

Metformin Improves Myocardial Fibrosis in Spontaneously Hypertensive Rats via the TGF- β /Smads Signaling Pathway

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Abstract: *Objective:* Effects of metformin on myocardial fibrosis in spontaneously hypertensive rats. *Method:* Twenty spontaneously hypertensive rats (SHR) were randomly divided into a model group and a metformin treatment group, with 10 rats in each group. The control group consisted of 8 Wistar-Kyoto (WKY) rats. In the treatment group, metformin was administered intragastrically for six months at a dose of 100 mg/kg/d, and the rats were fed a high-fat, high-sugar diet. The model group and the control group were administered gastric irrigation with the same amount of physiological saline. During the period, body weight was monitored every week, and blood pressure was recorded every seven weeks. Echocardiography was performed before sampling. The heart tissue of rats was taken, and a part of the heart tissue was fixed in formalin. The paraffin wax embedding method was used. Sirius red staining of pathological sections was performed to observe myocardial cells and calculate myocardial collagen volume fraction (CVF). In the other part of the heart tissue, TGF- β 1, Smad2, Smad3, and Smad7 proteins were analyzed by Western blotting. *Results:* In the statistical comparative analysis of SBP, DBP, MBP, HR, SV, aortic blood flow velocity, pulmonary artery flow velocity, and mitral blood flow velocity in the metformin group, there was no significant difference between the treatment group and the SHR group. However, metformin intervention in SHR significantly improved EF, FS, heart weight, myocardial fibrosis, and myocardial cell hypertrophy. In the comparison map of FS, EF, and heart weight, there were significant differences between the metformin group and the SHR group. EF and FS of hypertensive rats were significantly lower than those of healthy rats, and SHR rats in the metformin treatment group were significantly higher than those in the model group. It can be seen from the comparison results of cardiac weight and pathological sections that metformin inhibits hypertension-induced myocardial hypertrophy, cardiac mass gain, and myocardial fibrosis. Western Blot results show that metformin can inhibit TGF- β / Smads signaling pathway. *Conclusion:* Metformin ameliorates hypertension-induced myocardial fibrosis in SHR rats by inhibiting TGF- β /Smads signaling pathway.

Keywords: Metformin; SHR; Myocardial fibrosis

Online publication: September 30, 2026

1. Introduction

Chronic hypertension constantly stresses the myocardium, causing cardiomyocytes to grow larger and leading to an uneven mix of proteins called collagen, resulting in damaged myocardial tissue that is unable to function properly during beats, and it is the main source of all the trouble when there is hypertensive heart failure ^[1]. Transforming growth factor- β (TGF- β) / Smads signaling pathway plays an important role in the regulation of myocardial collagen synthesis and the fibroproliferative process, with its excessive activation causing myocardial interstitial remodeling ^[2]. Research has demonstrated that metformin has a protective effect against cardiovascular disease, which has also been confirmed in non-T2DM patients ^[3]. Metformin can regulate AMPK phosphorylation, which inhibits TGF- β 1 expression ^[4]. Moreover, metformin induces excessive repair damage in cardiomyocyte fibrosis ^[5]. Smad proteins are the main downstream activating proteins of TGF- β , which affect the proliferation of the extracellular matrix (ECM), such as tissue fibroblasts and collagen ^[6]. Also, many other studies have found that it actually has some sort of cardioprotective effect, such as lowering, you know, blood sugar levels, but no. But this still does not mean that, if metformin can reduce high blood pressure-caused myocardial cell fibrosis without lowering your hypertension, there isn't any direct evidence from an in vivo experiment done yet to see if metformin could work that way or not. We use Spontaneously Hypertensive Rats (SHR) as our animal model, control group, Hypertension Model group, and metformin intervention group. By using echocardiography, Sirius Red histopathological staining, and Western blotting, we will try to find out how good an impact metformin has on hypertensive myocardial fibrosis, as well as see if it has something to do with the modification of TGF- β / Smad. It aims to provide a non-antihypertensive experimental pharmacological prevention and cure for hypertensive myocardial injury.

2. Materials and methods

2.1. Animal strains

In total, twenty SHR rats and eight WKY rats were utilized. The experimental animals were sourced from Beijing Vital River Laboratory Animal Technology Co., Ltd., Laboratory Animal Production License Number: SCXK (Beijing) 2021-0006. They were male, SPF-grade, and aged 49- 55 days.

