Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Processed *Rehmanniae radix* can Improve Cold Syndrome Damage of Rats by Regulating Glycolipid Metabolism

Mengmeng Wang, Yingying Ke, Yangyang Wang, Tong Liu, Yage Li, Zengfu Shan, Wangyang Mi, Ning Zhou, Weisheng Feng, Xiaoke Zheng

School of Pharmacy, Henan University of Chinese Medicine, Zhengzhou, China

Submitted: 04-Jul-2020 Revised: 12-Aug-2020 Accepted: 10-Mar-2021 Published: 24-Jan-2022

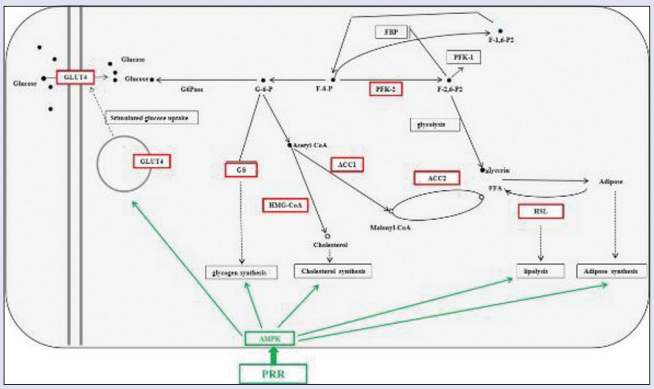
ABSTRACT

Background: The traditional chinese medicine have medicinal property,and the property of the drug has been found to be mostly related to the body's substance and energy metabolism. **Objectives:** The present study intended to assess the drug property of Rehmanniae Radix Praeparata (PRR), called Shudihuang in traditional Chinese medicine and to discover its mechanism of cold syndrome in rats. **Materials and Methods:** Through the creation of a typical cold syndrome animal model, the appearance score, body weight, rectal temperatures, and survival rate of the animals treated were evaluated at different time points. Several indices *in vivo* correlated with glycolipid metabolism (glucose transporters (GLUT- 4), fructose phosphate kinase (PFK- 2), glycogen synthetase (GS), acetyl- CoA carboxylase 1 (ACC1), acetyl- CoA carboxylase 2 (ACC2), hydroxy methylglutaryl coenzyme A (HMG CoA) and hormone-sensitive lipase (HSL) were determined; Western blot is used to analyze of phosphorylated amp- dependent protein kinase (p- AMPK) appearance in liver tissues. **Results:** Compared with the normal group, the levels of GLUT- 4, PFK- 2, GS, ACC1, ACC2, HMG- CoA, and HSL in the model group all reduced significantly and p- AMPK protein expression diminished. Compared with the model group, PRR can significantly relapse to GLUT- 4, PFK- 2, GS, ACC1, ACC2, HMG- CoA, and HSL ($P < 0.01$ or $P < 0.05$) and rise the expression of p- AMPK. RR could suggestively diminution the level of these indices ($P < 0.01$ or $P < 0.05$). **Conclusion:** Drug property of PRR was inferred as trending to "heat and warm," which still essential for further study. PRR may recover the metabolic function of the cold syndrome model rats by distressing the process of glycolipid metabolism which is by triggering the AMPK signaling pathway.

Key words: Adenosine 5'-monophosphate (AMP)-activated protein kinase signaling pathway, cold syndrome, glycolipid metabolism, medicinal properties, *Rehmanniae Radix*

SUMMARY

- Drug property of PRR was inferred as trending to "heat and warm"
- PRR may recover the metabolic function of the cold syndrome model rats by distressing the process of glycolipid metabolism



Abbreviations used: RR: *Rehmanniae Radix;* PRR: *Rehmanniae Radix Praeparata;* TCM: The theory system of traditional Chinese medicine; AMPK: Adenosine 5'-monophosphate (AMP)-activated protein kinase; GLUT-4: Glucose transporters; PFK-2: Fructose phosphate kinase; GS: Glycogen synthetase; ACC1: Acetyl-CoA carboxylase-1; ACC2: Acetyl-coa carboxylase-2; HMG CoA: Hydroxy methylglutaryl coenzyme A; HSL: Hormone-sensitive lipase; eEF–2: Eukaryotic cells peptide chain extension factor-2 kinase; UBE3: Ubiquitin ligase E3.

Correspondence:

Prof. Xiaoke Zheng,
Henan University of Chinese Medicine, Zhengdong New District,
Zhengzhou 450 000, China.
E-mail: zhengxk. 2006@163.com

DOI: 10.4103/pm.pm_282_20

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

The theory of medicinal property of Traditional Chinese Medicine (TCM) is a high-level swift of the efficacy of TCM and is a property related to the definite effect of clinical disease syndrome.[1] Its main contents can be abridged as four natures, five flavors, channel tropism, ascending and descending, and poisonous and toxicity. The four natures of drugs is also known as the four properties referred to cold, cool, hot, and warm, is the essential content of the theory of TCM.[2] The purpose of the cold, cool, hot, and warm of TCM is from the cause and the cold or hot nature of the disease treated by the drug, which is, the drug is cold or cool that can lessen and eradicate the heat syndrome, the drug has hot or warm properties that can diminish and remove the cold syndrome. Modern pharmacological studies have found that Chinese medicine's cold and hot properties are carefully related to body energy metabolism.[3] Cold drugs can constrain the body's energy metabolism and hot drugs can promote the body's energy metabolism.[4] For instance, the cold medicine Scutellaria baicalensis can apply its medicinal effects by hindering metabolic pathways such as lipid metabolism, sugar metabolism, and amino acid metabolism.[5]

Cite this article as: Wang M, Ke Y, Wang Y, Liu T, Li Y, Shan Z, *et al*. Processed *Rehmanniae radix* can improve cold syndrome damage of rats by regulating glycolipid metabolism. Phcog Mag 2021;17:728-34.

