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The role of cepharanthine as an anti-atherosclerotic agent in rats

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Abstract: This investigation assessed the potential of cepharanthine (CEP), a compound from *Stephania cepharantha* Hayata, in mitigating atherosclerosis in a hyperlipidemic rat model. Using Wistar rats, four distinct diet and drug treatment groups were established: a high-fat high sucrose diet (HFHS), HFHS supplemented with intraperitoneal cepharanthine (HFHS-C) or oral atorvastatin (HFHS-A) from the 8th week, and a normal-fat diet (NFD). The study aimed to evaluate diet and drug impact on aortic histopathological changes over 16 weeks. Our results revealed significant atherosclerosis prevention in the aorta of the HFHS-C group, marked by preserved endothelial integrity, absence of inflammation, and lack of atherosclerotic plaques. Additionally, CEP demonstrated a crucial role in preventing the emergence of cholesterol clefts and foamy macrophages. These findings suggest that CEP effectively curbs atherosclerosis progression in hyperlipidemic rats, reducing arterial fat deposition and offering a potential natural preventative strategy against this disease.

Keywords: ABCC10, atherosclerosis, cepharanthine, foamy macrophages, hyperlipidemia.

INTRODUCTION

Lipids play a vital role in biological existence and are key components of cellular structures. They are involved in numerous hormonal and metabolic processes. Diseases such as atherosclerosis and coronary artery disease, leading causes of death in urbanized countries, are associated with lipid imbalances. Atherosclerosis, a chronic condition resulting in lipid-rich plaques forming along blood vessels and obstructing blood flow, is influenced by both environmental and genetic factors (Khoo *et al*., 2003). The development and progression of this complex disease are mainly driven by lipids and inflammation, with sphingolipid alterations potentially playing a significant role in the pathogenesis of cardiovascular disease (Borodzicz *et al*., 2015; Sasset *et al*., 2016).

Sphingomyelin levels in plasma have been identified as independent risk factors for human coronary heart disease due to their association with increased atherosclerosis (Jiang *et al*., 2000). Elevated blood and aortic levels of ceramide, similar to sphingomyelin, are connected with higher risks of cardiac diseases (Kasumov *et al*., 2015). Additionally, individuals with a higher likelihood of developing atherosclerosis have increased plasma glycosphingolipid concentrations (Glaros *et al*., 2007). Glycosphingolipids, including glucosylceramides, have been found to accumulate in atherosclerotic lesions in both Apoe-/- mice and humans (Glaros *et al*., 2008).

Lipids, being insoluble in water, are transported in the blood as lipoproteins, which are soluble protein complexes. Lipids can be obtained from food (exogenous) or produced within the body (endogenous). Dietary lipids are absorbed and broken down in the intestine into chylomicrons, a type of least-dense lipoprotein (Mahmood Hussain *et al*., 2005; Mahmood Hussain, 2000). In contrast, Very Low-Density Lipoproteins (VLDLs), which transport endogenous lipids from the liver to peripheral cells, are the primary source of endogenous lipids (Hussain *et al*., 2012; Hussain *et al*., 2003). According to Naukkarinen *et al*. (2006), the main defect in individuals with familial combined hyperlipidemia is increased hepatic apoB-lipoprotein production, a crucial factor in dyslipidemia related to diabetes and obesity. Alterations in the metabolism of triglyceride-rich lipoproteins (VLDL & chylomicron) are becoming increasingly recognized as a significant contributor to the development of atherosclerosis and its clinical manifestations (Dallinga-Thie *et al*., 2010). Atherogenic apoB-lipoproteins interact with the artery wall, leading to atherosclerosis. There is a strong correlation between atherosclerosis and apoB-lipoproteins. These lipoproteins are believed to penetrate the endothelium and become trapped on sub-endothelial extra cellular matrix proteins (Curcio *et al*., 2009). This retention causes damage, including oxidation, aggregation, endothelial and extracellular matrix alterations, macrophage chemotaxis, foam cell formation, and smooth muscle cell migration (Witztum & Steinberg, 2001).