2.2. Drugs and reagents

Bristol Myers Squibb makes metformin (Glucophage) in Shanghai, China. Running buffer (Cat. No. P0014B), PVDF membranes, PVDF membrane activation solution (Cat. No. P0021S), and ECL hypersensitive chemiluminescence kit (Cat. No. W0001) were from Beyotime Biotechnology Co., Ltd. Anti-TGF- β 1 rabbit polyclonal antibody, Anti-Smad3 recombinant rabbit monoclonal antibody, Anti- β -actin antibody, Anti-Smad2 recombinant rabbit monoclonal antibody, and Anti-Smad7 recombinant rabbit monoclonal antibody all were from HuaAn Biotechnology Co., Ltd.

2.3. Instruments

Color Doppler ultrasound device(Mindray Medical International Limited), Softron's smart BP monitor, Thermo Fisher cold spinning equipment(4 degrees C), LABGIC TH - Mini portable grinding mixer, ChemiScope fluorescence & chemiluminescence imaging setup(Clinx Science).

2.4. Experimental methods

2.4.1. Experimental protocol

The disease in this trial is myocardial fibrosis after hypertension in SHR—Wistar-Kyoto (WKY) rats: Normal control group. After two weeks of acclimation, feedings would then occur, with all of the rats being ranked based on their current weights in order for them to fit within one specific diet according to strict criteria set via a random assignment process through a number sequence generator system (Random Number Generator) known as complete randomization. The WKY rats do not have any more subgroups; however, the SHR rats were separated into the model group that got no treatment, as well as the group given only metformin through gavage. Blood pressure, echocardiogram, and HE were taken to determine if hypertension was created in the hearts, as we would also observe metformin for heart health. After a 2-week adaptation period, the rats were randomly assigned and treated with their respective dose via gavage for 6 months. Monitor changes in body weight and blood pressure weekly; perform an echocardiogram before collecting the sample. At the end of administration, measure blood pressure again and perform an Echocardiogram again after tissue has been sampled. Heart tissues were obtained for Sirius Red staining and pathological examination; Western blot was carried out for TGF- β 1, Smad2, Smad3, and Smad7 protein quantification. Anesthesia with sodium pentobarbital by intraperitoneally injected Echocardiography and tissue collection were done under anesthesia induced by intraperitoneal injection of sodium pentobarbital.

(1) Grouping, drugs, and dosage

Rat grouping is shown below.

Table 1. Experimental grouping and dosage

Group	Rat Strain	Sex	Body Weight	Number (n)	Drug	Dosage
SHR group	SHR	Male	182–215 g	10	Distilled water	Equal volume
Treatment group	SHR	Male	182–215 g	10	Metformin	100 mg/kg/day
Control group	WKY	Male	182–215 g	8	Distilled water	Equal volume

(2) Drugs and administration

- Myocardial fibrosis model group: 10 SHR rats received an equivalent volume of saline as metformin, administered by daily gavage for 6 months.

- Metformin treatment group: 10 SHR rats received metformin at 100 mg/kg/day, administered by daily gavage for 6 months.

- Blank control group: 8 WKY rats received an equivalent volume of saline as metformin, administered by daily gavage for 6 months.

2.4.2. Blood pressure measurement method

Rats that were acquired between 49–55 days of age had 4 weeks of getting used to eating there. The first BP was measured @ 11W of age. The second was measured 7 weeks later, at 18 weeks of age. The third measurement was taken 7 weeks later, at 25 weeks of age.

In this research, blood pressure was taken indirectly from the rats' tail artery by using a non-invasive sensor cuff and a smart sphygmomanometer. The cuff would be used on the tail to block off the flow, then slowly released. Measure them each week, taking three measurements at once on the conscious but minimally stressed animal, and take the mean. This way there won't be any experimental injuries for our rats, and also

it's very simple to carry out; therefore we can do multiple readings over seven-week periods if necessary. However, it does not provide for continual real-time monitoring. Also, the different measurements at various times will have a bit of a difference. And the measuring process might bring about some stress responses too, which might influence our blood pressures. To reduce errors in the experiment, the mean of three consecutive readings is adopted as the blood pressure reading of each session. Each one was measured at one o'clock on their own body when doing those directions which came along with these RBP-1's rat-tail-artery blood-pressure/heart-rates.

