Artemisinin combined with allicin on improving cardiac function, fibrosis, and NF-κB signaling pathway in rats with diabetic cardiomyopathy

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Research Article

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Abstract

Background

This study aimed to see how artemisinin & allicin affected heart function, myocardial fibrosis, and regulating the nuclear factor-B (NF-B) signaling pathways in the myocardial tissue of diabetic cardiomyopathy rats.

Methods

50 rats were selected, 10 of which were kept normally without any intervention as the rest 40 were in the normal group injected intraperitoneally 65 μg/g streptozotocin at one time to construct diabetic cardiomyopathy model. 37 rats meeting the criteria for successful model establishment were randomly divided into ten rodents in the model category, 9 rats each in the Artemisinin, Allicin, & Combination groups. For four weeks, the Artemisinin group received 75 mg/kg of artemisinin, the Allicin group received 40 mg/kg of allicin, & the combination group received the same doses of artemisinin & allicin gavage as the Artemisinin & Allicin groups. We investigated the cardiac function, myocardial fibrosis, and the aberrant expression of protein levels in NF-κB signaling pathways in each group after the end of the intervention.

Results

The model group, Artemisinin group, Allicin group, and combination group showed significantly greater cardiac function indexes, including LVEDD, LVESD, LVEF, FS, and E/A with higher expression of NF-B signaling pathway proteins NF-B-p65 and p-NF-B-p65 than the normal group (P < 0.05). LVEDD, LVESD, LVEF, FS, E/A, and NF-B signaling pathway protein NF-B-p65, and p-NF-B-p65 were significantly decreased in the Artemisinin, Allicin, and combination groups when compared with the modeled group (P < 0.05). The combined group had significantly lower expression of LVEDD, LVESD, LVEF, FS, E/A, NF-B signaling pathway protein NF-B-p65, and p-NF-B-p65 than the Artemisinin and Allicin groups (P < 0.05). For comparing the cardiac function indicators LVEDD, LVESD, LVEF, FS, E/A, NF-B signaling pathway protein NF-B-p65, & p-NF-B-p65 expressions, there was no statistical difference here between Artemisinin & Allicin groups (P > 0.05). After observing the myocardial fibrosis in each group, we found the collagen fibers-associated disorder arrangement of the proliferative network in the modeled group, formation of the fibrous scar with large volume, cardiac hypertrophy, inconsistent coloration, nucleus consolidation, disintegration, and even removal. When compared to the model group, the Artemisinin group, Allicin group, & combined group all demonstrated various degrees of improvement in the problematic structure with more intact muscle fibers, neater arrangement, more normal cell morphology, and more homogeneous staining, with the most significant improvement in the combined group.
Conclusion

Compared with artemisinin and allicin alone, artemisinin combined with allicin improved cardiac dysfunction and reduced myocardial fibrosis in rats with diabetic cardiomyopathy, and both may act via promoting the inactivation of the NF-κB signaling cascade.

Introduction

Diabetic cardiomyopathy is crucially associated with mortality in diabetes mellitus and is marked by myocardial fibrosis and myocardial inflammation, with cardiac hypertrophy, arrhythmias, and heart failure as the main manifestations [1]. Diabetic cardiomyopathy is complex and involves several factors, so there is no specific drug to treat this disease. Allicin, the active component of garlic, has been clinically shown to have an anti-cardiac fibrosis effect, and it has been reported [2] that allicin is effective in diabetic cardiomyopathy, but a single drug may not completely inhibit the progression of diabetic cardiomyopathy. Artemisinin belongs to the extract of the Chinese herb Artemisia annua, which was found to attenuate myocardial fibrosis and ventricular remodeling via inhibition of the cascade of NF-κB [3].

In human astrocytoma T67 cells, artemisinin is associated with the inhibition of inducible nitric oxide synthase (iNOS) production and the activation of NF-κB. In MOG-reactive splenocytes, artemisinin derivative SM933 is correlated with the inhibition of NF-κB by suppressing its dissociation via elevation of its suppressing protein kappa B alpha (IkB-α). It indicates that artemisinin is substantially regulated the immune responses which in turn reduces the inflammatory response. However, the artemisinin-mediated anti-inflammatory effects on microglial activation remain lacking [4]. In the current research, for inhibiting diabetic cardiomyopathy progression to the maximum extent and reduce the degree of fibrosis, the combination of allicin and artemisinin was used in the study of diabetic cardiomyopathy to analyze the effect of the combination of the two and to analyze whether they act together on the NF-κB cascade and act, and clinical management of this disease provides a reference.

Materials And Methods

1.1 Materials

Animals to study: Hangzhou Qizhen Research Animal Technology Co. provided 50 SPF pure standard Wistar male rats (6–8 weeks old) with a body mass of 220 ± 20 g.: SYXK (Zhejiang) 2022-0006. 7 d of acclimatization under constant temperature (22 ± 2 °C), constant humidity (55 ± 5%), and artificial light and dark for 12 h. All were free to move, drink and take food. Our hospital's ethics committee gave its approval to the research (Approval number: FEH83456).