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The ABCC10 gene encodes the ATP-binding cassette sub-family C member 10 protein, also known as multidrug resistance protein 7 (MRP7), which is part of the ATP-binding cassette (ABC) transporter family. ABCC10 is expressed in various arterial wall cells, including endothelial cells, smooth muscle cells, and macrophages, all of which are involved in the development of atherosclerotic lesions. ABCC10 has been implicated in the transport of lipids and other molecules that contribute to the formation of atherosclerotic plaques, and its over expression has been linked to an increased risk of atherosclerosis and cardiovascular disease (Kathawala *et al*., 2014).

Foamy macrophages play a crucial role in the immune response against pathogens and foreign substances, particularly in the context of atherosclerosis. While their accumulation is a characteristic of advanced atherosclerotic lesions, they also contribute to the ongoing inflammatory response, potentially leading to plaque instability and other complications (Barrett, 2020).

Cepharanthine (CEP), an unusual biscoclaurine alkaloid extracted from the Stephania cepharantha Hayata plant, has demonstrated effectiveness in inhibiting the ABCC10 transmembrane protein (Zhou *et al*., 2009). For over seven decades, Japan has embraced and applied this natural compound to tackle a range of health issues, including malaria, hair loss, and snakebite poisoning, with minimal reported adverse reactions. CEP is thought to exhibit a variety of biological properties that underpin its clinical success. Originating from Taiwan and China, the plant has a long-standing history in traditional Chinese medicine. The purpose of this research is to delve into CEP's involvement in atherosclerosis and to assess its viability as a therapeutic intervention for such disorders.

MATERIALS AND METHODS

Male Wistar albino rats weighing between 150 and 175 grams were procured from Dow Medical College Ojha campus animal house in Karachi for the purpose of this study. Upon acquisition, the rats were acclimatized at a temperature of 25±2°C and a humidity level of 50±15%. They were housed in polypropylene cages, with a maximum of six rats per cage, under standard laboratory conditions (12-hour light/dark cycles). The rats were provided with a standard diet and unlimited access to water. The National Research Council (NRC) guidelines were adhered to while working with these animals (Council, 1997) and the research was approved by the University of Karachi's Advanced Studies and Research Board (ASRB/No.04681/ Pharm).

Sucrose, Carboxymethyl Cellulose (CMC), and CEP with a purity of ≥95% were sourced from Sigma-Aldrich, USA. CEP was suspended in phosphate buffer saline (PBS) containing 0.5% CMC. Atorvastatin (Lipiget) was procured from Getz Pharma. All other chemical reactions employed analytical-grade materials and distilled water was used to test biological reactions.

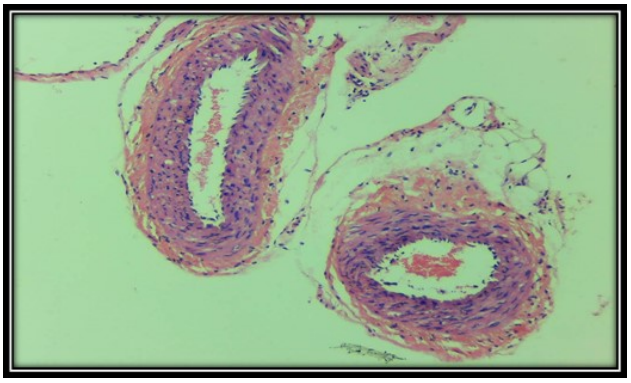

Fig. 1: Section of artery of NFD rat shows intact endothelium and architecture. No inflammation, foamy macrophages, cholesterol clefts, or atherosclerotic plaques are seen (10x).

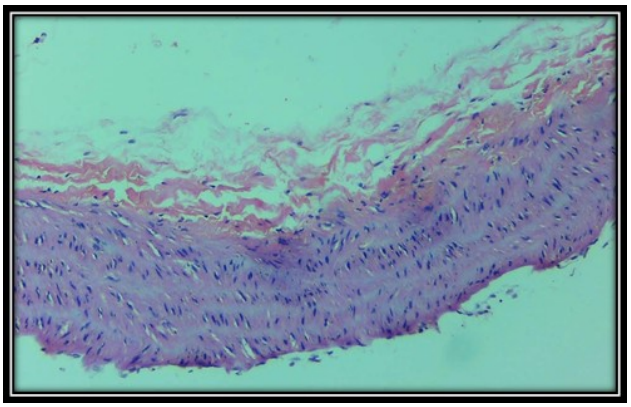

Fig. 2: Section shows the artery of an NFD rat with intact endothelium and architecture. No inflammation, foamy macrophages, cholesterol clefts, or atherosclerotic plaques seen (20x).