The theory of TCM considers that cold syndrome and heat syndrome reproduce the different reaction states of the body under the effect of internal and external pathogenic factors. The animal model of cold syndrome is the rudimentary model of common syndromes in the clinical practice of TCM. Stimulation of rats with ice water bath will cause chills, less movement, curling, unresponsiveness, dark red ears, like to get together, weak breathing, drowsy, dull fur, less drinking water, clear urine, and wet faces of the cold syndrome.[6] This is reliable with the cold syndrome of TCM and the method of modeling is modest, easy to learn and master, practical, and repeatable.[7] Therefore, this experiment used 0°C ice water swimming to form a cold syndrome model.

Rehmanniae Radix is the fresh or dry root of scrophulariaceae plant radix rehmanniae (*Rehmannia glutinosa Libosch*). *Dried Rehmanniae Radix* (DRR) and *Processed Rehmanniae Radix* (PRR) belong to dissimilar managed products of *Rehmanniae Radix*. DRR has sweet and cold properties, channel tropism to the heart, liver, and kidney of TCM; PRR has mild sweet properties, channel tropism to the liver and kidney of TCM.[8] According to the modern pharmacological, it has not been described that the effect of *Rehmanniae Radix* on its drug taste and glucose and lipid metabolism has been discovered. In this experiment, the typical cold syndrome model was recognized by swimming in 0°C ice water to reconnoiter the effect of *Rehmanniae Radix* on cold syndrome rats and discover the mechanism to lay a foundation for illuminating the medicinal properties of *Rehmanniae Radix* and its numerous handled products.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley (SD) rats (weighing 180 – 220 g) were acquired from Weitonglihua experimental animal technology Co., Ltd.(Beijing, China; qualification number SCXK2016-0006). The animals were kept in an air-conditioned room (temperature, 22°C; relative humidity, 55%) and fed *ad libitum* with standard feed and water during the entire progression of the present study. The examination conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The animals were employed for experiments after adaptive feeding for 7 days.

Plant material and extraction

Preparation of DRR: DRR (10 kg) was procured from Chinese herbal medicines of Henan Shunkang Pharmaceutical Co., Ltd. (China) and was qualified as authentic by Professor Chen Suiqing and Professor Dong Chengming of the Henan University of Traditional Medicine. Take a suitable amount of DRR, eradicate impurities, rinse with water, and dry pickle according to the ratio of DRR: Rice wine (2:1). Refer to the steaming method of the 2015 edition of the Chinese Pharmacopoeia (General Principles 0213),[8] and steam until it develops black and moisten, take out and bake at 55°C until 80% dry, cut into thick slices, and dry, then we get the Chinese Herbal Medicine PRR.

Extraction of DRR and PRR:[9] The crude herbs of DRR and PRR were distinctly immersed with ten-fold distilled water for 1 h and then boiled for 1 h, filtered and repeat the above three times. The filtrates were combined and followed by concentration under abridged pressure at 45°C to get the gross extract.

Reagents and instruments

Euthyrox (levothyroxine sodium tablets, MerkSerono, 253359), Whole protein extraction kit (Solarbio, bc3710-100), BCA protein concentration assay kit (Solarbio, PC0020), Prestained protein marker (Thermo, 26619-6), Phosphorylated amp-dependent protein kinase Adenosine 5‘-monophosphate (AMP)-activated protein kinase (p-AMPK) antibody (CST, #2535), Amp-dependent protein kinase AMPK antibody (CST, 18167-1-ap), Glyceraldehyde-3-phosphate dehydrogenase GADPH antibody (ABclonal, AC002), Goat anti-rabbit IgG secondary antibody (licor, 925-68071), Glucose transporters (GLUT-4), fructose phosphate kinase (PFK-2), Glycogen synthetase (GS), Acetyl-CoA carboxylase 1 (ACC1), Acetyl-CoA carboxylase 2 (ACC2), Hydroxymethyl 2 glutaric acid reductase (HMG CoA), and Hormone-sensitive lipase (HSL) kits (Jiangsu Calvin biotechnology Co., Ltd. Batch Numbers are ck-e93837r, ck-e90709r, ck-e30744r, ck-e30726r, ck-e30727r, ck-e93241r, and ck-e93380r).

Aria computer workstation (BD, USA), trans-blot® Plus transfer groove (BIO-RAD), Odyssey CLx two-color infrared laser imaging system (LI-COR, USA), Multiskan MK3 enzyme marker (Thermo Fisher), 5840R high-speed freezing centrifuge (Eppendorf), AdvantageA10 ultra-pure water device (Sartorius), BT25S 100 ppm precision analytical balance (Sartorius) and TS-2 shaker (Kylin-bell Lab Instruments), Thermo, BIO-RAD, VC independent air supply isolation cage (SuzhouFeng experimental animal equipment Co., Ltd.), BCD-206TAS low-temperaturerefrigerator(Haier),micro-pipipiant(Gilson)andDZF-6050B vacuum drying box (Beijing Hengtaifengke experimental Equipment Co., Ltd.).

Model preparation sampling

After 7 days of adaptive breeding, the animals were arbitrarily separated into Normal Control (NC) group, Model (M) group, DRR (7.0 g/kg/day, Convert according to the Chinese Pharmacopoeia, the high dose of the human daily dose of DRR) and PRR group (7.0 g/kg/day, Convert according to the Chinese Pharmacopoeia, the high dose of the human daily dose of PRR) according to the principle of weight balance. Each administration group was given by oral gavage (I. G.) every morning, while the blank group was directed with the equivalent volume of distilled water. The method of establishing the cold syndrome model is that the rat swims in a 0°C water bath every day at 3 pm until the hind limb of the rat is inflexible and cannot travel, the tip of the nose sinks into the water and cannot swim.[7]

Spontaneous activity

After 15 days, measured body weight, rectal temperature, and the number of spontaneous activities within 5 min, then the mice were forfeited.

Sample collection

After the animals were sacrificed, serum was attained by centrifugation (3000 rpm for 10 min at 4°C) and stored at −80°C until analysis. The liver and other tissues were rapidly detached and the surrounding fat and connective tissue were detached and the organ index was weighed and planned. Mouse liver was speedily frozen in liquid nitrogen and transferred to the refrigerator at −80°C for future use.

