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Effects of Rehmannia 14 on Hormone Levels and Renal Function in Geriatric and Kidney *Yang* Deficiency Mice

Aituan Ma*, Xufeng Qian, Haiyan Wang, Shuo Li

ABSTRACT
The objective of this study was to investigate Rehmannia 14 (R14), a Chinese herbal medicine, reported to be effective in treating endocrine and renal function disorders in geriatric and Kidney *Yang* Deficiency cases. Using a geriatric and Kidney *Yang* Deficiency mouse model (GKDM) with a control group, 60 male Kunming mice expressing clinical signs of GKDM (weight loss, lethargy, cool limbs, decreased rectal temperature) were treated with R14 to investigate its impact on serum concentrations of triiodothyronine (T3), tetraiodothyronine (T4), thyroid stimulating hormone (TSH), adrenocorticotropic hormone (ACTH), cortisol (COR), creatinine (CRE), blood urea nitrogen (BUN) and the content of nitric oxide (NO) in renal homogenates. Study results demonstrated that GKDM model mice had decreased body weight/rectal temperatures and lethargy while R14 treated animals had improved weight gains/normal rectal temperature and improved activity levels. The GKDM mice also had decreased T3/T4/ACTH with increased TSH while the R14 dosed animals had increased T3/T4/ACTH and decreased TSH levels. In addition, R14 exerted protective effects on the kidneys with decreased NO, CRE and BUN concentrations. Study findings concluded that R14 improved serum concentrations of key endocrine indicators of disorders of the hypothalamic-pituitary-adrenal axis and has a protective effect on renal function in a GKDM mouse model. The findings from this study provide a basis for the clinical use of R14 in the treatment of endocrine and renal disorders associated with aging and Kidney *Yang/Qi* Deficiency in animals.

Keywords: endocrine, geriatric, herbal therapy, Kidney *Yang* Deficiency, Rehmannia 14, renal function, traditional Chinese veterinary medicine

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ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
BUN	Blood urea nitrogen
BW	Body weight
COR	Cortisol
CRE	Creatinine
GKDM	Geriatric and Kidney *Yang* Deficiency mouse model
HPA	Hypothalamic-pituitary-adrenal
IG	Intra-gastric gavage
IM	Intramuscular
JGSQ	*Jin Gui Shen Qi*
KYD	Kidney *Yang* Deficiency
NO	Nitric oxide
PAA	Pituitary-adrenal axis
R14	Rehmannia 14
SC	Subcutaneous
T3	Triiodothyronine
T4	Tetraiodothyronine
TCM	Traditional Chinese medicine
TCVM	Traditional Chinese veterinary medicine
TSH	Thyroid stimulating hormone

Rehmannia 14 (R14) is a modification of the classical traditional Chinese medicine (TCM) formula, *Jin Gui Shen Qi* (JGSQ), which was originally described in *Jin Gui Yao Lue Fang Lun* (Synopsis of Prescriptions from the Golden Cabinet) written by Zhang Zhong-Jing and published in 220 C.E.[1] The individual ingredients for JGSQ include *Fu Zi* (Aconite), *Gui Zhi* (Cinnamomum), *Di Huang* (Rehmannia), *Shan Zhu Yu* (Cornus), *Shan Yao* (Dioscorea/Chinese Yam), *Mu Dan Pi* (Moutan/Peony Bark), *Ze Xie* (Alisma) and *Fu Ling* (Poria) having the therapeutic intent to warm and tonify Kidney *Yang* and to treat Kidney *Qi/Yang* Deficiency (Figure 1).[1,2] Kidney *Yang* Deficiency is characterized by a damaged hypothalamic-pituitary-adrenal (HPA) axis that incorporates diseases of multiple metabolic pathways which include the adrenal and thyroid glands, as well as the gonads.[3-5]

