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INFLUENCE OF BOKHI ON KIDNEY-YANG-DEFICIENCY SYNDROME IN RATS

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ABSTRACT

In Mongolian folk medicine, Bokhi, which comes from male camel occipital gland secretions, is used to treat Kidney-Yang-Deficiency Syndrome (KYDS) which has similar clinical signs as glucocorticoid withdrawal syndrome. Model KYDS rats were established by multipoint subcutaneous injection of hydrocortisone for 14 successive days and then the rats were treated by oral administration of Bokhi from two regions in China and at both a high and a low dose for a further 14 successive days. The growth rate, food intake, urine volume, vesicula seminalis, spleen, kidney, testes and the general condition of rats were recorded. The levels of serum creatinine, blood urea nitrogen, testosterone, thyroid stimulating hormone, superoxide dismutase and nitric oxide in rat serum were quantified. Results demonstrated that the symptoms of KYD were gradually alleviated by the administration of Bokhi, which also affected urine volume. Bokhi increased the growth rate and levels of testesterone, superoxide dismutase and nitric oxide. The levels of serum creatinine, blood urea nitrogen, thyroid stimulating hormone were reduced. However, Bokhi had little effect on food intake or organ indices. This experiment demonstrated that high doses of Bokhi could improve KYDS.

Key words: Camel, Bokhi, KYDS, Hydrocortisone, Occipital gland secretion

Kidney-Yang-Deficiency Syndrome (KYDS) is a term used in traditional Chinese medicine (TCM) to describe an illness characterised by paleness, chills, cold extremities, a weak and slow pulse, poor semen production and general spiritual malaise (Hao *et al,* 2008). In modern medicine it is recognised that KYDS is related to problems in the neuroendocrine immune system. Specifically, KYDS results from dysfunction of the hypothalamus-pituitary-adrenal axis, the thyroid axis, the gonadal axis (to varying degrees), but also organs involved in metabolism and the immune system resulting in hypofunction and pathological change (Lu and Wo, 2007). Experimentally, a valid method to establish KYDS in a model animal (e.g. rats), is by subcutaneous injection of high doses of an exogenous glucocorticoid such as corticosterone or hydrocortisone, which induces atrophy of the hypothalamic-pituitary glands and reduced secretion from those glands (Gou *et al,* 2009; Li *et al,* 2013; Zhao *et al,* 2013). Following injection these animal models show the same symptoms as KYDS and are widely employed in the evaluation of the mechanisms for establishment of KYDS and the therapeutic effects of curative drugs (Huang *et al,* 2013).

The Alxa League and Bayan Nur areas in the Autonomous Region of Inner Mongolia, have more bactrian camels than elsewhere in China. To adapt to the harsh desert and semi-desert conditions (extremes of heat and cold, arid conditions, poor grazing), camels have evolved many special abilities and attributes over a long period of natural selection (Jirimutu *et al,* 2012). Amongst these characteristics, camels secrete particular sex hormones during the rut. The sexual season in camels is relatively stable and sexual activity only occurs during the period of the rut. Female camels come into 'heat', or oestrus, between the end of one year and the spring of the next year. As with Asian elephants, during this time, male camels secrete a light brown foul smelling sticky liquid (Gosling, 1985) from their occipital glands (Yagil and Etzion, 1980), known locally as Bokhi (Mongolian transliteration). The volatiles produced by Bokhi are the male camels' sex hormone and they induce females into oestrus (Tingari and George, 1984). During this time the males' occipital glands increase in size secreting more Bokhi and then, during the summer, they shrink again in size. Bokhi production is a distinctive feature of male camels and the size of occipital glands and associated rate of

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Bokhi secretion is a measure of likely sexual activity and mating success.

In Mongolia Bokhi is used for the treatment of KYDS. When Bokhi from a male camel's mane is dipped in water and the resulting solution eaten over a period of a week, the positive effects on alleviation of KYDS is obvious. However, this is a folk method and the most effective dose of Bokhi has not been quantified. Here we describe an experiment to study the influence of Bokhi (from camels from two regions in China) on KYDS in a rat model in which the KYDS has been induced using hydrocortisone. This provides pharmacological evidence for the effects of Bokhi on KYDS.

