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# STUDY ON ANTI-ATHEROSCLEROTIC EFFECT OF SUXIAO JIUXIN PILL AND ITS MECHANISM

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## Abstract

**Background:** Suxiao Jiuxin Pill is composed of Ligusticum wallichii, Borneolum Syntheticum and other drugs; it has qi promoting and blood circulation activating, meridian dredging and pain relieving efficacies. The objective of this paper is to study the effect of Suxiao Jiuxin Pill (quick-acting heart reliever), in atherosclerosis (AS) rat model and explore the mechanism for its prevention and treatment of AS.

**Materials and Methods:** AS rat model was established by high cholesterol diet and single intra-peritoneal injection of increased dose of vitamin D3.

**Results:** Compared with the model group, Suxiao Jiuxin Pill medium- and high-dose groups and atorvastatin group can effectively regulate lipid metabolism.

**Conclusion:** We conclude that Suxiao Jiuxin Pill has a good hypo-lipidemic effect, and can inhibit the occurrence and development of AS.

**Keywords :** Suxiao Jiuxin Pill; atherosclerosis; atorvastatin

## Introduction

Atherosclerosis (AS), is a common and frequently-occurring disease of the vascular system; it is one of the diseases that portends serious harm to human health in China, and globally. Its morbidity and mortality rate rank top among various diseases (Ross et al., 1973; Lataliiadejj et al., 2000; Sandra et al., 2000). The incidence of AS is mainly due to the degenerative and proliferative lesions of arteries caused by a variety of reasons, leading to thickening and hardening of vascular walls, loss of elasticity and narrowing of lumen (Wei et al., 2006). The lesions mostly occur in the heart, brain, kidneys and other vital organs; in severe cases, they may cause ischemia and necrosis of organs, and even threaten the lives of patients.

A significant characteristic of AS lesions is lipid infiltration and accumulation; hence, prevention and control of hyperlipidemia by means of diet control, drug regulation and physical exercise is the foundation and basis for controlling this disease (Lu et al., 2000; Xing et al., 2008; Chen et al., 2008).

In modern medicine, AS lesions are often controlled by regulating blood lipids. Traditional Chinese medicine alleviates its symptoms by promoting blood circulation, and dissolving stasis, softening hard lumps and dispelling nodes, and to effect a permanent cure is achieved by invigorating spleen and supplementing qi, replenishing essence and benefiting marrow, by treating both symptoms and root causes, integrated control of AS is enabled (Luo et al., 2003; Zhou et al., 2004).

Suxiao Jiuxin Pill is composed of Ligusticum wallichii, Borneolum Syntheticum and other drugs, Which has qi promoting and blood circulation activating, meridian dredging and pain relieving efficacies, years of clinical applications have proved that Suxiao Jiuxin Pill has an anti-atherosclerotic effect, its active ingredient ligustrazine can effectively reduce the level of LDL, an AS-inducing factor, elevate the levels of HDL, an anti-AS factor, and its component HDLZ, and inhibit AS plaque formation, thus effectively alleviating AS lesions. This experiment aims at exploring the possible mechanism for prevention of AS using Suxiao Jiuxin Pill, through the observation of its effects on serum TC, TG, LDL and HDL levels in experimental AS rats.

## Materials and methods

### Animals

Clean healthy SD male rats, 2-3 months of age, weighing 256 ± 209, purchased from the Laboratory, Animal Center of China Medical

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University.

**Drugs and reagents**

Suxiao Jiuxin Pill (Sixth Factory of Zhongxin Pharmaceuticals, Tianjin, the country medicine accurate Z12020025); vitamin D3 injection (Shanghai General Pharmaceutical Co., Ltd.); atorvastatin (Pfizer Inc., USA).

**Establishment and grouping of AS rat model** (Yang et al., 2003)

After a week of adaptive feeding, the rats were weighed, numbered and randomly divided into normal group, model group. Suxiao Jiuxin Pill low-; medium-; and high-dose groups (50mg/kg.d, 100mg/kg.d, 1500mg/kg.d), and atorvastatin group (4mg/kg.d), each group contained 10, rats. Rats in the model group and each experimental group were given single intra-peritoneal injection of 700,000 U/kg vitamin D3 injection and fed with a high fat diet (formula: cholesterol 1%, sodium cholate 0.3%, 0.2% propylthiouracil, lard 10%). Rats in the normal control group were intra-peritoneally injected with the same volume of normal saline and fed with the basal diet. Body weight was weighed once a week, in order to adjust the administration dose and gavage amount; the rats were fed for a total of 12, weeks.