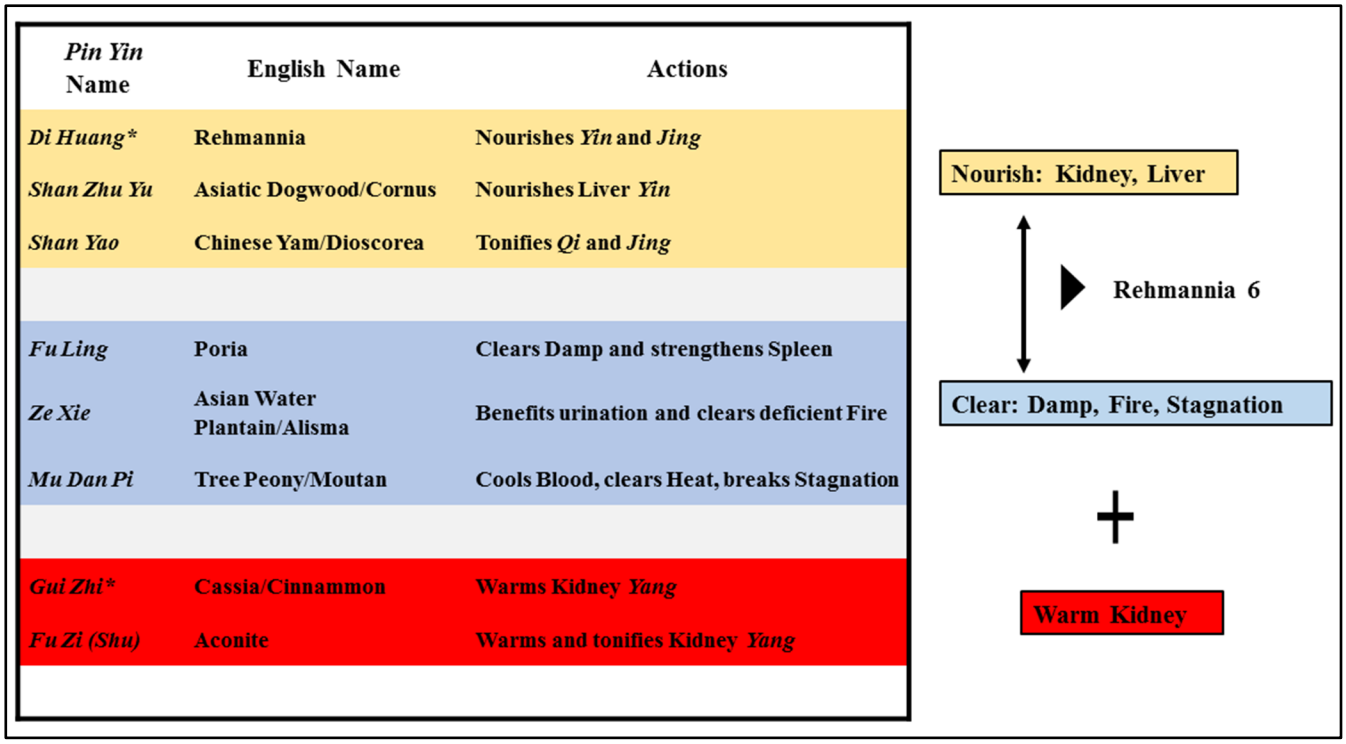


Figure 1: Ingredients of the Chinese herbal medicine *Jin Gui Shen Qi* and their actions; indications include renal failure, endocrine and immune diseases with *Qi*/*Yang* Deficiency. * = *Di Huang* (Rehmannia) in this formula can be *Shu Di Huang* (processed) if tonification of Blood is most important or *Sheng Di Huang* (unprocessed) if *Yin* tonification is most important. Cinnammon is another example where either *Rou Gui* (bark) can be used for more warmth or use *Gui Zhi* (twig) for less.

In addition, the HPA axis plays an important role in maintaining the structural stability of the central nervous system.[5] Kidney *Yang* Deficiency (KYD) is commonly seen in geriatric animals and is characterized by weakness/ lethargy, cool limbs and ear tips, painful/weak hindquarters, along with urinary incontinence and loose stools.[3,4]

Beneficial pharmacological effects have been demonstrated with JGSQ in pre-clinical laboratory animal models in endocrine and reproductive studies, as well as for anti-aging and as an immunostimulant. Human clinical trials have demonstrated its benefit for disorders such as diabetes, hypertension, low back pain, glomerulonephritis, urinary incontinence, asthma and infertility.[1] The use of JGSQ has shown similar benefit for a wide multitude of Kidney *Yang*/*Qi* associated diseases in veterinary medicine. These include endocrine disorders (diabetes mellitus/ insipidus, hypoadrenocorticism, hypothyroidism); sexual/ reproductive/genitourinary disorders; glomerulonephritis, proteinuria/urinary incontinence; and back pain/ intervertebral disease.[1]