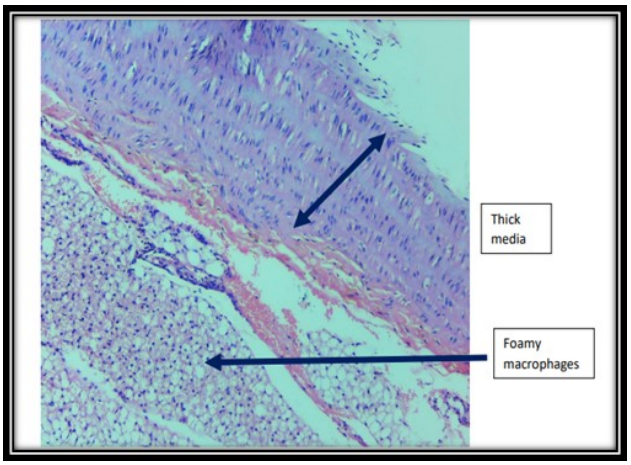

Fig. 3: Section of artery of HFHS group rat shows thickened media. Foci of cholesterol clefts and foamy macrophages are noted (20x).

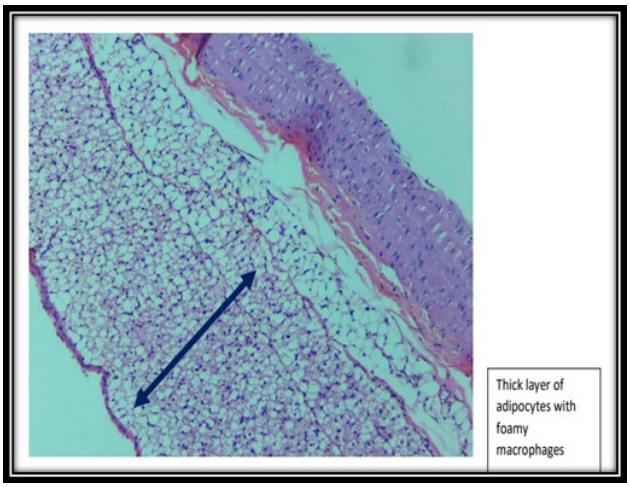

Fig. 4: Section shows the artery of the HFHS group with a thick layer of adipocytes and foamy macrophages (20x).

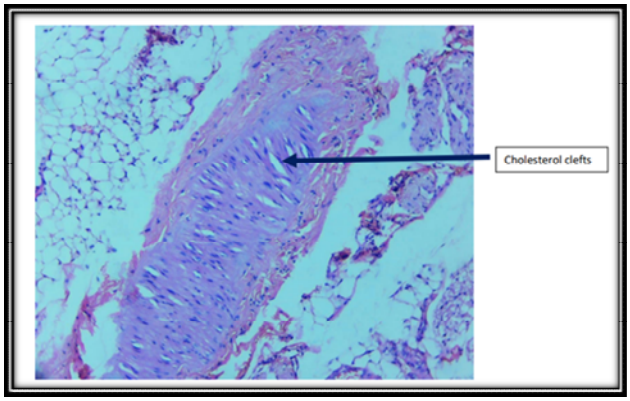

Fig. 5: Section shows artery of HFHS group with foci of cholesterol clefts (20x).