Table 2. Blood pressure measurement parameters

Abbreviation	Full Name	Chinese Full Name	Measurement Dates
HR (beats/min)	Heart Rate	心率	21/9/30, 21/11/25, 22/1/18
SBP (mmHg)	Systolic Blood Pressure	收缩压	
DBP (mmHg)	Diastolic Blood Pressure	舒张压	
MBP (mmHg)	Mean Blood Pressure	平均动脉压	

2.4.3. Echocardiography method

After anesthesia, rats were shaved, placed, and fixed in place. Coupling gel was applied around the cardiac area. Echocardiography and other parameters were carried out by medical personnel in the Department of Ultrasound and the related parameter department at North China University of Science and Technology Affiliated Hospital to record the results.

2.4.4. Rat heart collection

Rats were weighed and given a lethal dose of anesthesia via intraperitoneal injection of sodium pentobarbital (0.04 g/kg). The chest was shaved, and animals were held on a foam board by their nails. A thoracotomy was performed to access the heart. Post-extraction, the heart was washed under chilled physiologic saline to wash away the blood inside all the chambers. Blotted dry using filter paper and weighted right then. Then the heart was sectioned across using a scalpel along the coronal plane. Take a part with a full-cut surface & place it within a prearranged fixative liquid (quantity ranging between ten-fold to thirty-fold as compared to the quantity of tissue) designed mainly for preserving its properties before further staining procedure. The second half, after snapping, was quickly frozen in liquid nitrogen and put into -80 degrees freezer for storage before Western blot.

2.4.5. Paraffin sectioning and sirius red staining of rat heart tissue

The heart tissue was immersed in a 4% paraformaldehyde fixative solution overnight, at least 24 h (fixative ratio: 10-30:1 w/v). Tissues were then graded in alcohols as follows: 80% ethanol, 24 h; 95% ethanol, 1 h; 100% ethanol I, 1 h; 100% ethanol II, 10min; xylene I, 30min; xylene II, 30min; soft paraffin, 1 h; hard paraffin, 30min. We embedded the myocardium in paraffin such that the coronal plane was centered on the embedding cassette. We sectioned it using a microtome. We baked the sections for 1 hour at 56°C. We performed Sirius Red staining as follows: paraffin section was deparaffinized with xylene I (30 min), xylene II (30 min), absolute alcohol I (30 min), absolute alcohol II (30 min), 95% ethanol (5 min), 90% ethanol (5 min), 80% ethanol (5 min), 70% ethanol (5 min), distilled water. Mayer's hematoxylin & Sirius Red stain was prepared according to the manufacturer's instructions, and sections were stained as per instructions.

2.4.6. Western blot detection method

- (1) Western Blot: A protein immunoblotting technique where certain antibodies find those special bits of stuff (the targets) inside your body or tissue cells. It's an extremely accurate as well as easy way of performing a qualitative and quantitative measure. Detection steps include extracting protein, denaturing it, SDS-PAGE Electrophoresis, membrane transfer, immunoreaction, and developing.
- (2) Total protein isolation: About 40mg of tissue is placed inside a 1.5 mL centrifuge tube with lysis buffers. To avoid quantification being impacted by proteolytic decay, a blend of protease inhibitors was introduced. The tissue was mashed up, homogenized, and lysed for 20 minutes; then the material was spun. Take supernatants (200µL) from each and transfer them into the next tubes 1.5 ml. Use the BCA method to determine the total protein concentration. Add 50 µL of 5×protein loading buffer to each supernatant. Samples were boiled at 100 degrees Celsius for 5 min to denature completely, stored at -20°C.
- (3) SDS - PAGE electrophoresis: The electrophoresis was carried out on ice. The stacking gel was run at 80V for 30 minutes and the separating gel at 120 V for 60 mins.
- (4) Membrane transfer: The PVDF membranes were activated by immersion in the activation solution and then soaked in the transfer buffer for 5 min. After gel cutting, protein was transferred onto the PVDF membrane using 110v for 1.5 hours.
- (5) Immunoreaction:
Block: On the shaker, the membrane was blocked for 2 hours at RT.
Washing: Membranes were washed 3 times using 1x TBST in a shaking incubator for 10 mins x 3 at RT.
Primary antibody incubation: The membrane was incubated with Anti-TGF-β1 Rabbit Polyclonal Antibody, Anti - Smad3 Recombinant Rabbit Monoclonal Antibody, Anti-Beta Actin antibody, Anti-Smad2 Recombinant Rabbit Monoclonal Antibody, and Anti - Smad7 Recombinant Rabbit Monoclonal Antibody.
Washing: Same as step (2).
Secondary antibody incubation was done at RT for an hour, and half of the secondary antibodies were diluted 1:5000 with TBST; after the incubation, membranes were washed with TBST, shaking at RT, for another 3 times of 10 minutes each.
A chemiluminescent reaction was performed.
- (6) Development: The blots were developed by adding HRP substrate, and the gel was imaged. Gray values obtained with ImageJ were used to quantify TGF-β1, Smad2, Smad3, and Smad7 protein expression.