The veterinary specific herbal formula, R14[a], has modified JGSQ by adding *Huang Qi* (Astragalus), *Wu Wei Zi* (Schisandra), *Mai Men Dong* (Ophiopogon), and *Bai Shao Yao* (Paeonia) having the therapeutic intent to tonify *Qi* and nourish *Yin*. Additionally, *Huang Bai* (Phellodendron) and *Zhi Mu* (Anemarrhena) were added to clear Heat and nourish *Yin* (Figure 2).[2] This results in retaining the primary clinical use of JGSQ, which is to warm and tonify Kidney *Yang*, while also tonifying Kidney *Yin* (Table 1).[2] Clinical observations in veterinary practice have documented its successful use in treating endocrine, neurological and immune disorders, such as hyperadrenocorticism (dogs and horses), degenerative myelopathy, diabetes and hypothyroidism as well as lameness in geriatric animals with KYD.[6-10]

Non-Western medical systems such as TCM and traditional Chinese veterinary medicine (TCVM) originate from an ancient medical system with a unique cultural background. Based on ancient texts, TCM is the result of a continuous process of critical thinking, as well as extensive clinical observation.[11] The philosophy, logic, sensibility and habits of this civilization, which form the roots of the system are however, foreign to Western thought. There is a growing recognition by more Western countries that this unique medical system has therapeutic efficacy; however, safety and clear pharmacological mechanisms of action are still unknown. Due to the potential application of TCM/TCVM in healthcare, there is a desire to construct an evidence-based scientific evaluation system with TCM characteristics benchmarked by standards of Western medicine. Model organisms play an important role in the understanding of basic biological processes and bring modern scientific standards into ancient Chinese medicine.[11] The aim of the present study was to investigate whether R14 could improve impaired renal and HPA axis disorders, in an appropriate TCM animal model (geriatric and Kidney *Yang* Deficiency mouse) by documenting changes in endocrine parameters and renal function.

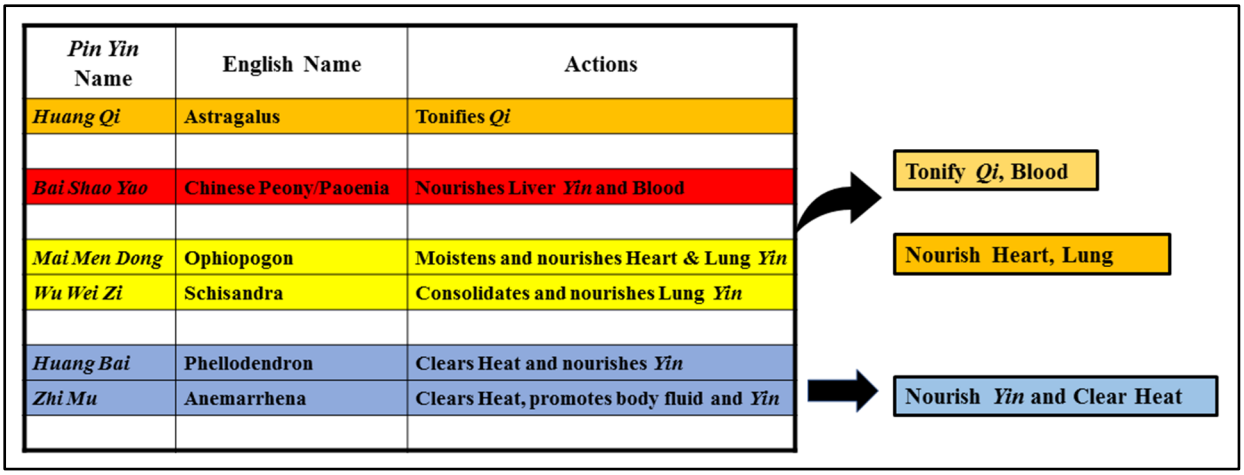


Figure 2: Additional herbal ingredients and their actions, added to the classical Chinese herbal medicine, *Jin Gui Shen Qi*, to make the Chinese herbal medicine, Rehmannia 14; indications include renal failure, immune and endocrine metabolic diseases with a more balanced *Yang* with *Yin* herbal formula.