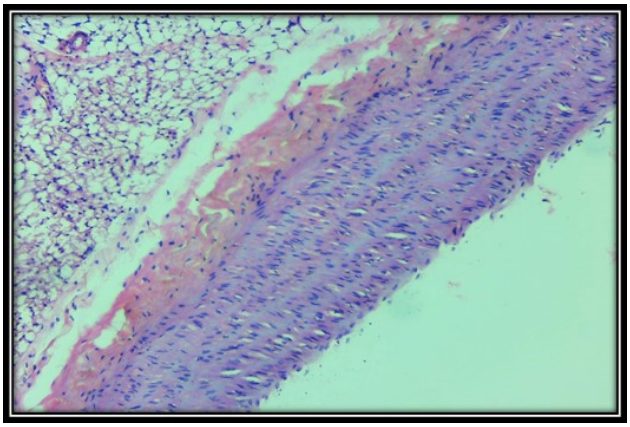

Fig. 6: Section shows the artery of a rat treated with atorvastatin with intact endothelium and architecture. No inflammation, foamy macrophages, cholesterol clefts, or atherosclerotic plaques are seen (20x).

Experimental rats were given a high-fat and high-sucrose (HFHS) diet that included three milliliters of Banaspati ghee, one milliliter of coconut oil per 10 milliliters per kilogram and 25% sucrose-sweetened water. Control rats were fed a normal fat diet (NFD) comprising 70% carbohydrates, 20% proteins, and 10% fat (Nissankara Rao *et al*., 2021). The NFD diet consisted of 20% crude protein, 10% crude oil, 10% ash, 0.15% sand silica, and 4% crude fiber (Solanki & Bhatt, 2010).

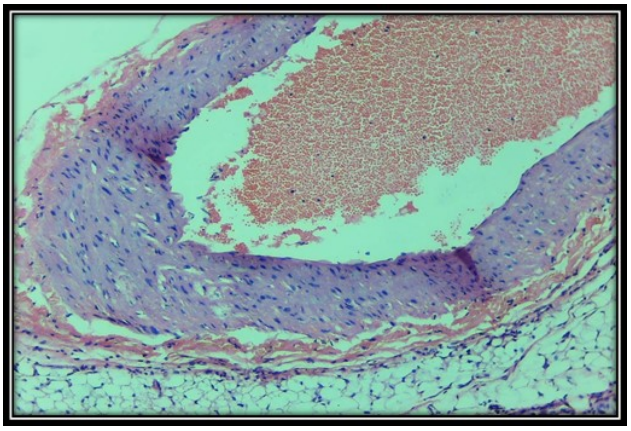

Fig. 7: Section shows the artery of a rat treated with CEP with intact endothelium and architecture. No inflammation, foamy macrophages, cholesterol clefts, or atherosclerotic plaques are seen (20x).

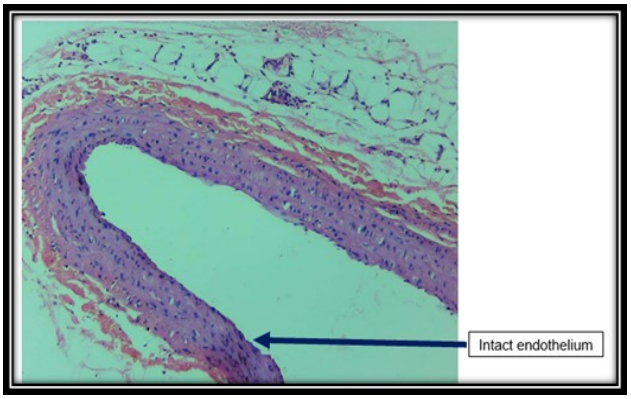

Fig. 8: Section of artery of rat treated with CEP shows intact endothelium and architecture (20x).

The rats were further divided into four groups, each containing ten animals, and subjected to the following treatments:

- Group I: served as healthy controls, consuming the NFD diet for 16 weeks (NFD group).
- Group II: received HFHS diets for 16 weeks (HFHS group).
- Group III: fed the HFHS diet for 16 weeks and administered CEP 10 mg/kg/d intraperitoneally from the eighth week of HFHS for 60 days (HFHS-C group).
- Group IV: fed the HFHS diet for 16 weeks and administered oral atorvastatin at 10 mg/kg/d from the eighth week of HFHS for 60 days (HFHS-A).