**Blood sampling**

After the last gavage, all the rats in the experimental groups were fasted for 12 hrs, and sacrificed on the next morning, after the blood sample specimens were centrifuged at 3000rev/min., for 20min., serum was separated, which was then placed in a -20℃, refrigerator for later use.

**Preparation of specimens**

The experimental rats were immediately dissected after been killed, the whole aorta in the bifurcation of aortic arch to iliac artery was separated, extra-vascular fat and connective tissues were carefully removed, aortic arch was cut and taken; bloodstains on the inside and outside of vessel walls were washed away with cold saline, three l, 1~3, arterial rings were cut, taken and placed in 4% glutaraldehyde for pre-fixation, then stored in a 4℃, refrigerator.

**Observation of histological morphology**

After 1hr, of pre-fixation, the specimens were washed in 0.1M PBS, post-fixed for 2hrs, and then washed trice again in 0.1M, PBS. After gradient dehydration with ethanol and acetone, clearing, infiltration, embedding, sectioning and staining, histological morphology was observed.

**Statistical methods**

The experimental results were analyzed with SPSS 11.0, data of each group were expressed as $\bar{x}\pm s$, test criterion was $\alpha = 0.05$.

## Results

**Effects of Suxiao Jiuxin Pill on serum TC, TG, LDL and HDL levels in rats**

As can be seen from the experimental data, serum TC, TG, HDL and LDL levels of rats in the model group were all higher than those of the normal control group; and the differences were statistically significant ($P<0.01$). Serum TC, TG and LDL levels in rats of each Suxiao Jiuxin Pill dose group and atorvastatin group were all lower compared with the model group, serum HDL levels in rats of various groups were all higher than the model group except for the low- and medium-dose groups, see Table 1.

It can be seen from Figure 1 that, serum TC levels of rats in the model group were significantly higher than the normal control group,

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which were about eight times that of the normal control group, and the differences between the groups were statistically significant (P<0.01). Compared with the model group, TC levels in each treatment group decreased in varying degrees, of which the measured serum TC values in Suxiao Jiuxin Pill medium-; and high-dose groups, and atorvastatin group were all significantly lower than the model group, which decreased 43.6%, 61.6% and 51.0%, respectively; the measured serum TG values in Suxiao Jiuxin Pill high-dose group and atorvastatin group decreased 33.6% and 41.2%, respectively; the differences between each group and model group were all statistically significant (P<0.05).

**Table 1:** Effects of each experimental group on serum TC, TG, LDL and HDL levels in rats (mmol/L, $\bar{x}\pm s$, n=10)

| Group | TC | TG | LDL | HDL |
|---|---|---|---|---|
| Normal control group | 1.51±0.22 | 0.91±0.24 | 0.55±0.13 | 0.59±0.13 |
| Model group | 12.12±1.42* | 1.49±0.37* | 7.84±0.65* | 2.11±0.13* |
| Suxiao Jiuxin Pill group (low) | 10.75±0.57** | 1.48±0.38** | 7.49±0.52** | 1.79±0.27** |
| Suxiao Jiuxin Pill group (medium) | 6.84±0.49*** | 1.25±0.19** | 5.13±0.39*** | 1.98±0.31** |
| Suxiao Jiuxin Pill group (high) | 4.13±0.75*** | 0.99±0.31*** | 3.35±0.42*** | 2.34±0.33*** |
| Atorvastatin group | 3.35±0.61*** | 0.87±0.17*** | 3.27±0.33*** | 2.15±0.41*** |

- Comparison with N, P<0.05; ** comparison with M, P>0.05; *** comparison with M, P<0.05