Table 1: Ingredients of the Chinese herbal medicine, Rehmannia 14[a], and their actions[2]

Pin Yin Name	English Name	Actions[2]
Sheng Di Huang	Rehmannia	Nourishes *Yin* and *Jing*
Shan Yao	Chinese Yam	Tonifies *Qi*, nourishes Kidney *Jing*
Shan Zhu Yu	Cornus	Nourishes *Yin*
Fu Ling	Poria	Drains Damp, nourishes Spleen
Fu Zi	Aconite	Tonifies Kidney *Yang*
Mu Dan Pi	Peony Bark	Cools Liver
Gui Zhi	Cinnamomum	Tonifies Kidney *Yang*
Ze Xie	Alisma	Drains Damp, clears Kidney False Fire
**Huang Qi*	Astragalus	Tonifies *Qi*
**Mai Men Dong*	Ophiopogon	Moistens and nourishes Heart and Lung *Yin*
**Wu Wei Zi*	Schisandra	Consolidates and nourishes Lung *Yin*
**Bai Shao Yao*	Paeonia	Nourishes Liver *Yin* and Blood
**Huang Bai*	Phellodendron	Clears Heat and nourishes *Yin*
**Zhi Mu*	Anemarrhena	Clears Heat, promotes Body Fluid and *Yin*

*=Additional herbal ingredients added to *Jin Gui Shen Qi* to make Rehmannia 14

MATERIALS AND METHODS

Animal Model

The investigation of R14 was conducted with the TCM animal model, geriatric and Kidney *Yang* Deficiency mouse. These animals present with accelerated aging changes (increase production of reactive oxygen species, increased senescence and memory loss); and characteristics of KYD, which features HPA axis dysfunction metabolic disorders and clinical signs such as lower body temperatures/cool limbs, poor body weight (BW) gain, loose body hair, arched back and slow responses.[3,12] The geriatric and Kidney *Yang* Deficiency mouse model (GKDM) has been established by preliminary trials in the principal author's laboratory based on an established rat model.[12]

Groups and Treatment

Sixty, healthy, 5-week-old Kumming male mice were randomly divided into 5 groups (A-E) with 12 mice in each group. The Group A mice remained as normal controls and only received saline during the study. To establish clinical changes consistent with the GKDM model, mice in Groups B-E were given a daily subcutaneous (SC) injection of D-galactose[b] (200 mg/kg/day), from Day 1 through Day 40 to create an accelerated aging effect. This was followed by a daily intramuscular (IM) injection in the hindlimb of hydrocortisone[c] at 25 mg/kg/day, from Day 41 through Day 50 to create HPA perturbations. In addition, mice in Groups C, D, E were respectively treated during the entire study (days 1-68) with either a low (0.80 g/kg/day),

Table 2: Summary table of groups, treatments and outcome data for the Rehmannia 14 study

Groups (n=12)	Geriatric & Kidney *Yang* Deficiency Model (GKDM) Daily Treatment	Rehmannia 14 (R14) Daily Treatment	Study Outcome Data
A – Normal Control	Days 1-40: saline, SC Day 41-50: saline, IM	Days 1-68: saline, IG	Daily activity Body/Hair coat condition Food consumption Water consumption Body weight T3, T4, TSH ACTH, Cortisol BUN, Creatinine Nitric oxide
B – GKDM Model	Days 1-40: D-galactose, SC Days 41-50: Hydrocortisone, IM	Same as Group A	
C – Low Dose R14	Same as Group B	Days 1-68: 0.80 g/kg R14, IG	
D – Medium Dose R14	Same as Group B	Days 1-68: 1.60 g/kg R14, IG	
E – High Dose R14	Same as Group B	Days 1-68: 3.20 g/kg R14, IG	

SC=subcutaneous injection, IM=intramuscular injection, IG=oral intragastric, T3= triiodothyronine, T4= tetraiodothyronine, TSH=thyroid stimulating hormone, ACTH= adrenocorticotropic hormone, BUN= blood urea nitrogen

medium (1.60 g/kg/day) or high (3.20 g/kg/day) dose of R14[a] once daily by intra-gastric gavage (IG). The test substance (R14 powder) was suspended in boiling water. After liquid cooling, all groups received an equal gavage volume. Group A mice were given a daily SC saline injection (days 1-40), an IM saline injection (days 41-50) and IG saline (days 1-68). The GKDM mice (Group B), did not receive R14 and instead received an equal daily IG dose (days 1-68) of normal saline (Table 2).