Materials and Methods

Preparation of Bokhi. At the peak of the male camel rutting season, 60 Bokhi samples (each sample containing approximately 100 camel hairs) were collected from the neck of 60 mature, domesticated, bactrian camels from the western region of Inner Mongolia; 30 samples came from camels in Alxa League and 30 samples came from camels in Bayan Nur. From each sample 20 camel hairs were selected and placed in 200 mL of distilled water and soaked for 24 h. The resulting 'Bokhi solution' was filtered through filter paper and the filtrate freeze-dried to produce a black solid powder.

Chemicals. Hydrocortisone injections were purchased from Zhengzhou Lingrui Pharmaceutical Company (Henan, China). Sodium chloride (control) injections (0.9 %) were purchased from Jilin Kelun Connell Pharmaceutical Company (Jilin, China). Sildenafil citrate was purchased from Pfizer Pharmaceuticals Limited (Liaoning, China). Serum creatinine (SCR), blood urea nitrogen (BUN), testosterone (T), thyroid stimulating hormone (TSH), superoxide dismutase (SOD) and nitric oxide (NO) reagent kits were purchased from Nanjing Institute of Biological Engineering (Jiangsu, China).

Experimental animals and groups. All protocols were approved by Animal Care and Use Committee at Inner Mongolia Agricultural University. Seventy healthy adult male Spague Dawley (SD) rats (weighing 200-240 g, Specific Pathogen Free, animal licence No. SCXK (Jing) 2006-0009) were supplied by Vital River Laboratory Animal Technology Company Limited (Beijing, China). The rats were maintained under standard laboratory conditions (temperature of 21-23°C, relative humidity of 45-55 % and 12 h/12 h light/dark cycle) with food and water freely available. After one-week of acclimation to standard laboratory conditions, cardiac blood samples were taken from all animals. The blood was centrifuged (3,000 g centrifugation for 15 min) and the serum isolated. The levels of SCR, BUN, T, TSH, SOD and NO in the serum from each rat was determined using the ELISA kits; there was no significant difference ($P > 0.05$) in the levels of these compounds amongst the rats. The 70 rats were randomly divided into seven groups as follows: Control, Model, AHDB, BHDB, ALDB, BLDB and SC (Sildenafil citrate), ten rats per group.

Treatment administration. The experimental design followed the recommended methods from published work (Shen *et al,* 2007; Zhou *et al,* 2007) and is fully described in Fig 1. Rats in the Control group received multipoint subcutaneous injections of 25 mg/kg body weight of medical physiological saline daily for 14 consecutive days and the remaining rats received multipoint subcutaneous injections of 25 mg/kg body weight of hydrocortisone daily for 14 consecutive days. The rats were weighed every other day and, at the same time, their activity, hair gloss and shedding were observed and recorded. After the 14 days, the rats in the KYDS Model groups were shedding hair and appeared thin, dispirited, less dynamic and cold, which are clear symptoms of KYDS and demonstrated that the KYDS model had been established. On the 15th day the Control and Model groups were each given 2 mL sterilised tap water. In contrast the AHDB and BHDB groups were given Bokhi 50 mg/kg body weight, the ALDB and BLDB groups were given Bokhi 10 mg/kg body weight and the SC group was given SC 10 mg/kg body weight. The treatments were administered daily (9:00-11:00 a.m.) for 14 consecutive days. Every other day, at a fixed time, each rat was weighed and the figure used to adjust the dosage of the drugs. The food intake of each group rats was weighed at a fixed time every day. After the final treatment, food was withheld and all rats were immediately kept in individual metabolic cages to collect urine for 24 h. Then they were euthanised by bleeding from the femoral artery under anaesthesia. The serum was extracted from the femoral blood as described previously. The serum was used to quantify SCR, BUN, T, TSH, SOD, NO using the ELISA kits. The vesicula seminalis, spleen, kidney and testes were dissected from each rat and weighed.