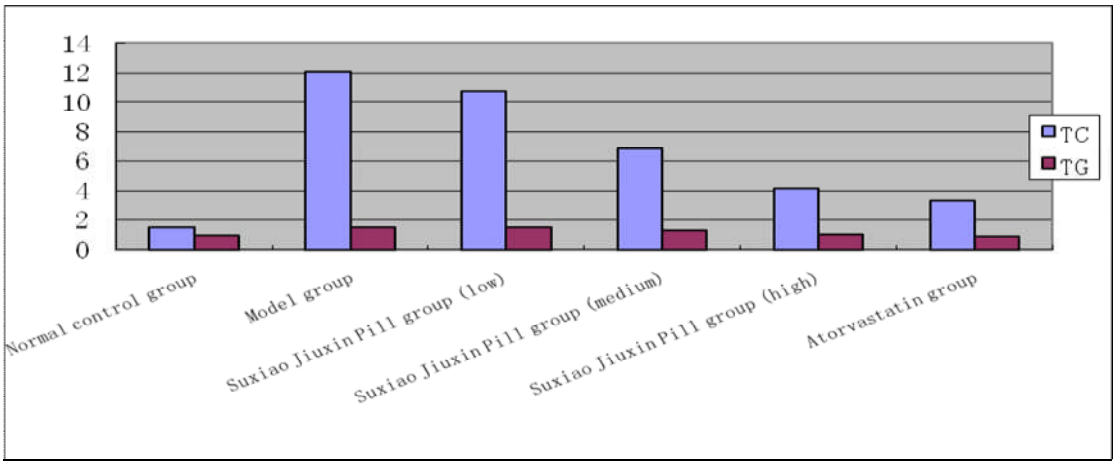


**Figure 1:** Effects of each experimental group on serum TC, TG levels in rats

The experimental data demonstrated that serum LDL, HDL levels of rats in the model group were far higher, than those of the normal control group, indicating that the modeling was successful. Viewing from the measured serum LDL data of rats, each treatment group (Suxiao Jiuxin Pill low-; medium- and high-dose groups, and atorvastatin group), was all lower than the model group, of which LDL level in Suxiao Jiuxin Pill low-dose group declined a bit numerically, but the decline was not statistically significant (P>0.05). Compared with the model group, HDL levels in Suxiao Jiuxin Pill low- and medium-dose groups showed declining trends, the differences were not statistically significant (P>0.05) compared with the model group; although HDL values in medium-dose group increased a bit, the difference was still not statistically significant (P>0.05) compared with the model group.

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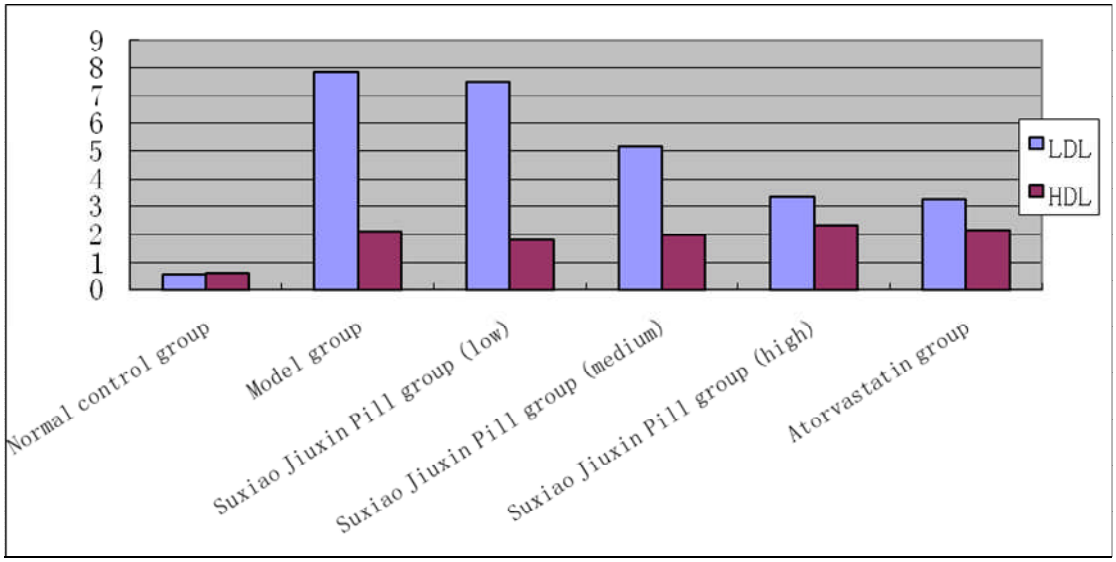


**Figure 2:** Effects of each experimental group on serum LDL, HDL levels in rats

**Results for transmission electron microscopic observation of aortas of AS rats**

The aortic intima of rats in the normal control group was thin, endothelial cells were flat, with relatively smooth surface, and in fusiform shape, organelle structure was normal, intercellular junction was tight, sub-endothelial space was relatively small, without lipid accumulation, internal elastic lamina was complete and continuous; SMC size in tunica media was uniform, nuclear morphology was regular, and organelle structure was normal. (See Fig. 3)

**Figure 3:** Normal control group

The aortic intima of rats in the model group was obviously thickened, endothelial microvilli were missing, large area of cell membrane was missing, cytoplasm spilled over, endothelial cell junction was destroyed, majority of cell membrane was damaged and missing, round or oval-shaped lipid droplets of varying sizes were seen in part of the endothelial cells and cytoplasm; and mitochondria in endothelial cells were mostly fused or disappeared (see Figures 4-5).