2.5. Statistical analysis

All Statistical Analyses were done using SPSS IBM STAT 25. For the three groups, WKY, SHR, and MET: 1 way anova for significant differences and variance analysis.

3. Results

3.1. Analysis of blood pressure outcomes

Figure 1A shows the three repeated measurements of systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean blood pressure (MBP) of rats. The first measurement was made before intragastric administration, and the other two measurements were made at week 7 post-intragastric treatment and week 14 post-intragastric treatment, respectively. As can be seen from **Figure 1A**, there is a positive relationship

between the SBP, DBP, and MBP values of rats and the rat's age in months. With respect to the control group composed of the WKY rats, it became very clear that there were significant differences between the metformin-treated group and the SHR model group ($P < 0.01$, $N = 5$). Both BP of the metformin group and SHRs were also higher than the normal level. However, no statistically significant difference was found between the metformin-treated group and the SHR Hypertensive Model Group, suggesting that metformin does not affect blood pressure in Hypertensive Rats.

SHR rats have higher HR than normal rats (**Figure 1B**, $P < 0.05$, $N = 5-6$). After 7 weeks of intragastric metformin, there were no big changes in heart rate for both the treated group and model group (**Figure 1A**). **Figure 1B**'s line from week seven through week fourteen indicated an inclination for heart rate in the metformin-treated group to go back toward typical values. But significance testing proved there wasn't any statistical difference. All with continuous 14 week of metformin dose is 100mg/kg/d had NO impact on heart rate in SHR rats. According to the curve shape of **Figure 1**, if we increase the amount of metformin or extend the treatment time, heart rate abnormalities in hypertensive rats can possibly be improved. But more metformin and longer treatment still fail to change blood pressure in SHR rats.

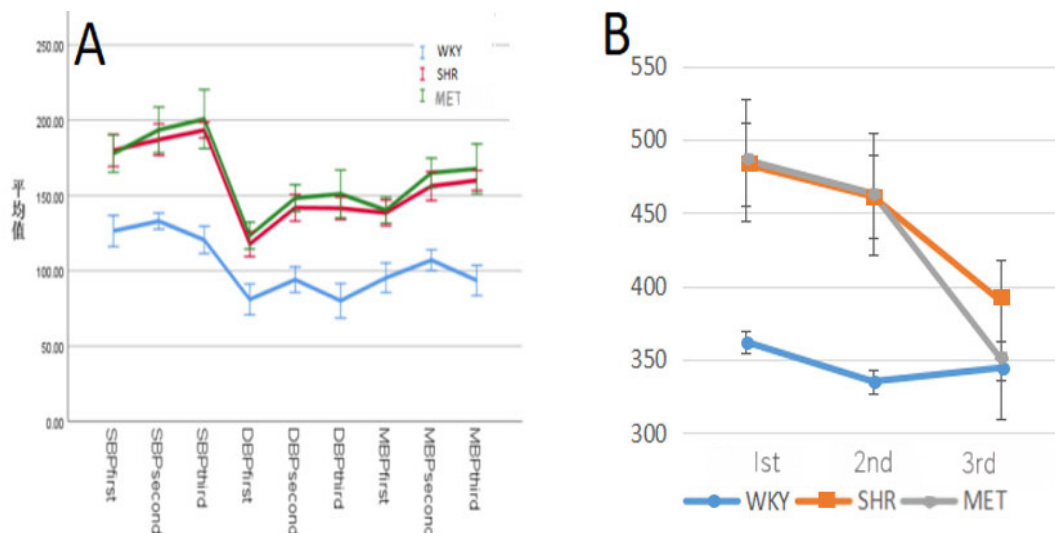


Figure 1. The comparison of blood pressure and heart rate measurements among three groups of rats over three time points.(A) is the comparison between the 3 measurement times (SBP, DBP & MBP) for all 3 rats, and (B) shows the difference between the 3 heart rate measurements for all three groups. One-way analysis of variance (ANOVA) was performed using SPSS. Results: No notable influence of metformin on SBP, DBP, MBP, and HR of SHRs was found.