Main reagents: streptozotocin (Beijing Ita Biotechnology Co., Ltd.), artemisinin (Nanjing Guanrun Biological Products Co., Ltd.), allicin (Zhengda Tianqing Group Co., Ltd.), NF-Kb-p65, and p-NF-κB-p65
antibody (primary) (Abcam, USA).

1.2 method

1.2.1 Modeling and intervention

The normal group was chosen at random from the 50 rats, while the remainder 40 rats were given a single injection of 65g/g streptozotocin intraperitoneally, and their blood glucose levels were evaluated one week later if the blood glucose value was \( \geq 16.7 \text{mmol/L} \), and there were polyuria, polyphagia, and polyphagia, then the modeling of diabetes was successful [5], and all 40 rats have successfully established the model of diabetes. After that, they were fed with normal chow until 16 weeks, and 3 rats were detected by ultrasound to have not become cardiomyopathy, and the remaining 37 were successfully established as diabetic cardiomyopathy models, The rats were split into four groups: 10 in the model group, 9 in the Artemisinin team, 9 in the Allicin team, and 9 in the group respectively.

At 16 weeks, the Artemisinin group was gavaged 75 mg/kg of artemisinin, the Allicin group was gavaged 40 mg/kg of allicin, the combination group was gavaged with the same dose of artemisinin and allicin as the Artemisinin and Allicin groups, and the saline was introduced into the normal and model groups with similar dose, all of which were intervened continuously for 4 weeks, and the changes of indexes in each group were observed at 20 weeks.

1.2.2 Measurement Of Cardiac Function

After the last pharmacological intervention, echocardiography was performed in each group to determine changes in cardiac function, and we measured left ventricular end-diastolic internal diameter (LVEDD), left ventricular end-systolic internal diameter (LVESD), left ventricular ejection fraction (LVEF), and short-axis shortening (FS), maximum mitral valve velocity in early dilatation (E), and maximum atrial systolic velocity (A) ratio to examine changes in cardiac function. We captured Echocardiographic image-based measurements from grayscale M-mode images with the parasternal short-axis view and also captured from B-mode images with parasternal long-axis and short-axis views. The measurements included LVEDD, LVESD, thicknesses of the anterior and posterior wall, FS, thicknesses of the wall, fractional area change (FAC), end-systolic and end-diastolic volumes, EF, and LV mass.

1.2.3 Observation Of Myocardial Fibrosis

After the completion of cardiac function measurement do rapid execution of rats, take myocardial tissue, do sectioning, and then perform HE, Masson staining, and microscopic observation of myocardial fibrotic lesions in each group. We dissected the tissues immediately and utilized 4% neutral buffered formalin for immersing those whole days and then utilized Masson's Trichrome for staining. We employed Image Pro Plus 6.0 for quantifying the fibrous tissues. We analyzed three areas in each slide with 100 squares of
each area. The blue point indicated collagen points with a numeric score of 1 (present) or 0 (absent). We expressed the fibrosis as the percentage of fibrosis area to the whole area.

1.2.4 Nf-κb Signaling Cascade Protein Expression Measurement

By using the Western blot approach, we quantified the level of NF-B signaling pathway proteins NF-B-p65 and p-NF-B-p65, myocardial tissue was taken, 80 µL of lysis buffer was added, tissue protein was extracted, 2×SDS buffer utilized to mix 50 µg of the protein. After electrophoresis, transferring to the membrane, mold taking, fixation and closure, NF-κB-p65 and p-NF-κB-p65 primary antibody diluted at 1:1000 ratio using TBST buffer was added and incubation treatment was done at 4°C. After 1d, TBST buffer was washed, utilized the HRP-labeled secondary antibody, washed the membrane, utilized diaminobenzidine method to develop color, and used the GAPDH as an internal reference for obtaining levels of NF-κB-p65, p-NF-κB-p65 protein.

1.3 statistical Analysis

We employed SPSS 22.0 computational software for processing the data and utilized one-way ANOVA for assessing the differences in multiple groups. For two-by-two comparisons among groups, the LSD t-test was utilized. After natural logarithm transformation and non-parametric test, the data did not adhere to normally distributed & were expressed as M (Qn). For data comparison, we considered P < 0.05 as the statistical threshold.