Organ index = viscera index (mg)/body weight (g) ×100%

Determination of the factors correlated with substance and energy metabolism

Enzyme-linked immunosorbent assay double antibody sandwich method was employed to perceive the factors linked with substance and energy metabolism, GLUT4, PFK-2, GS, ACC1, ACC2, HMG-CoA, and HSL. The sample or standard was added and bound to the corresponding antibody on the carrier. Further, the biotinylated antibody was added and bound precisely to the antigen which was verified on the carrier. Horseradish peroxidase-labeled avidin and biotin were precisely

combined to form an immune complex, which developed color and noticed OD value.

Western blotting

The total liver proteins of each group were mined according to the operation steps of the mammalian total protein extraction kit with BCA protein quantitative determination kit protein concentration. Separated by SDS-PAGE electrophoresis was transferred to the PVDF membrane. With 5% skimmed milk powder closed for 2 h and add p-AMPK (1:500). Incubate over the night in GADPH (1:1000) at 4°C. Rabbit and mouse source secondary antibodies (1:2000) were added and incubated at 37°C for 1 h, respectively. Protein bands were collected by the ultrasensitive multifunctional imager and examined by Quantity One software.

Statistical analysis

Measurement data were articulated as means ± standard deviation. Statistical analysis was achieved using one-way analysis of variance with the least significant difference test using SPSS version 17.0 (SPSS Inc, Chicago, IL, USA). $P < 0.05$ were measured to be statistically significant.

RESULTS

Effects on the general conditions

Compared with the normal group, the rats in the cold syndrome model group have less movement, fatigue, sneezing, unresponsiveness, crouching or recumbent lying state, abridged dietary water consumption, clear urine, clear stool, etc. After administration, the condition of the rats in the DRR group was lessened and there was no change in PRR and model groups.

Effects on body weight

According to Table 1 and Figure 1, compared with the normal group, the bodyweight of the model group was tremendously significantly abridged ($P < 0.01$). Compared with the model group, DRR and PRR can extremely meaningfully progress the weight loss of mice ($P < 0.01$).

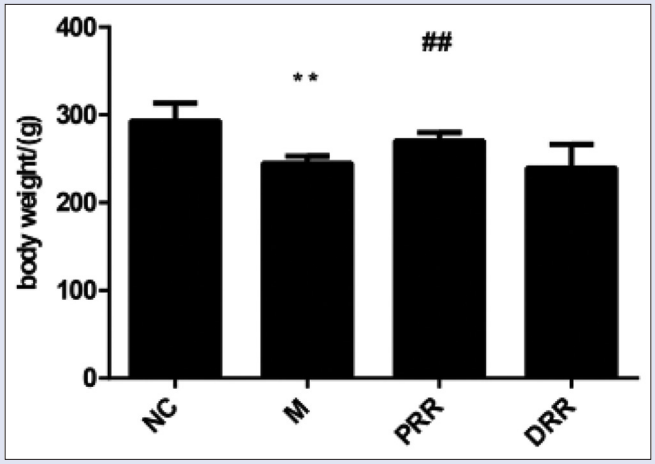


Figure 1: Effect of *Rehmanniae Radix* on body weight of rats. After administration 15 consecutive days, measured body weight. Data are mean ± Standard deviation, $n = 10$ rats per group. $^{**}P < 0.01$ versus normal group. $^{\#\#}P < 0.01$ versus model group. The method of establishing the cold syndrome model is that the rat swims in a 0°C water bath every day at 3 pm until the hind limb of the rat is stiff and cannot move, the tip of the nose sinks into the water and cannot swim;[7] PRR: Processed *Rehmanniae Radix aqueous* extract (7.0 g/kg/day); DRR: Dried Rehmanniae Radix aqueous extract (7.0 g/kg/day)

Effects on survival rate

According to Figure 2, compared with the normal group, the existence rate of the model group was enormously significantly abridged. Compared with the model group, DRR and PRR can really significantly improve rat survival.

Effects on body temperature

According to Table 2 and Figure 3, compared with the normal group, the body temperature of the model group was extremely significantly condensed ($P < 0.01$). Compared with the model group, DRR can extremely significantly recover the low temperature ($P < 0.01$).

Effects on spontaneous activities

According to Table 3 and Figure 4, compared with the normal group, the impulsive activities of the model group were extremely pointedly reduced ($P < 0.01$). Compared with the model group, PRR can awfully significantly surge the number of autonomous activities ($P < 0.01$), DRR can significantly reduce the number of autonomous activities ($P < 0.05$).

Effects on glucose metabolism

According to Table 4 and Figure 5, compared with that in the normal group, the expressions of glucose metabolism-related factors

Table 1: Effect of *rehmanniae radix* on body weight of rats ($\bar{X}$±s, n=10)

Group	Dose/(g/kg/day)	Weight/(g)
NC	-	292.99±20.23
Model	-	244.87±8.10**
PRR	7	270.15±9.69##
DRR	7	239.50±27.16

**P<0.01 versus normal group, ##P<0.01 versus model group. Data are mean±SD, n=10 mice per group. The method of establishing the cold syndrome model is that the rat swims in a 0°C water bath every day at 3 pm until the hind limb of the rat is stiff and cannot move, the tip of the nose sinks into the water and cannot swim.[7] PRR: Processed *rehmanniae radix* aqueous extract (7.0 g/kg/day); DRR: Dried *rehmanniae radix* aqueous extract (7.0 g/kg/day); NC: Normal control; SD: Standard deviation