Statistical Analysis. Data was expressed as mean ± standard deviation (SD). SPSS 17.0 software was used for all statistical analysis. Differences between mean values of normally distributed data

were assessed by one-way analysis of variance (ANOVA) followed by post hoc least significant difference (LSD) comparison tests. For comparisons between two groups, t-tests were used. Statistical differences were considered significant at $P < 0.05$. GraphPad Prism 5 software was used for all figures analyses. R language software was used for principal components analysis using the ggplot2 package for data visualisation.

Results

Changes in Growth Rate of rats

Before the experiment began the rats were randomly divided into groups and there was no significant difference in the growth rate of rats amongst the groups ($P > 0.05$). After the 14 days during which the KYDS model had been established, growth rate of the Model groups were significantly slower than the Control group ($P < 0.01$, Fig 2 A). There was no significant difference ($P > 0.05$) in growth rate amongst rats in each of the model groups. These differences in growth rate before and after establishing the model demonstrated that the KYDS model had established successfully.

After the 14 days during which the KYDS model groups had received treatment (Fig 2 B), growth rate in the treatment groups (AHDB, BHDB, ALDB, BLDB and SC) was significantly faster than the Control group ($P < 0.01$). The growth rates of AHDB and BHDB groups were the fastest.

Changes in food intake of rats

During the KYDS model establishment period rats in the Control group consumed significantly more food (and achieved a steady growth rate) than the other six groups ($P > 0.05$, Fig 3). This demonstrated that the rats had been stimulated by the drugs, resulting a significantly reduced food intake.

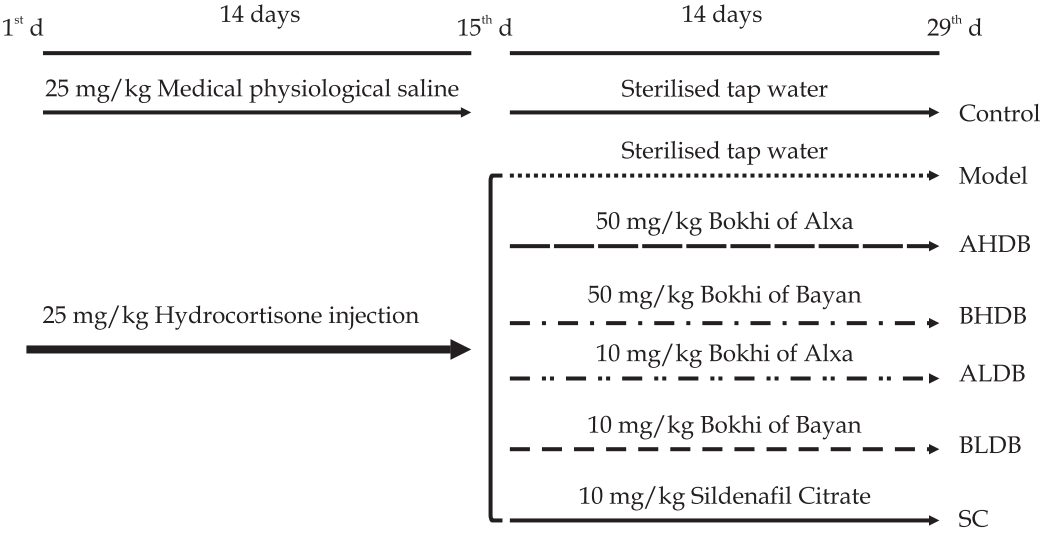

Fig 1. Experimental design.