3.2. Effects of metformin on echocardiographic parameters in rats

The detected index items are LVIDd, LVIDs, EDV, ESV, FS, EF, SV, pulmonary artery blood flow speed, maximum mitral blood flow speed, and maximum aortic blood flow speed. Formula for calculating FS and EF: $FS = (LVIDd - LVIDs) / LVIDd * 100\%$, $EF = (EDV - ESV) / EDV * 100\%$. To obtain the plot, one-way ANOVA was performed on the obtained FS and EF values in **Figure 2C**.

Statistical results between different groups of SHR and WKY were significant ($P < 0.01$, $N = 3$), which means myocardial ejection function declined in hypertensive rats compared with normal rats. Compared to the SHR disease model group, the metformin-treated group showed greatly improved cardiac function ($P < 0.01$, $N = 3$). Also, when comparing the treatment group with the control group (WKY), there was a very

obvious difference ($P < 0.05$, $N = 3$). Altogether, it shows that metformin gives heart protection and improves the squeezing ability of the heart muscles in SHRs.

From the information in other people's work: In hypertension, the rat myocardial pump decreases because of sustained myocardial fiber hypertrophy. Metformin helps with the hypertension-caused heart trouble in these SHR things, so it's like it could be doing some kind of guard thing for your heart. More importantly, we saw in our previous portion that we did find no signs that metformin was causing any reduction in blood pressure in these hypertensive animal specimens; therefore, this may also indicate that perhaps those cardiac-protecting actions were unrelated to lowering one's blood pressure. In the next parts, it is shown more on the effect and mechanism behind metformin in causing the changes in myocardial fibrosis with help from stained slides and western blots.

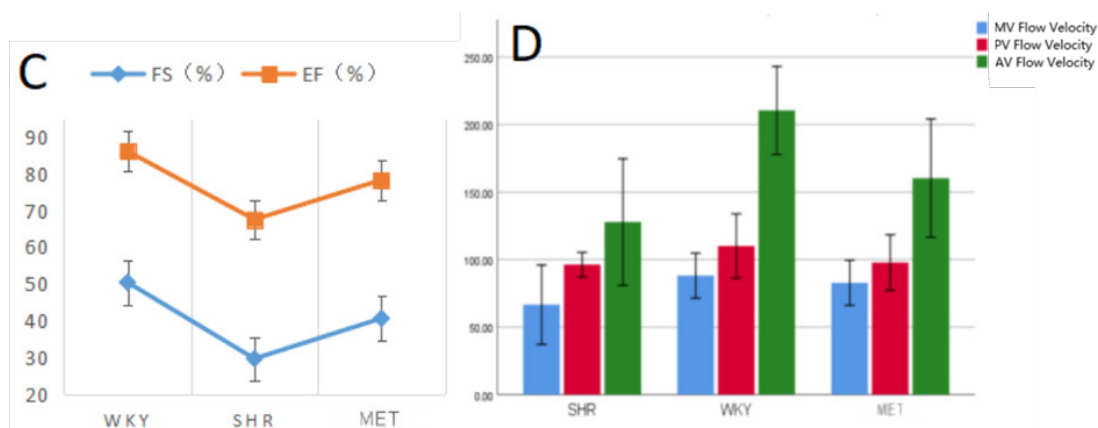


Figure 2. Comparison of Echocardiographic EF, FS, and flow velocities at the mitral valve orifice, aortic valve orifice, and pulmonary valve orifice among three groups of rats after treatment.

3.3. Effects of metformin on heart weight of SHR rats

When hypertension happens, we have these kinds of things happening to your heart muscle cells, where they start getting bigger in size because there are more cardiac myocytes around, as well as an increase in proliferation of those fibrous cells known as fibroblasts, overall leading towards increased heart mass. There is a large change in heart weight among hypertensive rats compared with normal control rats (**Figure 3F**, $P < 0.01$, $N = 5$). Compared to untreated hypertensive rats, metformin-treated hypertensive rats manifest a marked decrease in heart weight with statistically significant differences (**Figure 3F**, $P < 0.05$, $N = 5$); however, no distinct difference could be found between the treatment group and the normal control group. Metformin makes hypertension-induced cardiac weight gain less severe. Metformin did not decrease blood pressure, according to what we said earlier (**Figure 1A**). This indicates that Metformin's decrease in cardiomyocytes and extracellular matrix in SHR rats is not by way of decreasing blood pressure. Instead, metformin blocks the compensatory growth in cardiomyocytes and ECM expansion.