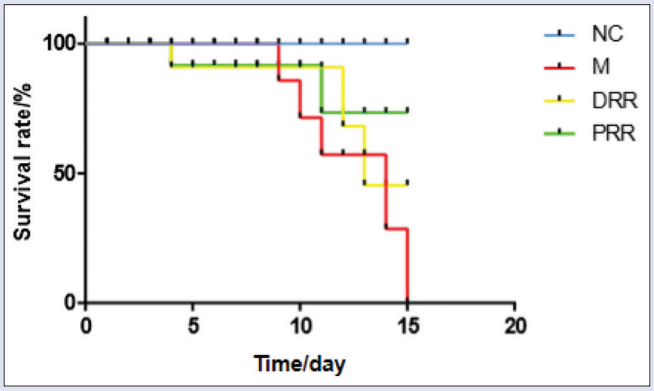


Figure 2: Effect of *Rehmanniae Radix* on survival rate of rats. Statisticaled analysis the survival rate of rats during administration 15 consecutive days. Data are mean ± Standard deviation, n = 10 rats per group. The method of establishing the cold syndrome model is that the rat swims in a 0°C water bath every day at 3 pm until the hind limb of the rat is stiff and cannot move, the tip of the nose sinks into the water and cannot swim;[7] PRR: Processed *Rehmanniae Radix aqueous* extract (7.0 g/kg/day); DRR: Dried Rehmanniae Radix aqueous extract (7.0 g/kg/day)

GLUT-4, PFK-2, and GS in the model group were very significantly abridged ($P < 0.01$). The expressions of GLUT-4, PFK-2, and GS in both the PRR and DRR groups were extremely significantly evoked. The GLUT-4 level in the DRR group extremely significantly diminished ($P < 0.01$).

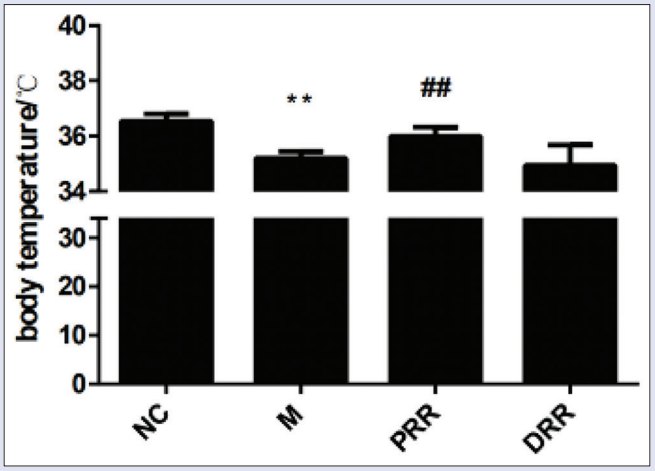


Figure 3: Effect of *Rehmanniae Radix* on body temperature of rats. After administration 15 consecutive days, measured Rectal temperature. Data are mean ± Standard deviation, $n = 10$ rats per group. $^{**}P < 0.01$ versus normal group. $^{\#\#}P < 0.01$ versus model group. The method of establishing the cold syndrome model is that the rat swims in a 0°C water bath every day at 3 pm until the hind limb of the rat is stiff and cannot move, the tip of the nose sinks into the water and cannot swim;[7] PRR: Processed *Rehmanniae Radix* aqueous extract (7.0 g/kg/day); DRR: Dried *Rehmanniae Radix* aqueous extract (7.0 g/kg/day)

Effects on lipid metabolism

According to Table 5 and Figure 6, compared with the normal group, the expression levels of ACC1, ACC2, HMG-COA, and HSL lipid

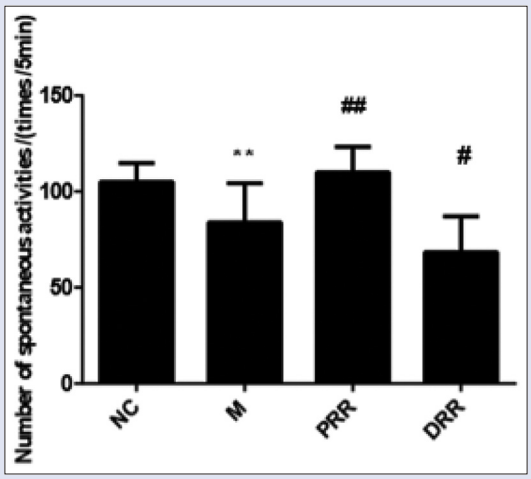


Figure 4: Effect of *Rehmanniae Radix* on autonomic activity of rats. After administration 15 consecutive days, measured the number of spontaneous activities within 5 min, then the mice were sacrificed. Data are mean ± Standard deviation, $n = 10$ rats per group. $^{**}P < 0.01$ versus normal group. $^{\#}P < 0.05$ versus model group. $^{\#\#}P < 0.01$ versus model group.The method of establishing the cold syndrome model is that the rat swims in a 0°C water bath every day at 3 pm until the hind limb of the rat is stiff and cannot move, the tip of the nose sinks into the water and cannot swim;[7] PRR: Processed *Rehmanniae Radix aqueous* extract (7.0 g/kg/day); DRR: Dried *Rehmanniae Radix* aqueous extract (7.0 g/kg/day)

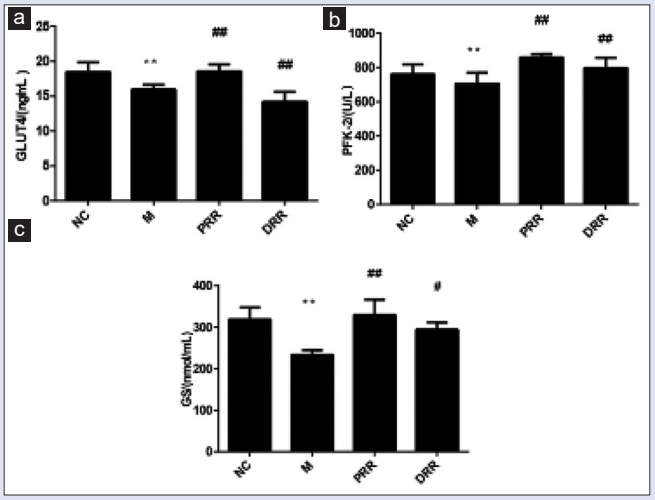


Figure 5: Effect of *Rehmanniae Radix* on glucose metabolism indexes of rats.Daily oral administration of DRR, and PRR occurred on 15 consecutive days, detect the expressions of GLUT4(a.), PFK-2(b.), GS (c.) according to the kit instructions. Data are mean ± Standard deviation, $n = 10$ rats per group. $^{**}P < 0.01$ versus normal group. $^{\#\#}P < 0.01$ versus model group. The method of establishing the cold syndrome model is that the rat swims in a 0°C water bath every day at 3 pm until the hind limb of the rat is stiff and cannot move, the tip of the nose sinks into the water and cannot swim;[7] PRR: Processed *Rehmanniae Radix aqueous* extract (7.0 g/kg/day); DRR: Dried Rehmanniae Radix aqueous extract (7.0 g/kg/day)