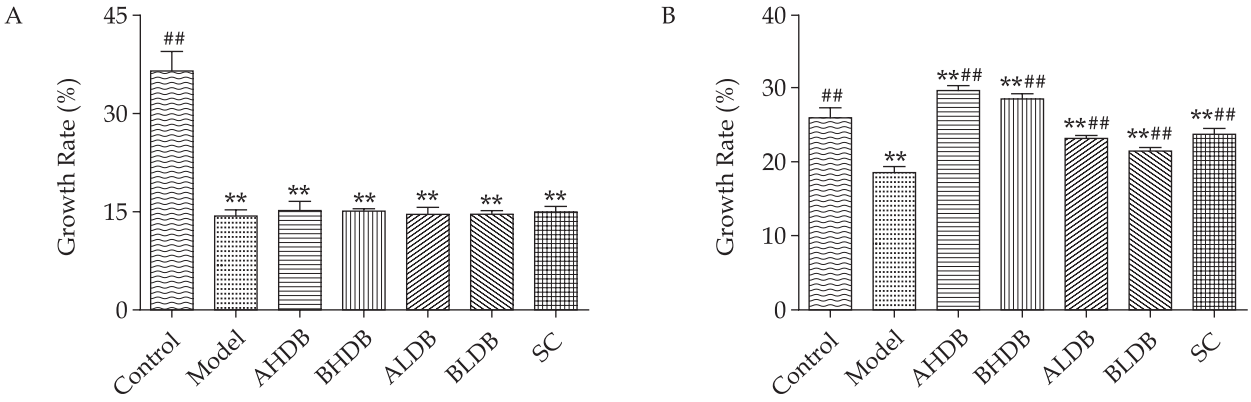

Fig 2. Changes in growth rate of rats: A. Growth rate of rats during the 14 days of KYDS model establishment. B. Growth rate of rats during the 14 days of treatment application. **P < 0.01 and *P < 0.05 compared with the Control group; ##P < 0.01 and #P < 0.05 compared with the Model group.

During the treatment period (days 15-28), rats in the Control group had the highest daily food intake and steady growth ($P < 0.05$, Fig 4 A). After hydrocortisone injections were stopped normal metabolic regulation returned to the remaining six groups of rats and there was a gradual recovery of physical condition and increased food intake (Fig 4 B-D). By the end of the experiment, the daily intake of food was the same as in the Control group of rats ($P > 0.05$). This showed that curative drug treatment resulted in no differences in food intake between the KYDS rats and the Control rats.

Effects of Bokhi on urine volume of KYDS model rats

After 14 successive days of Bokhi administration the Model group had obvious urinary dysfunction producing more urine than the other groups ($P < 0.01$, Fig 5). The urine volume level of AHDB group was close to that of the Control group ($P > 0.05$) and they regulated the urine volume better than the other four treatment groups (Fig 5).

Observations on General State and Condition of rats

Before the experiment began, the rats showed normal activity, shiny hair and there were no obvious differences between the groups. During model establishment (days 1-14), the model group rats: ate

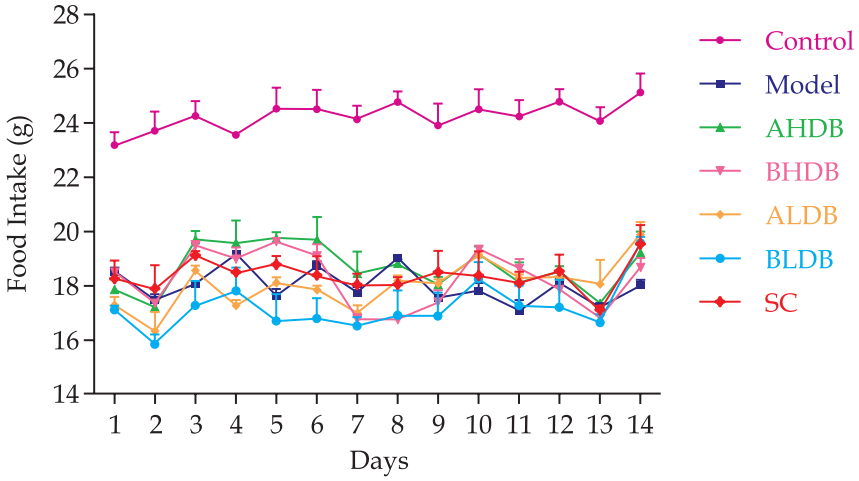

Fig 3. Changes in daily food intake during KYDS model establishment in rats.