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**Figure 4:** Model group (5.0k ×)

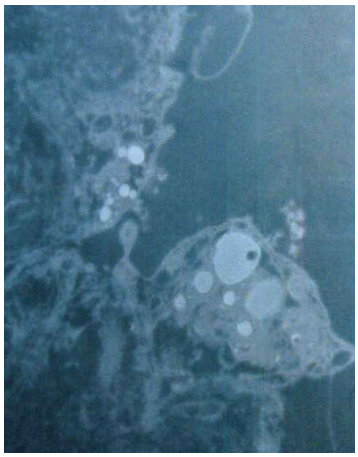

**Figure 5:** Model group (20.0k ×).

Similar to the model group, no significant improvement was observed in Suxiao Jiuxin Pill low-dose group (see Figure 6). Aortic intimal thickening in the medium- and high-dose groups and atorvastatin group significantly reduced, endothelial cells were relatively complete, sub-endothelial space was relatively small, a small number of lipid droplets were seen occasionally, internal elastic lamina was still continuous, and intercellular junction was visible (see Figures 7-9).

**Figure 6:** Low-dose group (7.0k ×)

**Figure 7:** Medium-dose group (7.0k ×)

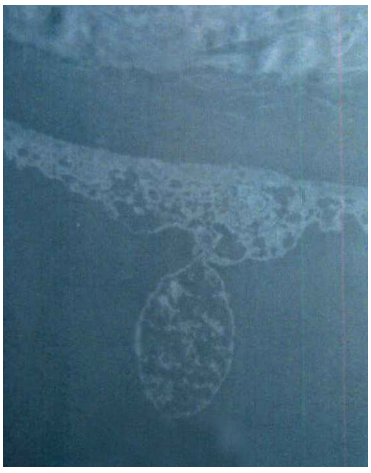

**Figure 8:** High-dose group (7.0k ×)

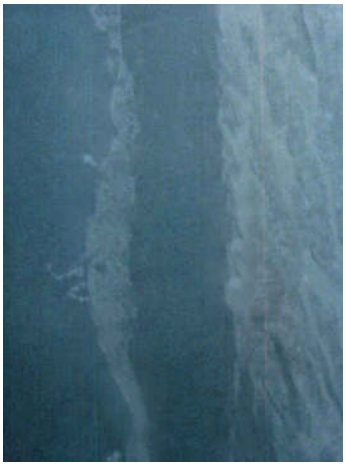

**Figure 9:** Atorvastatin group (7.0k ×)

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## Discussion

In this experiment, experimental SD rats were used as experimental animals, the experiment proved that feeding of high cholesterol level can lead to hyperlipidemia, vascular endothelial cells cause increased permeability of arterial endothelial cells, blood monocytes exclude junction, lipoproteins and mononuclear cells infiltrate the phagocytic lipids mediated by monocyte-macrophage scavenger receptor of vascular intimal cells and smooth muscle cells, in order to form foam cells, which were then stacked to form AS injury in rats.

AS mainly affects large and medium-sized muscular elastic arteries, which commonly occurs in the aortas, coronary arteries and cerebral arteries. It can cause loss of elasticity of thickened and hardened arterial walls, eventually leading to luminal narrowing. The affected vascular lesions generally start from intima, including local deposition of lipids and accumulation of complex carbohydrates, blood and thrombus formation, and fibrosis and calcification, accompanied by progressive degeneration and calcification of arteries. Modern molecular biotechnology suggests that (Libby et al., 2002), every AS is characterized by smooth muscle cell proliferation, formation of large amount of connective tissue matrices such as collagen fibers and proteoglycans, and extracellular lipid accumulation.

Our experimental results showed that: serum TC, TG and LDL lowered while HDL significantly increased in the atorvastatin group, and the differences were statistically significant ($P<0.05$) compared with the model group, indicating that atorvastatin has a significant lipid regulatory effect. Compared with the Suxiao Jiuxin Pill treatment groups, lipid metabolism in high dose of Suxiao Jiuxin Pill was not significantly different ($P>0.05$) with the atorvastatin.

Transmission electron microscopic observation showed that, compared with the model group, the aortic endothelial cells of rats in the Suxiao Jiuxin Pill medium- and high-dose groups were relatively complete, intercellular junction was smaller, subendothelial space was uniform, moderate amount of lipid droplets were seen occasionally, and the internal elastic lamina was still continuous.

The above experimental results indicate that Suxiao Jiuxin Pill has a good hypolipidemic effect, and can inhibit the occurrence and development of AS. Regulation of lipid metabolism disorders may be one of the mechanisms for Suxiao Jiuxin Pill's inhibition of AS.

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