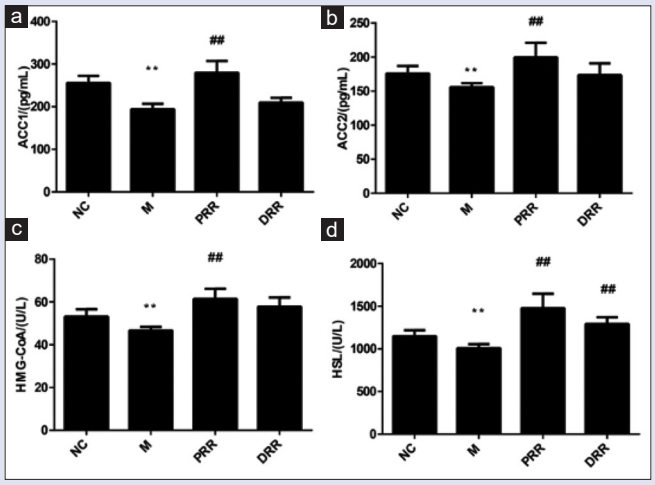


Figure 6: Effect of *Rehmanniae Radix* on lipid metabolism indexes of rats. Daily oral administration of RR, DRR, and PRR occurred on 15 consecutive days, detect the expressions of ACC1 (a.), ACC2 (b.), HMGCoA (c.), HSL (d.) according to the kit instructions. Data are mean ± Standard deviation, $n = 10$ rats per group. $^{**}P < 0.01$ versus normal group. $^{\#\#}P < 0.01$ versus model group. The method of establishing the cold syndrome model is that the rat swims in a 0°C water bath every day at 3 pm until the hind limb of the rat is stiff and cannot move, the tip of the nose sinks into the water and cannot swim;[7] PRR: Processed *Rehmanniae Radix aqueous* extract (7.0 g/kg/day); DRR: Dried Rehmanniae Radix aqueous extract (7.0 g/kg/day)

metabolism-related factors in the model group were enormously significantly abridged ($P < 0.01$). After administration, PRR can knowingly surge the expression levels of ACC1, ACC2, HMG-CoA, and HSL ($P < 0.01$); DRR can significantly upsurge the expression level of HSL ($P < 0.01$).

Table 2: Effect of *Rehmanniae Radix* on body temperature of rats ($\bar{X}$±s, *n*=10)

Group	Dose/(g/kg/day)	Temperature/(°C)
NC	-	36.54±0.27
Model	-	35.19±0.25**
PRR	7	35.98±0.35##
DRR	7	34.94±0.73

**$P<0.01$ versus normal group, ##$P<0.01$ versus model group. Data are mean±SD, *n*=10 rats per group. The method of establishing the cold syndrome model is that the rat swims in a 0°C water bath every day at 3 pm until the hind limb of the rat is stiff and cannot move, the tip of the nose sinks into the water and cannot swim.[7] PRR: Processed *rehmanniae radix* aqueous extract (7.0 g/kg/day); DRR: Dried *rehmanniae radix* aqueous extract (7.0 g/kg/day); NC: Normal control; SD: Standard deviation

Table 3: Effect of *Rehmanniae radix* on spontaneous activities of rats ($\bar{X}$±s, *n*=10)

Group	Dose/ (g/kg/day)	Number of spontaneous activities/(times/5 min)
NC	-	105.00±9.77
Model	-	83.90±20.47**
PRR	7	110.00±13.37##
DRR	7	68.38±18.66#

**$P<0.01$ versus normal group, #$P<0.05$ versus model group, ##$P<0.01$ versus model group. Data are mean±SD, *n*=10 rats per group. The method of establishing the cold syndrome model is that the rat swims in a 0°C water bath every day at 3 pm until the hind limb of the rat is stiff and cannot move, the tip of the nose sinks into the water and cannot swim.[7] PRR: Processed *rehmanniae radix* aqueous extract (7.0 g/kg/day); DRR: Dried *Rehmanniae Radix* aqueous extract (7.0 g/kg/day); NC: Normal control; SD: Standard deviation

Table 4: Effects of *rehmanniae radix* on glucose metabolism indexes of rats ($\bar{X}$±s, *n*=10)

Group	Dose/ (g/kg/day)	GLUT4/ ($ng \cdot mL^{-1}$)	PFK-2/($U \cdot L^{-1}$)	GS/ ($nmol \cdot mL^{-1}$)
NC	-	18.49±1.38	761.10±57.15	318.83±29.33
Model	-	15.94±0.71**	706.54±62.96**	233.56±11.29**
PRR	7	18.57±0.99##	858.74±19.29##	329.73±37.01##
DRR	7	14.22±1.42##	797.24±60.25##	294.58±17.09#

**$P<0.01$ versus normal group, #$P<0.05$ versus model group, ##$P<0.01$ versus model group. Data are mean±SD, *n*=10 rats per group. The method of establishing the cold syndrome model is that the rat swims in a 0°C water bath every day at 3 pm until the hind limb of the rat is stiff and cannot move, the tip of the nose sinks into the water and cannot swim.[7] PRR: Processed *Rehmanniae Radix* aqueous extract (7.0 g/kg/day); DRR: Dried *Rehmanniae Radix* aqueous extract (7.0 g/kg/day ay); NC: Normal control; SD: Standard deviation; GLUT4: Glucose transporters; PFK-K: fructose Phosphate kinase; GS: Glycogen synthetase

Results of western blot analysis

According to Figure 7, the p-AMPK protein expression level in the model group was extremely significantly reduced compared with that in the normal group ($P < 0.01$). Compared with the model group, the expression level of p-AMPK protein in liver tissues of the PRR group were extremely significantly augmented, the DRR group were extremely significantly lessened ($P < 0.01$) (GADPH was employed as internal reference protein).