Results

2.1 Comparison of cardiac functions among the groups

The model group, Artemisinin group, Allicin group, & combined group had significantly greater LVEDD, LVESD, LVEF, FS, E/A, and cardiac function indexes when compared with the control (P < 0.05). The cardiac function indicators LVEDD, LVESD, LVEF, FS, and E/A were significantly lower in the Artemisinin group, Allicin group, and combined group when compared with the modeled group (P < 0.05). The cardiac performance parameters LVEDD, LVESD, LVEF, FS, and E/A were significantly poorer in the combination group after comparing with the Artemisinin & Allicin groups (P < 0.05). In the comparison of LVEDD, LVESD, LVEF, FS, and E/A, the cardiac function indexes in the Artemisinin and Allicin groups were not significantly differentiated (P > 0.05). See Table 1 for more information.
Table 1
Comparison of cardiac functions among the group ()

<table>
<thead>
<tr>
<th>Group</th>
<th>LVEDD(mm)</th>
<th>LVESD(mm)</th>
<th>LVEF(%)</th>
<th>FS(%)</th>
<th>E/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group (n = 10)</td>
<td>5.70 ± 0.11</td>
<td>2.20 ± 0.08</td>
<td>78.75 ± 2.31</td>
<td>57.54 ± 2.10</td>
<td>2.23 ± 0.12</td>
</tr>
<tr>
<td>Model group (n = 10)</td>
<td>7.00 ± 0.23*</td>
<td>4.65 ± 0.10*</td>
<td>63.32 ± 2.45*</td>
<td>36.55 ± 1.04*</td>
<td>1.30 ± 0.09*</td>
</tr>
<tr>
<td>Artemisinin group (n = 9)</td>
<td>6.51 ± 0.11*#</td>
<td>4.00 ± 0.14*#</td>
<td>66.75 ± 2.11*#</td>
<td>40.62 ± 1.33*#</td>
<td>1.55 ± 0.10*#</td>
</tr>
<tr>
<td>Allicin group (n = 9)</td>
<td>6.48 ± 0.11*#</td>
<td>4.03 ± 0.16*#</td>
<td>66.98 ± 2.09*#</td>
<td>40.55 ± 1.54*#</td>
<td>1.58 ± 0.11*#</td>
</tr>
<tr>
<td>Combined group (n = 9)</td>
<td>6.00 ± 0.10*# &amp; ∆</td>
<td>2.98 ± 0.09*# &amp; ∆</td>
<td>73.42 ± 2.76*# &amp; ∆</td>
<td>53.39 ± 2.13*# &amp; ∆</td>
<td>2.07 ± 0.13*# &amp; ∆</td>
</tr>
</tbody>
</table>

Note: “*” indicates a meaningful comparison with the normal group; “#” indicates a meaningful comparison with the model group; “*&” indicates a meaningful comparison with the Artemisinin group; “∆” indicates a meaningful comparison with the Allicin group.

2.2 Myocardial Fibrosis Observation

In the normal group, Myocardium cells were almost in unchanged condition by microscopic examination of rat myocardial tissues with few collagen fibers and without pathophysiological alterations. In the model group, we found the collagen fibers-associated disorder arrangement of the proliferative network in the modeled group, formation of the fibrous scar with large volume, cardiac hypertrophy, inconsistent coloration, nucleus consolidation, disintegration, and even removal. Myocardial fibrosis was dramatically reduced in each treatment group as compared to the model group, cell morphology was gradually restored, staining was more uniform, and myofibers tended to be intact, In comparison to the Artemisinin and Allicin groups, pathological alterations were more clearly restored in the combination group. Figures 1 and 2 are examples of this.

2.3 Comparison Of Nf-kb Signaling Pathway Protein Expression Among Groups

The model group, Artemisinin group, Allicin group, and combined group had significantly increased levels of NF-B-p65 and p-NF-B-p65, the NF-B signaling pathway protein than the normal group (P < 0.05). The NF-B signaling pathway proteins NF-B-p65 and p-NF-B-p65 were expressed significantly at lower levels in the Artemisinin, Allicin, and combined groups after comparing with a modeled group (P < 0.05). The combined group had significantly lower levels of NF-B-p65 and p-NF-B-p65, the NF-B signaling pathway proteins, than the Artemisinin & Allicin groups (P < 0.05). In the Artemisinin and Allicin groups, there was
no statistical difference in the levels of NF-B-p65 and p-NF-B-p65 (P > 0.05). See Table 2 for more information.

<table>
<thead>
<tr>
<th>Group</th>
<th>NF-κB-p65</th>
<th>p-NF-κB-p65</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group(n = 10)</td>
<td>0.35 ± 0.04</td>
<td>0.56 ± 0.07</td>
</tr>
<tr>
<td>Model group(n = 10)</td>
<td>1.89 ± 0.22*</td>
<td>1.93 ± 0.24*</td>
</tr>
<tr>
<td>Artemisinin group(n = 9)</td>
<td>1.03 ± 0.15*#</td>
<td>1.12 ± 0.15*#</td>
</tr>
<tr>
<td>Allicin group(n = 9)</td>
<td>1.06 ± 0.12*#</td>
<td>1.14 ± 0.13*#</td>
</tr>
<tr>
<td>Combination group(n = 9)</td>
<td>0.70 ± 0.08*#&amp;∆</td>
<td>0.88 ± 0.09*#&amp;∆</td>
</tr>
</tbody>
</table>

Note: "*" indicates a meaningful comparison with the normal group; "#" indicates a meaningful comparison with the model group; "&" indicates a meaningful comparison with the Artemisinin group; "∆" indicates a meaningful comparison with the Allicin group.