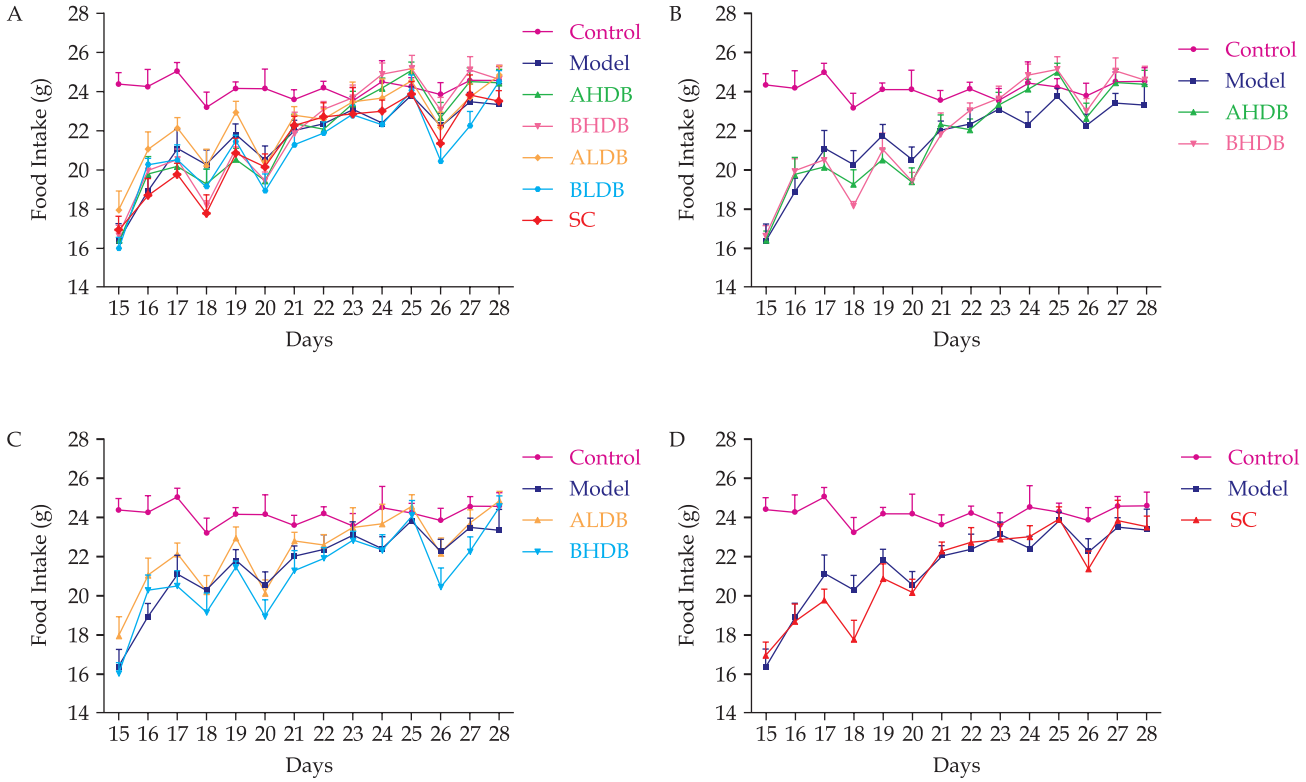

Fig 4. A. Changes in daily food intake during treatment of KYDS rats with Bokhi. B. Changes in daily food intake during treatment of KYDS rats with high doses of Bokhi (AHDB and BHDB). C. Changes in daily food intake during treatment of KYDS rats with low doses of Bokhi (ALDB and BLDB). D. Changes in daily food intake during treatment of KYDS rats with SC.

less; became unresponsive inactive and dispirited; their eyes became glazed; they appeared chilled; suffered hair loss, weight loss and anal pollution; engaged in arching their backs and twining. These symptoms caused by using large doses of hormones to induce KYDS were consistent with the research as discussed by Gou *et al* (2009) and matched the symptom diagnosis standard for an animal model of KYDS from the 'Reference standard for syndrome differentiation of traditional Chinese Medicine' (Shen and Wang, 1986).

