and cTnl is considered as a significant biomarker for estimation of the degree of cell membrane permeability and myocardial ischemic necrosis\textsuperscript{22, 27, 34, 36}. AS group rats showed an enhanced level of LDH and cTnl, due to expansion of necrosis. SM treatment significantly suppressed the level of cTnl and LDH, suggesting a protective effect against atherosclerosis. The results suggest that the SM may have a protective effect against atherosclerotic heart disease.

Creatine phosphokinase (CPK) is a biomarker of energy metabolism in the body that balances the high amount of intracellular ATP via phosphorylation and is thought to be a good indicator of atherosclerosis\textsuperscript{18}. LDH is a key marker for myocardial infarction and is used to determine the severity of the condition. The level of AST, also used as a diagnostic marker for myocardial infarction\textsuperscript{18}. ALT, LDH, CPK, and AST, which are key markers for the detection of cardiac injury, leak from necrotic heart cells into the blood during pathological conditions\textsuperscript{34, 38}. These enzymes aren’t just for cardiac damage. On the other hand, a combination of these enzymes could indicate myocardial injury. In reaction to oxidative stress, the level of these enzymes in the circulation has increased\textsuperscript{18, 37}. The activity of ALT, CPK, AST and LDH were boosted in the AS group rats and SM treated rats reduced the level of ALT, CPK, AST and LDH, suggesting a cardio-protective effect.

HMG-CoA reductase is considered as a significant marker of myocardial damage\textsuperscript{39, 40}. The ratio of HMG-CoA/ mevalonate in hepatic tissue was used to measure the activity of HMG-CoA reductase (primary site of action)\textsuperscript{40, 41}. In the hepatic tissue, suppression of HMG-CoA reductase (metabolic pathway) that generates isoprenoids and cholesterol\textsuperscript{42, 43}. HMG-CoA reductase inhibition is used to lower serum cholesterol levels and improve survival in people at risk of atherosclerotic vascular disease. The liver is an important organ in which all metabolic reactions originate\textsuperscript{42, 45}. Simvastatin is the most commonly used drug for the treatment of hyperlipidaemia. Simvastatin suppresses the HMG-CoA reductase enzyme in the hepatic tissue that converts the HMG-CoA into mevalonate (precursor of cholesterol biosynthesis)\textsuperscript{42, 46}. Atherosclerosis and aortic stenosis are two diseases in which arterial calcification is a common and clinically significant feature. Atherosclerosis
and aortic stenosis are commonly seen in patients with coronary artery disease (>90%) and vascular lesions (80 percent). Because aortic calcification reduces aortic compliance and elastic recoil, resulting in cardiac ischemia in severe cases due to diminished reverse aortic flow and coronary perfusion, arterial calcification is a key risk factor for heart attacks and strokes. Collagen, is a protein which consist of various genetically distinct molecular species and is involved in the tissue functioning, expansion and differentiation. AS group rats showed the reduction of HMG CoA/mevalonate ratio and increased level of collagen, calcium and protein level and SM treated rats reduced the level of calcium, collagen, protein and increased the ratio of HMG CoA/mevalonate.

Previous research suggest that the inflammatory reactions are significantly involved in the different stages of AS expansion. Pro-inflammatory cytokines are the significant markers for inducing the AS disease. The increased level of inflammatory cytokines such as IL-6, IL-1β and TNF-α, that is the primary stage of inflammatory reaction. TNF-α plays a dual role in innate immunity and inflammatory illness, IL-6 facilitates inflammatory response negative feedback, and macrophages produce IL-1β in response to inflammatory stimuli. In this experimental study, AS group rats exhibited an increased level of inflammatory cytokines and SM treatment significantly reduced the level of inflammatory cytokines.

Previous reports suggest that the TLR4/NF-κB signaling pathway is involved in the activation of the inflammasome during AS. We measured the expression of TLR4/NF-B signalling pathways to determine the underlying mechanism of SM in atherosclerosis. Toll-like receptors regulate both the innate and adaptive immune systems. According to previous study, TLR4 may have a role in chronic inflammatory illnesses like AS through modulating MyD88-dependent and independent signal transduction pathways. The MyD88 pathway is involved in the binding of interleukin-1 receptor-associated kinase (IRAK) -1, IRAK-4, and TLR4. IRAK-1 dissociates from the receptor complex after auto-phosphorylation and binds and activates TNF receptor-activated factor-6 (TRAF-6). TRAF-6 activates the inhibitor of nuclear factor kappa-kinease (IKK) complex via activating TRAF-1, which promotes IB phosphorylation and NF-B release into the nucleus, leading in the production of pro-inflammatory cytokines. SM treated group rats showed the suppression of MyD88, TLR4, NF-κB and IkB phosphorylation and suggesting an anti-inflammatory effect. Taken together, SM treatment showed a suppressive effect against high fat induced AS via TLR4/NF-κB signaling pathway.

5 Conclusion

Sinomenine significantly increased the bodyweight and altered the level of various organ such as liver, heart and kidney. The current investigation showed the sinomenine significantly reduced the level of total cholesterol, LDL-c, TG, VLDL and atherogenic index (LDL-c/HDL-c and TC/HDL-c ratio) in AS rats. The current investigation showed that the sinomenine considerably maintain the lipoprotein and lipid status via HMG-CoA reductase (significant metabolic enzyme) along with maintain the level of calcium and collagen, which play a key role in atherosclerosis. Sinomenine significantly improved the endogenous antioxidant level along with suppressed the level of hepatic markers. Sinomenine significantly reduced the level of heart markers and pro-inflammatory cytokines. Sinomenine significantly altered the mRNA expression of TRL4, MyD88, NF-κB and NLRP3 and suggesting the protective effect against atherosclerosis via alteration of TLR4/NF-κB signaling pathway. Further molecular level investigation required for the estimation the possible mechanism.

Author Contribution

Pengbo Geng performed the experimental study. Xiaohui Xu and Zhao Gao interpretate the biochemical data. All the experimental study was designed by the Zhao Gao. All the authors proof read the manuscript.

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