All animals were sourced from the Laboratory Animal Center of Hebel Medical University. The mice were group housed with 6 mice in a polypropylene cage with cellulose fiber chip bedding[d] at a temperature of 25ºC and 30-40% humidity along with a 14-hour light and 10-hour dark cycle. All animals were given free access to rodent feed[d] and room temperature water (boiled first) in glass bottles with rubber stoppers for an adaptive period of 7 days. These identical study parameters were then maintained for the entire study. All animal care and use were in accordance with the guidelines of the Chinese Council for Animal Care.

Physical Activities, Hormone and Renal Function Tests

The amount of time, during a 5-minute observation period, that mice maintained spontaneous physical activity was evaluated and documented daily. This observation period occurred 15 minutes after IG treatment each day for all study mice. Their mental state was also observed along with changes in hair coat and temperature of limbs. Daily food and water consumption were recorded while BW was measured every 4 days and rectal temperature measured on days 0, 40, 50 and 68.

Serum samples were obtained on Day 68 prior to euthanasia. At necropsy, a kidney was collected to be used for renal homogenate measurements. The serum concentrations of triiodothyronine (T3), tetraiodothyronine (T4), thyroid stimulating hormone (TSH), adrenocorticotropic hormone (ACTH) and cortisol (COR) were measured by radioimmunoassay[e]. Serum concentration of creatinine (CRE) and blood urea nitrogen (BUN) were measured by CRE and BUN test kits[f] respectively. The concentration of nitric oxide (NO) was detected in the renal homogenates using the nitrate reductase assay kit[f].

Statistical Analysis

Statistical analysis was conducted using SPSS 20 Software[g]. All data are presented as Mean±SD. After testing for homogeneity of variance, the 5 groups were compared using one-way analysis of variance (ANOVA). Differences were considered at $p<0.05$ and significant differences were present when $p<0.01$.

RESULTS

Physical Parameters

When compared to the normal controls, study findings documented the spontaneous physical activity times within the 5-minute observation period in GKDM mice (Group B) were decreased. These mice would crowd together with dulled responses and cool limbs, while mice in the three R14 groups (C-E) had greater spontaneous activity times (5-minute observation period) and had general observations of being more alert and responsive.

The normal controls had a steady BW increase over the study with a 40.41% BW gain (compared to Day 0) by study termination. During the D-galactose injection period (days 0-40), Groups B-D had lower BW gain when compared to controls while the high dose R14 mice (Group E) equaled and slightly bettered control mice BW gain. All four model induction groups (B-E) had significant BW reduction when hydrocortisone was injected from day 40 to 50. The R14 dose groups' average BW started to increase from days 50 to 68 with the R14 groups improving to a greater extent than the model group by the end of the study. Significant differences, however, between the test groups (C-E) and normal controls (Group A) remained through the end of the study (Table 3).

Table 3: Effect of Rehmannia 14 on group mean body weight at study assessment time points with percent weight gain compared to Day 0

Groups (n=12)	Day 0 Body Weight	Day 40^ Body Weight (% weight gain)	Day 50+ Body Weight (% weight gain)	Day 68 Body Weight (Total % body weight gain)
A – Normal Control	38.53±0.82	49.65±2.58 (28.9%)	50.62±2.35 (31.38%)	54.10±3.38 **(40.41%)**
B – GKDM Model	38.53±1.03	49.19±1.87 (27.7%)	45.57±1.69*	48.94±1.48* **(27.02%)**
C – Low Dose R14	38.28±1.15	48.09±1.91 (25.6%)	44.95±1.17*	50.33±1.24* **(31.48%)**
D – Medium Dose R14	38.85±1.13	48.84±2.34 (25.7%)	45.40±2.07*	49.41±4.49* **(27.18%)**
E – High Dose R14	37.79±1.81	50.01±2.94 (32.3%)	46.15±2.49*	49.42±2.81* **(30.78%)**

Note: ^ = Groups B-E received D-galactose (days 1-40); + = Groups B-E received hydrocortisone (days 41-50), Note only control had BW gain during this period; * = $p<0.05$ (compared to controls); # = $p<0.05$, ## $p<0.01$ (compared to Group B model)