Following the establishment of the model (days 15-28), the weight of the Model group rats did increase, but there was no improvement in the other symptoms and indeed in some cases they were even worse. Symptoms in the drug therapy groups receiving Bokhi or SC improved: urine volume normalised, their hair gradually regained luster, depilation reduced, their spirits improved and their activity increased. From the changes in appearance of the drug therapy groups it could be seen that, while the KYDS model had established successfully, that Bokhi had a positive effect on alleviating the symptoms of KYDS.

Effects of Drug therapy on SCR and BUN in KYDS model rats

After 14 successive days of administration of drug therapy, SCR levels in the AHDB, BHDB and ALDB groups were not significantly different to the Control group (P > 0.05, Table 1). However, levels of SCR in the AHDB, BHDB and ALDB groups were all significantly different to levels in the Model group (P < 0.01, Table 1). This showed that in the AHDB, BHDB and ALDB groups that Bokhi had a good effect on SCR regulation compared with KYDS rats.

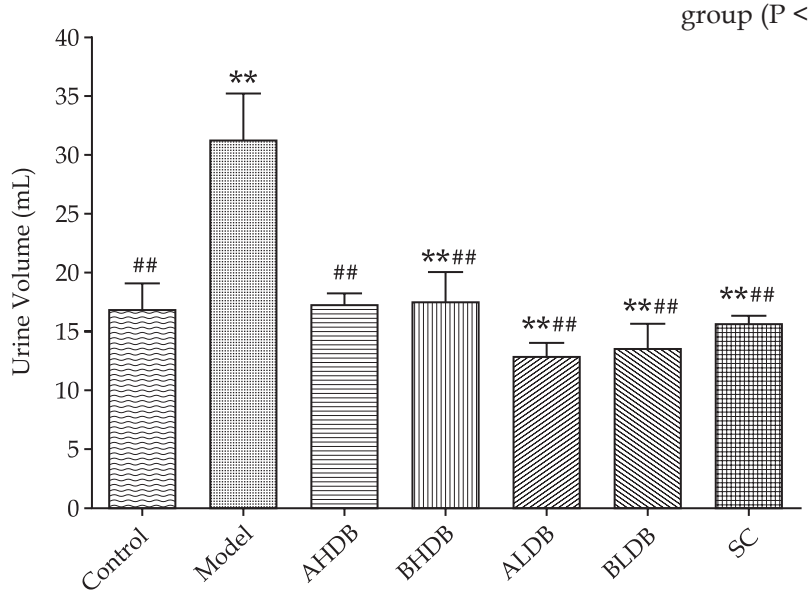

Fig 5. Effects of 14 successive days of Bokhi administration on urine volume (24 h) of KYDS model rats. $^{**}P < 0.01$ and $^{*}P < 0.05$ compared with the Control group; $^{\#\#}P < 0.01$ and $^{\#}P < 0.05$ compared with the Model group.

Table 1. Effects of Bokhi on SCR and BUN in KYDS model rats compared with control rats and rats receiving drug therapy.

Group	SCR (umol/L)	BUN (mmol/L)
Control	68.174±0.736$^{\#\#}$	4.592±0.158$^{\#\#}$
Moldel	76.916±0.827**	6.699±0.279**
AHDB	67.612±0.835$^{\#\#}$	4.861±0.238$^{\#\#}$
BHDB	68.324±0.921$^{\#\#}$	4.856±0.222$^{\#\#}$
ALDB	67.821±0.647$^{\#\#}$	5.256±0.079$^{*\#\#}$
BLDB	70.248±0.931$^{*\#\#}$	5.130±0.585$^{\#\#}$
SC	76.810±0.000**	5.370±0.066$^{*\#\#}$

The data shown are mean scores ± SD, n = 10. $^{**}P < 0.01$ and $^{*}P < 0.05$ compared with the Control group; $^{\#\#}P < 0.01$ and $^{\#}P < 0.05$ compared with the Model group.