DISCUSSION

In the basic theory of TCM, the property of cold, heat, warm, and cool are inimitable standards that specify the efficacy of the medicine. Current studies have shown that the medicinal properties are primarily related to the energy metabolism of the body and it mainly contains the anabolic and catabolic metabolism of the three major constituents of sugar, lipids, and protein in the body.[10] Studies have exposed that the cold Chinese medicine *phellodendron chinensis* can lessen energy metabolism in normal rats,[11] the hot Chinese medicine aconite could endorse the energy metabolism of mitochondria in rats.[12] Cold and hot herbs can move the lipid metabolism of mice at gene and protein levels.[13] This is reliable with the hypothesis "medicinal properties can affect substance and energy metabolism of the body through dissimilar ways, and cold (or cool) TCM can hinder substance and energy metabolism, hot (or warm) TCM can help substance and energy metabolism of the body." So the other way around, Can we apply the code of clinical medication "cold disease usages hot Chinese medicine to treat" to prove the cold and hot property of Chinese medicine? Basis on the code, we employ the classical cold syndrome model to authenticate the medicinal properties of *Rehmanniae Radix* and its treated products and intricate the scientific connotation of their medicinal properties.

First, the basic physical indicators can straight imitate the inclusive state of the animals. Compared with the normal group, the weight of the rats in the cold syndrome model group was meaningfully abridged, and the rectal temperature and autonomous actions were significantly condensed, which is similar to the cold syndrome of TCM.[14] After the administration of PRR, the bodyweight returned, the rectal temperature and voluntary activities augmented, that is, it enhanced the cold syndrome model of rats after the administration. This can also circuitously infer that the property of PRR is "hot."

Under normal conditions, the energy metabolism of the body is in an active balance. When the body is in a pathological state such as a cold syndrome, this steadiness will be broken. Studies have established that in the cold state, most of the gene expressions related to energy metabolism pathways are downregulated.[15] Hot property medicines can help the body's glycolipid metabolism to precise the cold syndrome imbalance,

Table 5: Effect of *rehmanniae radix* on lipid metabolism indexes of rats ($\bar{X}$±s, *n*=10)

Group	Dose/(g/kg/day)	ACC1/($pg \cdot mL^{-1}$)	ACC2/($pg \cdot mL^{-1}$)	HMG-CoA/($U \cdot L^{-1}$)	HSL/($U \cdot L^{-1}$)
NC	-	255.80±16.65	176.04±10.93	53.18±3.39	1148.59±70.61
Model	-	194.46±12.50**	155.89±5.62**	46.61±1.74**	1008.49±46.89**
PRR	7	279.86±27.40##	199.75±21.17##	61.41±4.75##	1477.30±169.98##
DRR	7	209.97±10.99	173.55±17.09	57.81±4.31	1292.36±79.15##

**$P<0.01$ versus normal group, ##$P<0.01$ versus model group. Data are mean±SD, *n*=10 rats per group. The method of establishing the cold syndrome model is that the rat swims in a 0°C water bath every day at 3 pm until the hind limb of the rat is stiff and cannot move, the tip of the nose sinks into the water and cannot swim.[7] PRR: Processed *Rehmanniae Radix* aqueous extract (7.0 g/kg/day); DRR: Dried *Rehmanniae Radix* aqueous extract (7.0 g/kg/day); NC: Normal control; SD: Standard deviation; ACC1: Acetyl-coa carboxylase 1; ACC2: Acetyl-coa carboxylase 2; HMG-CoA: Hydroxy methylglutaryl coenzyme A; HSL: Hormone sensitive lipase

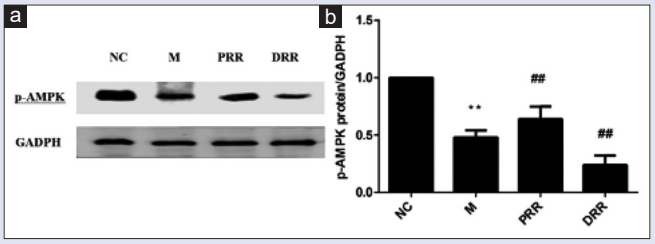


Figure 7: Effect of *Rehmanniae Radix* on p- AMPK in liver of rats (a. , b. is its Quantized histogram). Western blotting was used to detect the expression of p-Adenosine 5'-monophosphate-activated protein kinase proteins. The protein bands were analyzed by Quantity One image analysis software. Data are mean ± Standard deviation, n = 10 rats per group. $^{**}P < 0.01$ versus normal group. $^{\#\#}P < 0.01$ versus model group. The method of establishing the cold syndrome model is that the rat swims in a 0°C water bath every day at 3 pm until the hind limb of the rat is stiff and cannot move, the tip of the nose sinks into the water and cannot swim;[7] PRR: Processed *Rehmanniae Radix aqueous* extract (7.0 g/kg/day); DRR: Dried Rehmanniae Radix aqueous extract (7.0 g/kg/day)

such as Li Yi found that after treatment with warm Chinese medicine, augmented sugar and lipid metabolism and enlarged protein synthesis lead to active energy metabolism.[16] The processed Epimedium mostly establishes in the upregulation of the expression of enzymes related to the aerobic oxidation pathway of sugar and upregulation of glycogen synthesis, decomposition pathways and the expression of related enzymes in lipid metabolism pathways.[17] *Morinda officinalis* improving rats with Yang deficiency and the internal cold syndrome is connected to the inhibition of glucose and fat metabolism.[18]