Table 4: Effect of R14 on group mean rectal temperature in mice at study assessment time points

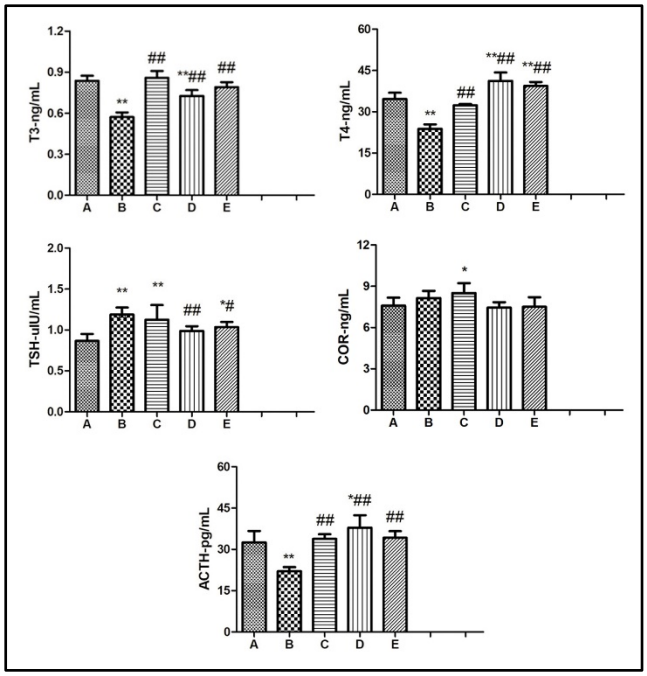
Groups (n=12)	Day 0	Day 40^	Day 50+	Day 68
A – Normal Control	37.25±0.32	37.23±0.20	37.96±0.26	38.12±0.39
B – GKDM Model	37.39±0.30	37.01±0.35	37.32±0.55*	37.04±1.09*
C – Low Dose R14	37.37±0.42	37.11±0.42	37.32±0.41*	37.84±0.46#
D – Medium Dose R14	37.55±0.26	37.09±0.36	37.24±0.25*	37.18±0.43
E – High Dose R14	37.65±0.15	37.76±0.43#	37.32±0.44*	37.62±0.58

^ = Groups B-E received D-galactose (days 0-40); + = Groups B-E received hydrocortisone (days 41-50) with all groups having lower temperature than controls; * = $p<0.05$ (compared to controls); # = $p<0.05$ (compared with model Group B)

The mean group rectal temperature was decreased in all groups at Day 40 except for the R14 high dose group. These mice had a slight increase in temperature that was statistically significant when compared to the GKDM model (Group B). At Day 68, the GKDM model still had a decreased mean rectal temperature compared to normal control while the rectal temperatures in the three R14 groups had equaled or exceeded the normal controls with the average rectal temperature in Group C (R14 low dose) significantly increased relative to the GKDM model mice (Table 4).