The SHRE Group differs from the WKY Group and metformin groups (Panel C, $P < 0.01$, $N = 3$). Metformin has no impact on PA flow velocities. For MV and AOA flow velocities (Panel D), there are no apparent variations between the metformin group and the SR or WKY groups; however, there is an obvious distinction between the SR group and the WKY group ($P < 0.05$, $N = 5$).

Comparative statistical analyses of stroke volumes (SV), it can be seen that it does not change cardiac

stroke volume (Panel E) under effect of metformin. But the metformin group's SV values are still a little bit higher when compared with SHRs'. As the Met-treated group can largely increase myocardial EF and FS, it may be due to the short duration or lower drug level, which is why we do not find much SV difference among these groups.

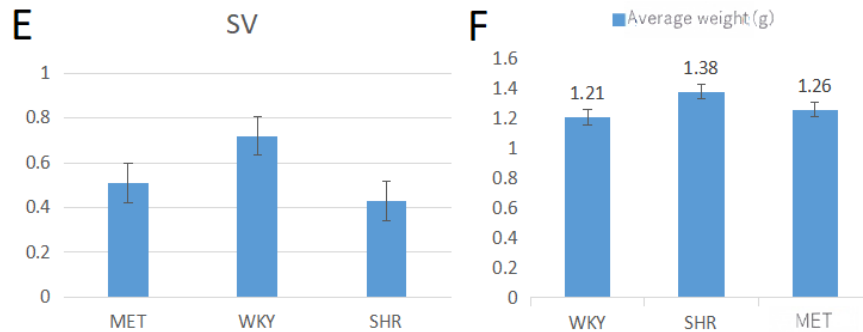


Figure 3. Comparison of SV and mean heart weight among three groups of rats after treatment

Metformin showed no changes in cardiac stroke volume (SV) of SHR rats (E). Metformin lessened heart weight growth in SHR rats. Comparison of treatment group with model group (F, $P < 0.05$, $N = 5$); comparison between treatment group and control group $P < 0.05$, $N = 5$). Comparison of model group vs control group (F, $P < 0.01$, $N = 5$).

3.4. Histopathological slice observation

Sirius Red staining helps determine how big your heart muscle cells are, as well as how things are arranged within them. If there's fibrous scar tissue (heart disease), we should be able to see it with a special kind of microscope called polarized microscopy after some time has passed since the problem began. A 400 $\times$ magnification microscopic view of the myocardium from all three groups showed obvious cardiomyocyte hypertrophy and severe myocardial fibrosis in untreated SHR and treated SHR with metformin, in contrast to healthy control rat cardiomyocytes with hypertrophy and fibrosis. Although the metformin-treated group had slightly less cardiomyocyte hypertrophy and decreased fibrotic lesions than the untreated SHR group, this also verified that metformin has great protective effects against myocardial fibrosis in hypertension-induced rats.

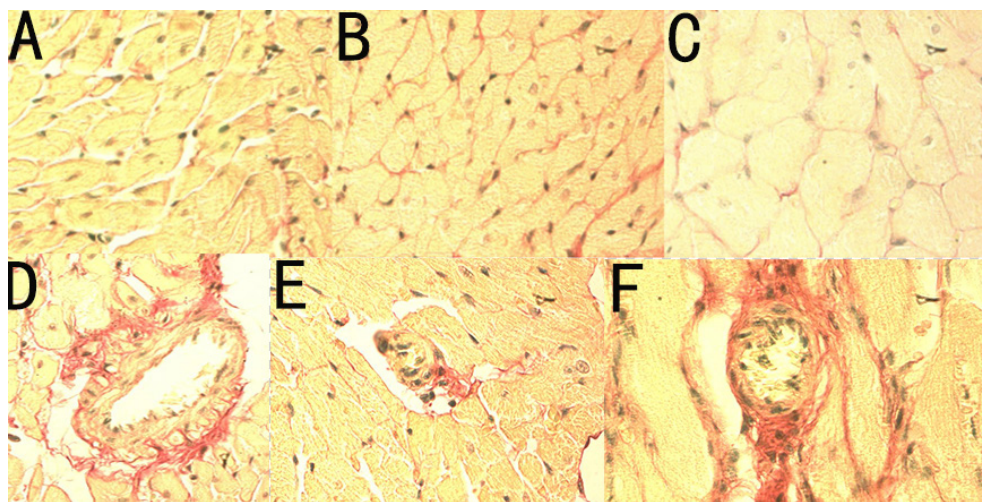


Figure 4. Polarized light micrographs of Sirius Red staining (magnification $\times 400$).