Levels of BUN were not significantly different in the AHDB, BHDB and BLDB groups compared with the Control group (P > 0.05, Table 1). However, levels of BUN in the AHDB, BHDB and BLDB groups were all significantly different to levels in the Model group (P < 0.01, Table 1). This showed that in the AHDB, BHDB and BLDB groups that Bokhi had a good effect on BUN compared with KYDS rats.

Effects of drug therapy on hormones in KYDS model rats

Levels of the hormone, T, in the AHDB and BHDB were significantly different to the Control group (P < 0.01, Fig 6). Levels of T were significantly different in the AHDB and BHDB (P < 0.01, Fig 6) compared with the Model group. Provision of 14 successive days of drug therapy to rats after the establishment of KYDS did not restore T levels to that of the Control group. However, the greatest improvements were found in the high dose Bokhi groups (i.e. AHDB and BHDB, Fig 6).

Levels of TSH in the AHDB and BHDB groups were not significantly different to levels in the Control group (P > 0.05, Fig 7). Levels of TSH in the AHDB and BHDB groups were significantly different to the Model group (P < 0.01, Fig 7). Levels of TSH in the Model group were seriously unbalanced, while in the drug therapy groups there was a regulatory effect on

TSH. The effects of AHDB and BHDB were the best as TSH levels in KYDS rats returned to normal levels.

Effects of Bokhi on SOD and NO in KYDS model rats

Levels of SOD in the AHDB, BHDB and SC groups were not significantly different to the Control group ($P > 0.05$, Fig 8). Levels of SOD were significantly different in the AHDB, BHDB and SC groups ($P < 0.01$) compared with the Model group. The effects of the high dose Bokhi were similar to the effect of SC. Both the high dose Bokhi and SC returned the level of serum SOD in rats with KYDS to near normal values.

Levels of NO in all six groups were significantly different to the levels in the Control group ($P < 0.01$, Fig 9). Levels of NO were significantly different in the Control, AHDB, BHDB and ALDB groups ($P < 0.01$) and the BLDB and SC groups ($P < 0.05$) compared with the Model group (Fig 9). Provision of 14 successive days of drug therapy to rats after the establishment of KYDS, did not restore NO levels to that of the Control group. But the best effects were found in the AHDB, BHDB and ALDB groups.

Effects of Bokhi on the Organ Indices of KYDS model rats

The vesicula seminalis index was not significantly different in the AHDB and BHDB groups ($P > 0.05$, Table 2) compared with the Control group. The vesicula seminalis index was significantly different in the AHDB and BHDB groups ($P < 0.01$) compared with the Model group (Table 2).

All treatment groups ($P < 0.01$, Table 2) were a significant difference in the spleen index compared with the Control group and the Model group. Drug therapy to rats after the establishment of KYDS did not restore the Spleen index to the levels of the Control group.

There was no significant difference in the kidney index in the AHDB group compared with the Control group ($P > 0.05$, Table 2). There was a significant difference in the kidney index of the AHDB group ($P < 0.01$) compared with the Model group (Table 2).

All treatment groups ($P < 0.01$, Table 2) were significantly different in the testes index compared with the Control group and the Model group. Drug therapy to rats after the establishment of KYDS did not restore the testes index levels to that of the Control group.