Most of the body's energy derives from glycolipid metabolism.[19] After glucose is absorbed into the blood, GLUT first transports glucose into the cells to comprehensive the glucose metabolism procedure in the body.[20] Under normal circumstances, glucose mainly delivers energy to the body through aerobic oxidation.[21] About 70% of the glucose consumed by the body is transported to muscle tissue via GLUT4 for energy supply or synthesis of muscle glycogen and 7% glucose is transported to fat cells via GLUT4 and endures a series of transformations to form lipids.[22] Glycogen synthase (GS) is a key enzyme in glycogen synthesis. GS attaches the glucosyl group to the glycogen primers so that the straight chain of the glycogen molecule is unceasingly protracted to finally produce glycogen. When being in hypoglycemia or cold and hypoxia for a short time, the expression of GLUT4 will increase, which will consume more glucose for the body.[23] At the same time, the body is mostly powered by glycolysis. Phosphofructokinase-2 (PFK-2) is a key enzyme that controls the process of glycolysis. Amplified levels of PFK2 catalyze the hastening of glycolysis and produce ATP increase. Acetyl CoA produced by sugar metabolism can be employed as a basic raw material to contribute in lipid metabolism. Part of acetyl CoA is reduced under the catalysis of enzymes to form HMG-CoA, which finally produces cholesterol and part of it is catalyzed by ACC1 to yield fatty acids, which are significant raw materials for fat synthesis. Further, the body can slowly hydrolyze the fat stored in adipose tissue cells through fat mobilization, releasing free fatty acids into the blood to other tissues and organs to be oxidized and employed in the mitochondria. HSL and ACC2 are chief enzymes for fat mobilization and can control the rate of fatty acid transport to the mitochondria. Results presented that the expressions of GLUT4, PFK-2, GS, ACC1, ACC2, HMG-CoA, and HSL reduced. After treatment, PRR can expressively upsurge GLUT-4, PFK-2, GS, ACC1, ACC2, HMG-CoA, and HSL. It designated the levels of glucose and lipid metabolism were declined in the cold syndrome animals. PRR could increase glycolipid metabolism, promote the body to ingest more glucose, transport to muscle tissue and fat cells to augment glycogen synthesis, glycolysis, fat mobilization, and produce ATP increase.

The AMPK signaling pathway is thoroughly related to the metabolism of glycolipids in the body.[24] When the glucose content in the body is too high, glucose will promote the production of GLUT-4 under the regulation of AMPK, help the transport of glucose, upsurge the level of GS, ACC2, HMG-CoA, and promote the synthesis of glycogen, fatty acid, cholesterol for storage energy; when the body is famished, the body's energy is expended, intracellular ATP levels are abridged, AMP levels are amplified and AMPK is phosphorylated to activate, by promoting the production of GLUT4 on the one hand, thereby augmenting the body's glucose uptake and transport. On the one hand, by increasing PFK-2, ACC2 and HSL levels to promote glycolysis, fat mobilization, fatty acid oxidation and other catabolic pathways, upsurge ATP production.[25-28]

From the experimental consequences, the model rats with cold syndrome presented a low level of metabolism of glucose and lipid. The administration of PRR can meaningfully improve the pathological state of the model rats. Compared with the normal group, rats in the model group displayed abridged activity, burnout, mental fatigue, loose stools, reduced rectal temperature and weight loss, demonstrating that the model was effectively established. After being treated with PRR, the state of the rats improved and indicators such as body weight and the rectal temperature rose, showing that PRR can release the symptoms of cold syndrome; compared with the normal group, p-AMPK protein expression declined, GLUT-4, PFK-2, GS, ACC1, ACC2, HMG-CoA, HSL, and other factors related to glycolipid metabolism exposed a significant lessening. After treatment, PRR can significantly rise GLUT-4, PFK-2, GS, ACC1, ACC2, HMG-CoA, and HSL expression level; while the GLUT-4 level in the DRR group diminished even more. This displays that PRR is a warm Chinese medicine, which can treat cold syndrome diseases by increasing the expression level of p-AMPK in the state of heat syndrome and snowballing the metabolism of glucose and lipid; DRR is a cool Chinese medicine, it can constrain the level of GLUT-4 in rats with cold syndrome inhibits glucose metabolism in the body and exacerbates the state of cold syndrome.

CONCLUSION

PRR can advance the low level of material and energy metabolism of cold syndrome model, which specifies that the properties of PRR are warm. PRR could improve the injury of cold syndrome rats by distressing the process of glucose and lipid metabolism.

Acknowledgements

We would like to thank the Engineering and Technology Center for Chinese Medicine Development of Henan Province and the Collaborative Innovation Center for Respiratory Diseases Diagnostics, Treatment and New Drug Research and Development, Zhengzhou, China (2013638).

Financial support and sponsorship

This study was supported by The National Key Research and Development Project (The Major Project for Research of the Modernization of TCM): Key Technology Research for the Characteristic Chinese Medicine Industry Chain of Rehmannia glutinosa (2017YFC1702800), The Major Science and Technology Projects in Henan Province: Study on the key technology for quality control and the key characteristics of Rehmannia glutinosa, Dioscorea opposita Thunb and Achyranthes bidentata Blume from Henan Province (171100310500) , Henan province hige-level personnel special supprot: ZhongYuan One Thousand People Plan - Zhongyuan

Leading Talent (ZYQR201810080), The National Key Research and Development Project :The Major Project for Research of the Modernization of TCM (2019YFC1708802), High-level talent scientific research ability training projec : Study on the medicinal properties of Rehmannia glutinosa, Dioscorea opposita Thunb , Achyranthes bidentata Blume, chrysanthemum and their processed products (00104354-2018-18) and Key Scientific Research Project of Colleges and Universities in Henan Province (20B360010).