### Discussion

Allicin is critically associated with cell death and limiting cell growth in cancer cells by interacting with thiol groups of glutathione and proteins. In cancer cells, Allicin inhibits the proliferation of bacteria and fungus or kills them totally in a dose-dependent manner. Allicin is correlated with health-promoting qualities, including effects on lowering cholesterol and maintaining blood pressure to benefit the cardiovascular system. Allicin is associated with the reduction of higher-glucose-stimulated cardiomyocyte apoptosis through lowering the NADPH oxidase-associated ROS content and its subsequent NK/NF-B signaling cascades, according to Kuo et al [7], & may have therapeutic promise for diabetic cardiomyopathy.

Artemisinin belongs to the sesquiterpene lactones with peroxy bridges, which were first used in the treatment of malaria and have anticancer, antiviral, antiparasitic, & anti-inflammatory properties in vitro & in vivo, in addition to their antimalarial effects [8–9]. With extensive clinical studies on artemisinin, it has been found that it can alter fibrosis via several pathways, TGF, MAPK, Wnt/-catenin, PI3K/AKT/mTOR, FRX & Notch, NF-B signaling pathways, as well as BMP-7 & cellular autophagy, contribute to the anti-fibrotic process, as do its anti-inflammatory effects.

The results of this paper showed that compared with the artemisinin and allicin alone, the cardiac function of rats with combined administration of both interventions improved more significantly. It was found that the myocardium with diabetic cardiomyopathy showed obvious fibrosis after staining of rat tissue, as well as the severity of cardiac was relieved after the intervention of artemisinin and allicin, the myocardial fibers were more intact and neatly arranged, the cell morphology tended to be normal, and the
staining was more homogeneous, but the improvement was more obvious with artemisinin combined with allicin, indicating that the myocardial damage was more serious in the occurrence of diabetic cardiomyopathy, and the cardiac function of rats with artemisinin and allicin administration improved significantly and the myocardial fibrosis was relieved.

The myocardial structure is altered when the body is in a state of prolonged hyperglycemia, and the NF-κB signaling cascade is initiated during this process. NF-κB is secreted by vascular endothelium, vascular smooth muscle cells, etc., and is associated with the degradation of IB by utilizing the inflammatory mediators which ultimately stimulate NF-κB, and when NF-κB is activated it can increase to some extent the binding to nuclear DNA, which in turn acts on cardiomyocyte behavioral processes and leads to apoptosis [12–14]. In addition, NF-κB activates inflammatory cytokines including interleukins and tumor necrosis factor and further circulates to activate NF-κB itself, amplifying the local cascade response in this circulatory pattern and promoting the progression of myocardial injury [15–17]. The effects on the NF-B signaling pathway in diabetic cardiomyopathy rats were examined in this work. Transartemisinin combined with allicin reduced NF-B-p65 expression and NF-B-p65 phosphate activation level in diabetic cardiomyopathy rats, which inhibited over-stimulation of the NF-B signaling cascade, augmented the physiological function of cardiac tissue, and lowered the extent of fibrosis in cardiac tissue, according to the findings. In summary, the present study found a rat model of diabetic cardiomyopathy & confirmed that artemisinin combined with allicin intervention improved cardiac function, myocardial fibrosis, & the NF-B signaling pathway in diabetic cardiomyopathy rats, implying that artemisinin conjunction with allicin intervention may perform a role in improving cardiac function & inhibiting myocardial fibrosis via the NF-B signaling pathway, which has positive implications.

However, this study also has its limitation in that the development of diabetic cardiomyopathy is associated with multiple factors and involves several mechanisms, and further studies are needed regarding whether both act via other pathways.

**Conclusion**

Compared with artemisinin and allicin alone, artemisinin combined with allicin improved cardiac dysfunction and reduced myocardial fibrosis in rats with diabetic cardiomyopathy, and both may act via promoting the inactivation of the NF-κB signaling cascade.

**Declarations**

**Data Availability**

The data will be provided upon request.

**Conflicts of Interest**

We have no conflict of interest
Funding:

No external fund was collected for this study

Acknowledgments:

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References


Figures

Figure 1
HE staining observation of myocardial tissue (×200)

Figure 2
Myocardial tissue Masson staining observation map (×200)

Note: A: Normal group; B: Model group; C: Artemisinin group; D: Allicin group; E: Combination group