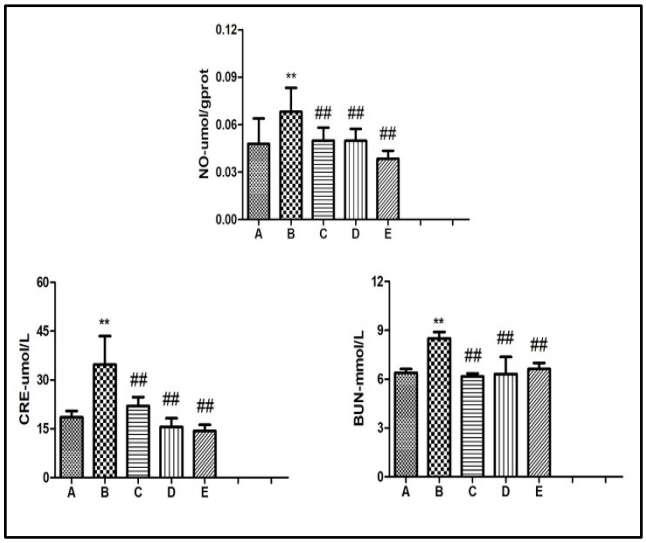
Serum Blood Chemistry Parameters

In the GKDM model (Group B), the serum concentrations of T3, T4 and ACTH decreased 31.67%, 31.33%, 37.27% respectively, when compared with controls ($p<0.01$) thus establishing the validity of the TCM mouse model for these parameters (Table 5). All R14 dose groups increased the serum concentrations of T3, T4, ACTH ($p<0.01$). The low dosage of R14 elevated T3, T4 and ACTH by 50.09%, 36.02%, and 53.15% respectively. The medium dosage elevated T3, T4 and ACTH by 26.97%, 73.41%, and 71.62%, whereas the high dosage increased T3, T4 and ACTH by 38.11%, 65.71%, and 55.07%. The TSH increased 37.27% ($p<0.01$) in GKDM mice in response to the hypothyroid condition present in these mice. The TSH serum concentration in mice receiving the medium dosage of R14 decreased by 16.94% ($p<0.01$). There were no statistically significant changes in COR serum concentrations, except the low dose R14 group was elevated when compared with the control group (Figure 3). All NO, BUN and CRE concentrations, when compared to normal controls, increased significantly ($p<0.01$) for GKDM mice thus establishing the validity of the TCM mouse model for these parameters. Study findings demonstrated all three R14 groups had significantly ($p<0.01$) reduced NO, BUN and CRE concentrations compared to the GKDM model mice (Figure 4).



A – Normal Control group, B – GKDM Model group, C – Low Dose R14 group, D – Medium Dose R14 group, E – High Dose R14 group.

Figure 3: Effects of Rehmannia 14 on T3 (triiodothyronine), T4 (tetraiodothyronine), TSH (thyroid stimulating hormone), ACTH (adrenocorticotropic hormone) and cortisol (COR) serum concentrations at Day 68 in study groups A-E; * p<0.05 (compared with control), ** p<0.01 (compared to control); # p<0.05 (compared with GKDM); ## p<0.01 (compared with GKDM).



A – Normal Control group, B – GKDM Model group, C – Low Dose R14 group, D – Medium Dose R14 group, E – High Dose R14 group.

Figure 4: Effects of Rehmannia 14 on serum levels of BUN (blood urea nitrogen), CRE (creatinine) and renal homogenate of NO (nitric oxide) at Day 68; * p<0.05 (compared with control), ** p<0.01 (compared to control); # p<0.05 (compared with GKDM); ## p<0.01 (compared with GKDM).

Table 5: Group percentage serum concentration changes with statistical significance for Group B (GKDM model) when compared to normal controls (Group A) to establish the validity of the model; Groups C to E are compared to Group B (GKDM model) on Day 68 (p<0.01)

Groups (n=12)	T3	T4	TSH	ACTH	BUN	CREAT	NO
B – GKDM Model	↓ 31.67**	↓ 31.33**	↑ 37.27**	↓ 37.27**	↑ 33.07**	↑ 87.33**	↑ 42.31**
C – Low Dose R14	↑ 50.09##	↑ 36.02##		↑ 53.15##	↓ 27.55##	↓ 36.75##	↓ 26.69##
D – Medium Dose R14	↑ 26.97##	↑ 73.41##	↓ 16.94##	↑ 71.62##	↓ 25.83##	↓ 55.23##	↓ 26.89##
E – High Dose R14	↑ 38.11##	↑ 65.71##		↑ 55.07##	↓ 22.11##	↓ 58.77##	↓ 43.59##

↓ = percent decrease, ↑ = percent increase; T3= triiodothyronine, T4= tetraiodothyronine, TSH=thyroid stimulating hormone, ACTH= adrenocorticotropic hormone, BUN= blood urea nitrogen; * p<0.05 (compared with control), ** p<0.01 (compared to control); # p<0.05 (compared with GKDM); ## p<0.01 (compared with GKDM)

DISCUSSION

Jin Gui Shen Qi is a classic traditional Chinese herbal medicine for the treatment of KYD. According to TCM/TCVM theory, Kidney *Yang* is the source of global *Yang*, and the geriatric animal is prone to Kidney *Yang* Deficiency; particularly when showing "Cold" clinical signs. Rehmannia 14 was formulated from JGSQ which is the basic formula for Kidney *Qi* or *Yang* Deficiency patterns.[2] After adding herbs to not only tonify Kidney *Yang*, but also nourish Kidney *Yin*, and thereby avoid consumption of the body *Yin* by the hot *Yang* tonics; R14 is *Yin* and *Yang* balanced. The aim of the present study was to investigate the effects of R14 on a geriatric KYD mouse model.