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

1. Guo J, Wang J, Iino K, Tomita M, Soga T. Quantitative and Molecular Similarity Analyses of the Metabolites of Cold- and Hot-Natured Chinese.
2. Peng W, Zhenguo W. The origin of the formation and development of the four-property theory of traditional Chinese medicine. J Shandong Univ Tradit Chin Med 2010;34:5-7.
3. Na Y, Xue Z, Ting FW, AiMin L, ZiChen W, Chun V, *et al.* [Effect of Lepidium meyenii (Maca) on neurotransmitter level and neuroendocrine-immune network of deficiency-cold and deficiencyheat syndrome rats].[J]. Zhongguo Zhong yao za zhi Zhongguo zhongyao zazhi China journal of Chinese materia medica,2020,45(5).
4. Ying HX, Nan WY, Qiang DD. Regulatory effects of Poria on substance and energy metabolism in colddeficiency syndrome compared with heat-deficiency syndrome in rats. [J]. Chinese journal of natural medicines,2018,16(12).
5. Yanan Z, Pingping C, Hongyu W, Xin G, Shumin L. Effects of Scutellaria baicalensis split components on normal rats' material metabolism, energy metabolism, endocrine system and autonomic nervous system. J Tianjin Univ Tradit Chin Med 2018;37:137-40.
6. Jia Y, Zhang ZZ, Wei YH, et al. Metabolomics coupled with similarity analysis advances the elucidation of the cold/hot properties of traditional Chinese medicines [J]. Chin J Nat Med, 2017;15:0631-0640.
7. Huixuan L, Zhihao L, Jingchun T, Yuqi L, Bin W. Establishment and evaluation of animal models of cold syndrome and heat syndrome. Pharmacol Clin Chin Mater Med 2020;36:222-7.
8. Chinese Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China. Beijing: China Medical Science and Technology Press; 2015. p. 124-5.
9. Zhu L. Discussion on processing methods and clinical efficacy of raw and cooked Rehmannia glutinosa . Modern distance education of traditional Chinese medicine in China 2010;8:100-1.
10. Yingying K, Peipei Y, Xiaolan W, Weisheng F, Xiaoke Z. The effect of the chemical resolution components of Descurainia sophia on the substance and energy metabolism of normal rats. The first "Pharmaceutical Journal" Pharmacy Frontier Forum and the 2015 Chinese Pharmaceutical Association Chinese Medicine and Natural Medicine Professional Committee Meeting; Tianjin, China; 2015.
11. Shan X, Fan Z, Pengpeng L, Tianzhu J. The effects of processing on the drug properties of *Phellodendron chinensis* were studied based on substance and energy metabolism in rats. Chin Herb Med 2015;38:1835-41.
12. QuanFu Z. Objective authenticity study on "thermal properties" of Aconite based on mitochondrial energy metabolism : Chengdu University of TCM; 2015.
13. Yujiao Y, Qianqian W, Miao Z, Yin W, Zhongfeng S, Weiwei Z. Effect of cold and heat herbs on expression of UCP1 gene and protein in brown adipose tissue of mice. Chin J Tradit Chin Med 2019;34:1679-81.
14. Yuehua L. Review and prospect of research on the nature of cold and heat syndromes. Chin J Integr Med 2019;39:397-404.
15. Alvim RO, Cheuhen MR, Machado SR, Sousa AG, Santos PC. General aspects of muscle glucose uptake. An Acad Bras Cienc 2015;87:351-68.
16. Yi l, Zhen Y, Jian X, Lihua T, Xingde A, Dezhao L. Effect of warm traditional Chinese medicine on liver mitochondrial proteome of cold model rats. J Herb Med 2014;45:373-9.
17. Xiaoxu Y, Zhenai H, Yu W, Wanru D, Shumin L. Effects of epimedium on the basis and energy metabolism of experimental animals before and after processing. Chin Med Inform 2017;34:1-5.
18. Yuqiu H, Yanan F, Tianzhu J, Tongtomh G, Ji S. Comparative study of morinda officinalis and salt morinda officinaliss regulating energy metabolism in rats with yang deficiency and internal cold. Chin Herb Med 2016;39:1028-31.
19. Han X, YangY, Metwaly AM, Xue Y, Shi Y, Dou D. The Chinese herbal formulae (Yitangkang) exerts an antidiabetic effect through the regulation of substance metabolism and energy metabolism in type 2 diabetic rats. J Ethnopharmacol 2019;239:111942.
20. Hardie DG, Ashford ML. AMPK: Regulating energy balance at the cellular and whole body levels. Physiology (Bethesda) 2014;29:99-107.
21. Orskov L, Schmitz O, Bak JF, Lund S, Kaal A, Nyholm B, *et al.* Skeletal muscle glucose uptake, glycogen synthase activity and GLUT 4 content during hypoglycaemia in type 1 diabetic subjects. Scand J Clin Lab Invest 2001;61:371-81.
22. Wood IS, Wang B, Lorente-Cebrián S, Trayhurn P. Hypoxia increases expression of selective facilitative glucose transporters (GLUT) and 2-deoxy-D-glucose uptake in human adipocytes. Biochem Biophys Res Commun 2007;361:468-73.
23. Huang S, Czech MP. The GLUT4 glucose transporter. Cell Metab 2007;5:237-52.
24. Russell RR 3rd, Li J, Coven DL, Pypaert M, Zechner C, Palmeri M, *et al.* AMP-activated protein kinase mediates ischemic glucose uptake and prevents postischemic cardiac dysfunction, apoptosis, and injury. J Clin Invest 2004;114:495-503.
25. Hui L, Yaoshan D, Wenliang Z, Baiyang Y, Yuan L, Siqian F, *et al.* Exercise improves lipid droplet metabolism disorder through activation of AMPK-mediated lipophagy in NAFLD. Life Sciences. 2021;273.
26. Francisca TM, Mario P, Carlos W, Daniel L, Javier D, Sebastián R, *et al.* Testosterone activates glucose metabolism through AMPK and androgen signaling in cardiomyocyte hypertrophy. Biological Research. 2021;54.
27. Li L, Jiang Z, Yao Y, Yang Z, Ma H. (–)-Hydroxycitric acid regulates energy metabolism by activation of AMPK - PGC1α -NRF1 signal pathway in primary chicken hepatocytes. Life Sciences. 2020;254.
28. Zhe Z, Xi L, Lingjun T, Youyou L, Huaxi Y, Pimin G, *et al.* Effect of Inonotus obliquus (Fr.) Pilat extract on the regulation of glycolipid metabolism via PI3K/Akt and AMPK/ACC pathways in mice. Journal of ethnopharmacology. 2021;273.