At the end of the study, the rats were given a ketamine overdose of 75mg/kg (Molina *et al*., 2015) and euthanized

under aseptic conditions to expose their internal organs. The organs were rinsed with normal saline (0.9% NaCl) to prevent drying. The aorta was then removed, washed with sterile saline and prepared for histological analysis. Tissue samples were placed in pre-labeled glass vials and fixed in a 10% buffered neutral formalin solution for 24 to 48 hours. After staining with hematoxylin and eosin, the sections were examined under a light microscope to evaluate the microscopic structures of the tissues.

RESULTS

Upon examining hematoxylin and eosin (HE)-stained aortic tissue sections under a microscope, the rats in the NFD group displayed an intact endothelium and normal tissue architecture, with no signs of inflammation, foamy macrophages, cholesterol clefts, or atherosclerotic plaques. On the other hand, the untreated group fed a high-fat, high-sucrose diet exhibited a thickened medial layer, characterized by the presence of cholesterol clefts and foamy macrophages. However, when rats on the HFHS diet were treated with CEP, the formation of cholesterol clefts, foamy macrophages, and atherosclerotic plaques was effectively prevented.

DISCUSSION

Lipids play a significant role in the development and risks associated with Metabolic Syndrome (MetS). Elevated triglyceride and fatty acid levels lead to dyslipidemia, which involves lipid abnormalities such as alterations in atherogenic and antiatherogenic lipoproteins (Su *et al*., 2014). Atherogenic dyslipidemia is a term used to describe the increased presence of atherogenic lipoproteins during these abnormalities. Dyslipidemia arises when the production of Very Low-Density Lipoprotein (VLDL) and Low-Density Lipoprotein (LDL) is increased while High-Density Lipoprotein (HDL) production is reduced (Avramoglu *et al*., 2006; Christian & Su, 2014).

A critical factor in the development of atherosclerosis is the disruption of lipid homeostasis. Excessive lipid accumulation in macrophages and lipid deposition in the arterial wall are known factors in the complex chain of events that lead to atherosclerosis (Kotlyarov & Kotlyarova, 2021).

Risk factors for atherosclerosis include obesity, hypertriglyceridemia, hypertension, and hyper insulinemia. Previous research has shown that aortic sinus lipid deposits in mice result from a diet high in fat and sugar (Schreyer *et al*., 1998). Another study indicated that CEP significantly improved dyslipidemia and prevented the accumulation of fatty deposits in the liver tissue of rats fed an HFHS diet (Iqbal *et al*., 2022). In our investigation, histological analysis of the aorta in the HFHS rat group showed thickened media, cholesterol cleft foci and atherosclerotic plaques with foamy macrophages. Rats treated with CEP were protected from developing atherosclerotic plaques containing foamy macrophage cells, and their tunica media did not exhibit thickening. We hypothesize that by inhibiting ABCC10, atherosclerosis is prevented in these animals, as these medications are ABCC10 inhibitors (Iqbal *et al*., 2022). Disruption of transport processes mediated by ABC transporters has been suggested to contribute to atherosclerosis and other disorders. Previous research revealed that ABCC10 expression was most significantly increased in atherosclerotic samples (Kotlyarov & Kotlyarova 2021).

Both vascular smooth muscle cells (VSMC) and cancer cells display proliferation and migration. CEP has been found to inhibit cell growth and motility in both cases. CEP's anti-migratory effect on VSMCs, which inhibits matrix metalloproteinase (MMP)-9 expression, prevents the degradation of extra cellular matrix components. This outcome, combined with the reduction of inflammation and lipid per oxidation, provides CEP with anti-atherosclerotic properties (Paudel *et al*., 2016).

CONCLUSION

High plasma lipid concentrations are risk factors for various cardiovascular and metabolic conditions, including obesity, metabolic syndrome, and atherosclerosis. Statins, the standard treatment for reducing plasma lipids, are not effective for 20-40% of individuals due to poor response or sensitivity (Boekholdt *et al*., 2014). Thus, there is a need to develop new strategies or regimens to prevent atherosclerosis. The protective effects of CEP against dyslipidemia and fatty deposits in liver tissue, as well as its anti-migratory action on VSMCs, suggest that it may have potential as an alternative therapeutic strategy for atherosclerosis prevention. However, the exact mechanisms underlying the anti-atherosclerotic effects of CEP remain unclear, and further research is needed to elucidate the role of ABCC10 inhibition in this process.

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