In **Figure A** and **D**, we have WKY blank controls; in **Figure B** and **E**, we have our metformin-treated groups; and in **Figure C** and **F**, we have our SHR disease controls. Under a microscope, the WKY rat has normal heart tissue, but when looking at it, we see enlarged and disorganized muscle in the SHR rat, along with a lot of scar tissue. Compared to the SHR group, the metformin group showed that cardiomyocyte hypertrophy was relieved, with more orderly cell arrangements and a significant decrease in myocardial fibrosis.

3.5. Western Blot analysis of the effects of metformin on TGF- β 1, Smad2, Smad3 and Smad7 protein expression

As seen in **Figure 5**, it shows that Metformin reduces the Protein levels of TGF-Beta 1, Smad 2 & 3 but increases the expression of Smad7, $P < 0.01$, $N = 3$. TGF-beta1/Smad signaling mainly involves the phosphorylation of Smad2 and Smad3, and they bind together with Smad4 to regulate the inflammatory response and fibrosis. Smad7 functions as a negative regulator of this pathway. Metformin inhibits TGF- β 1 / Smads.

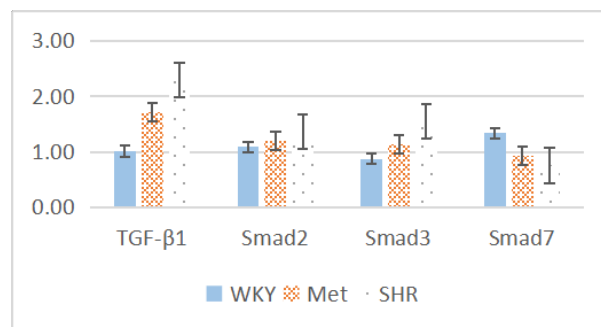


Figure 5. Western Blot detection of the effects of metformin on TGF- β 1, Smad2, Smad3, and Smad7 protein levels.

Western blot was performed to detect the protein levels of TGF- β 1, Smad2, Smad3, and Smad7, with β -actin as the internal reference, with units of ng/ml. One-way ANOVA analysis revealed that metformin downregulated the expression of TGF- β 1, Smad2, and Smad3 proteins while upregulating Smad7 protein expression ($P < 0.01$, $N = 3$).

4. Discussion

Myocardial fibrosis means too much healing that results from damage to the heart; it's like normal heart cells in parts that aren't hurt start changing into myofibroblast cells (special helper cells), and there's lots of lumpy stuff filling up the space between the heart muscles. This pathological process affects the heart's ability to pump, causes extra thickening and beating of the heart muscle, and further damage through an ongoing cycle of inflammation and more hardening of tissue, eventually leading to heart problems. The cause of myocardial fibrosis is many things; we know some risk factors like having high blood sugar, having high blood pressure, or changes in glands that make hormones called adrenaline and thyroxine. A heart attack leads to an inflammatory reaction, oxidative stress, and abnormal signals like the TGF- β cascade. But there is a lot about what contributes, so all of those specific answers at the very tiny level remain mostly unknown to people; therefore, more investigation has to occur as time moves forward. The current work wanted to figure out how

much effect was caused by metformin on hypertension-related myocardial fibrosis, mainly paying attention to what kind of regulatory effect would be exerted if metformin worked within the context of a TGF- β /smad system and also looking at whether this medication would provide improvement against myocardial fibrosis observed among SHR.