Findings demonstrated that the geriatric KYD mouse model (GKDM) had decreased BW gain along with exercise intolerance, consistent with the clinical presentation of *Qi* Deficiency in geriatric animals. The mice also exhibited cold extremities and lower rectal temperatures, which is consistent with *Yang* Deficiency. General daily observation of R14 dosed mice compared to

GKDM model mice gave the impression that R14 dosed at 0.80, 1.60, and 3.20 g/kg/day may improve *Qi* Deficiency signs such as decreased activity level and alertness. At Day 68, all R14 groups had normal rectal temperature compared with controls. The BW gain, however, in the R14 groups did not obtain the mean weight gain of their control counterparts by study completion. These results suggest that R14 improves the Kidney *Yang* Deficiency pattern, but is not as beneficial in supporting the Spleen, which is the dominant *Zang-fu* organ for BW.

In addition to beneficial effects on physical parameters for R14 dosed mice, there was a significant effect on the HPA axis perturbations. Studies have shown that one of the major characteristics of the KYD syndrome is functional disorder of the hormones of the HPA axis target glands.[4] Using the KYD rat model, one study reported that the pituitary-thyroid axis was affected with T3 and T4 significantly decreased while increasing TSH. Administration of *You Gui Wan*, the traditional herbal formula for treating KYD, reversed this and increased the concentrations of T3 and T4 while decreasing TSH.[13] The GKDM model was consistent with this, which was reflected in decreased levels of T3 and T4 accompanied by an increased TSH. This suggests the Chinese herbal medicine, R14, assists regulation of the pituitary-adrenal axis (PAA) which is similar to results reported by Zha.[14]

Regarding the PAA, a previous study revealed that KYD rats had decreased PAA function and that the herbal medicine *Dong Chong Xia Cao* (Hirsutella sinensis fungus) ameliorated KYD symptoms via enhancement of the PAA function as shown by increased concentrations of serum CRH, ACTH, and CORT.[5] The ACTH gene expression level was significantly elevated in KYD rats administered JGSQ.[15] The R14 treatments in the present study had similar hormone levels with increased ACTH levels in dosed animals from the deficient levels present in the GKDM mice.

Geriatric patients with Cushing's disease, hypothyroidism and diabetes as well as other metabolic disorders often present with decreased renal function and elevated serum NO, CRE and BUN concentrations. The present study demonstrated that R14 protected renal function in dosed animals with decreases in NO, CRE and BUN concentrations when compared with the elevated levels observed in the GKDM model mice.

Even though the present study demonstrated positive effects for R14 on clinical signs, endocrine parameters and renal function in the induced geriatric KYD mouse model, study limitations should be considered. This study only included a small number of animals of 1 sex (male). In addition, inadequacy of test indexes and/or insufficient information should always be considered. It is difficult to know the whole picture of R14 from this small study, thus further large, well-designed randomized controlled trials are required for a more definitive conclusion of its effectiveness. In addition, further studies are required for more complete understanding of R14's mechanism of action based on other animal models.

The utilization of a model organism, as done in the present study, in the construction of a TCM/TCVM syndrome model, highlights the relevance of modern medicine with TCM/TCVM diagnostic patterns. A good TCM syndrome model can help bridge the relationship between the essence of the relevant TCM/TCVM pattern observed in the clinic and experimental data describing its mechanism of action.[11] The TCVM syndrome model, as used in the present study, provided an effective basic tool for study of TCVM clinical signs and hormonal abnormalities seen in Kidney *Yang* Deficiency animals and relevant benefits of the Chinese herbal formula R14.

In summary, Rehmannia 14 improves both clinical signs and pathologic changes in Kidney *Yang* Deficiency mice expressing geriatric changes induced by D-galactose and hydrocortisone injections. It also helps restore normal function to disordered pituitary-thyroid axis, pituitary-adrenal axis and renal function in affected animals, thus proving efficacy for treating endocrine and renal disorders in clinical geriatric and Kidney *Yang* Deficiency cases.

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FOOTNOTES

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g. SPSS 20 Software, SPSS Inc., Chicago, Illinois, USA

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