In total, there are 20 SHRs; they were split evenly into 2 groups randomly using a random number table, with each group having 10 rats: the first group is referred to as 'the model group', while the second group stands for 'the metformin i.g.'. 8 Wistar Kyoto (WKY) rats are our controls. Rats ate a high-fat, high-sugar diet. After the adaptation period of two weeks, oral feeding was done intragastrically and administered at 100 mg/kg/day. The model group and control group were given normal saline (i.g.) at equal doses by volume. Take body weight before taking drugs and check it weekly after that; take blood pressure every 7 weeks. Echocardiography was done before collecting the tissue. Cardiac tissues were then cut up and split in half. One part was put in formaldehyde, wrapped up in wax, and cut into bits with a dye called Sirius red so we could see how bad the hardening and growth of the heart muscle was. The other half went through Western Blot analysis to figure out how much of the TGF- β 1, Smad2, Smad3, and Smad7 proteins were present. Parameters of 3 groups were compared statistically.

Compared with the SHR model group, a significant difference in fraction shortening(FS), ejection fraction(EF), and heart weight was found in the metformin group. The EF and FS of hypertensive SHRs are obviously below those of healthy WKY rats. After the metformin intervention, we saw an increase in SHRs EF and FS; however, they did not reach the same levels as the WKY rats, though they did reach much higher levels than SHRs before treatment. Heart weights also showed a lowering effect of metformin on hypertension-induced myocardial hypertrophy, as well as weight gain. Collectively, metformin improved myocardial hypertrophy and cardiac ejection function in SHRs.

There isn't a statistical difference in SBP, DBP, MBP, HR, SV, aortic blood flow, pulmonary artery flow, and mitral valve flow between the metformin group and the SHR model group. Many different studies mention that they have some kind of effect that can lower blood pressure in this hypertensive animal, but there is no decrease in blood pressure in this experiment we are doing right now. Using the line chart/column chart based on the raw data showed slight rising trends in post-metformin treatment SV/aortic/pulmonary artery/mitral blood flow velocities; however, all comparison results gave $P > 0.05$. Metformin didn't show any considerable impact on HR during the initial seven weeks of usage, but it began trending down toward regular values around week 14 without being statistically meaningful ($P > 0.05$). Previously, it had been found that administering continuous intragastric metformin (200 mg/kg/d) for 6 weeks could improve ischemia-reperfusion-induced HR, SV, and AFBV [7]. So even though it's not quite statistical but just looking at what I can see on the plot, seems like maybe more metformin dose or maybe if it goes on for some more time there would be like some big improvement in SBP and then DBP and also in MBP, also heart beat per second, also strokes per minute, and also those kind of arterial bloods flowing through all kinds of valves.

Under the microscope, using Sirius Red staining, we can see that metformin reduces myocardial fibrosis. Compared with the SHR model group, metformin-treated rats had neatly arranged and smaller cardiomyocytes. SHRs were organized and smaller compared to WKYs when examined, unlike SHRs, whose cardiomyocytes were disorganized with interstitial collagen accumulation and cardiomyocyte hypertrophy compared with WKYs. This confirmed the anti-fibrotic effects of metformin. In order for us to know more exactly how metformin reduced the hypertension-related myocardial fibrosis, we excluded a reduction in

blood pressure from being the cause because there was no decrease in blood pressure in the mice used. Liang et al. ^[8] thought that metformin protected the heart by stopping the TGF- β signaling pathway and, later, SMAD. So, Western blot is used here to measure the level of TGF- β 1, Smad2, Smad3, and Smad7.

TGF- β /SMAD signaling canonical pathway goes like this: TGF- β 1 binds transmembrane T β R II, after this event happens. T β RI is activated ^[9]. T β RI activates the phosphorylation of Smad2 & Smad3; these get transformed into different forms, then pair up together in something known as a master transcription factor. Smad7 becomes an inhibitor within this pathway. Inhibiting TGF- β 1, Smad2 and Smad3. Inhibiting interaction among all three of the above. We can find that we also have some evidence through our protein quantification for downregulating TGF- β 1 by Met and then Smad2, Smad3 up, as well as Smad7 up. So that's probably telling us that it must be doing its work with this modulation of the TGF - Beta slash Smads.

5. Conclusion

In short, hypertension induces myocardial fibrosis, cardiomyocyte enlargement, impairment of the heart's ejection function, as well as tachycardia. Metformin can reduce these kinds of hypertensive cardiac injuries through the way it controls TGF- β / Smads signals. Metformin reduces hypertension-induced myocardial fibrosis in SHR via TGF- β /Smads signaling cascade.

Funding

Hebei Traditional Chinese Medicine Joint Fund Cultivation Project (Project No.: H202309042)

Disclosure statement

The authors declare no conflict of interest.

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