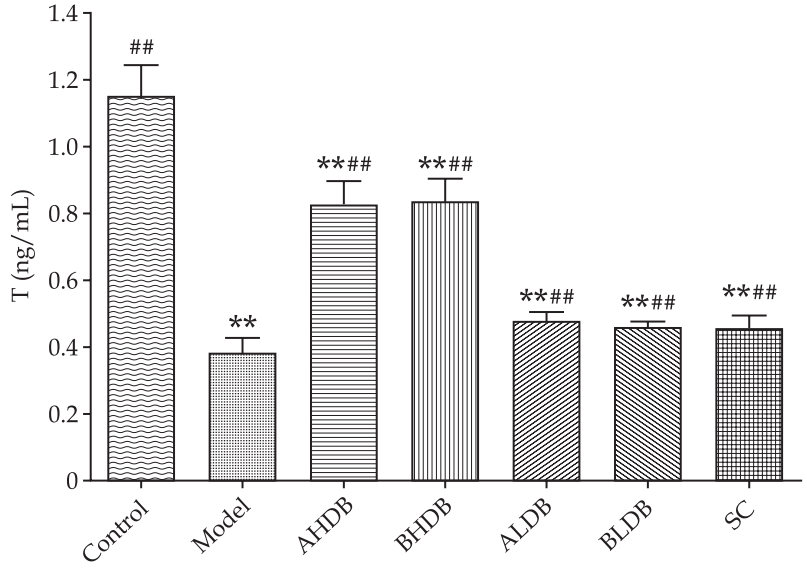

Fig 6. Effects of Bokhi on the hormone, T, in KYDS model rats compared with control rats and rats receiving drug therapy. $^{**}P < 0.01$ and $^{*}P < 0.05$ compared with the Control group; $^{\#\#}P < 0.01$ and $^{\#}P < 0.05$ compared with the Model group.

Table 2. Effects of Bokhi on the vesicula seminalis, spleen, kidney and testes indices of KYDS model rats compared with control rats and rats receiving drug therapy.

Group	Vesicula Seminalis (g/100g)	Spleen (g/100g)	Kidney (g/100g)	Testes (g/100g)
Control	$0.382\pm0.038^{\#\#}$	$0.191\pm0.012^{\#\#}$	$0.729\pm0.010^{\#}$	$1.010\pm0.055^{\#\#}$
Moldel	$0.333\pm0.038^{**}$	$0.287\pm0.022^{**}$	$0.707\pm0.011^{*}$	$1.239\pm0.095^{**}$
AHDB	$0.390\pm0.013^{\#\#}$	$0.223\pm0.003^{**\#\#}$	$0.745\pm0.015^{\#\#}$	$1.125\pm0.070^{**\#\#}$
BHDB	$0.374\pm0.012^{\#\#}$	$0.215\pm0.005^{**\#\#}$	$0.750\pm0.026^{*\#\#}$	$1.106\pm0.079^{**\#\#}$
ALDB	$0.342\pm0.050^{**}$	$0.251\pm0.004^{**\#\#}$	$0.778\pm0.020^{**\#\#}$	$1.151\pm0.051^{**\#\#}$
BLDB	$0.351\pm0.011^{**\#\#}$	$0.246\pm0.001^{**\#\#}$	$0.767\pm0.020^{**\#\#}$	$1.177\pm0.012^{**\#\#}$
SC	$0.409\pm0.017^{**\#\#}$	$0.389\pm0.008^{**\#\#}$	$0.816\pm0.012^{**\#\#}$	$1.151\pm0.070^{**\#\#}$

The data shown are mean scores ± SD, n = 10. $^{**}P < 0.01$ and $^{*}P < 0.05$ compared with the Control group; $^{\#\#}P < 0.01$ and $^{\#}P < 0.05$ compared with the Model group.

Principal Component Analysis (PCA)

The data (growth rate, urine volume, SCR, BUN, T, TSH, SOD, NO, vesicula seminalis index, spleen index, kidney index and testes index) was used in PCA (Fig 10). The Model group and the Control group was very distant from each other showing that the biochemical function of the Model group had been pathological changed. It also indicated that the establishment of the KYDS model rats had been successful. The Bokhi high dose groups (AHDB and BHDB) were the closest to the Control group and also distant from the Model group. The distance between the Bokhi low dose groups (ALDB and BLDB) and the Control group was slightly closer than the distance between Bokhi low dose groups (ALDB and BLDB) and the Model group. The SC group was distant from both the Control group and the Model group. SC had a therapeutic effect on erectile dysfunction caused by KYDS (Xu *et al,* 2010) but had little effect on the other symptoms of KYDS. Therefore, the treatment effect of high doses of Bokhi on KYDS rats was the most beneficial.

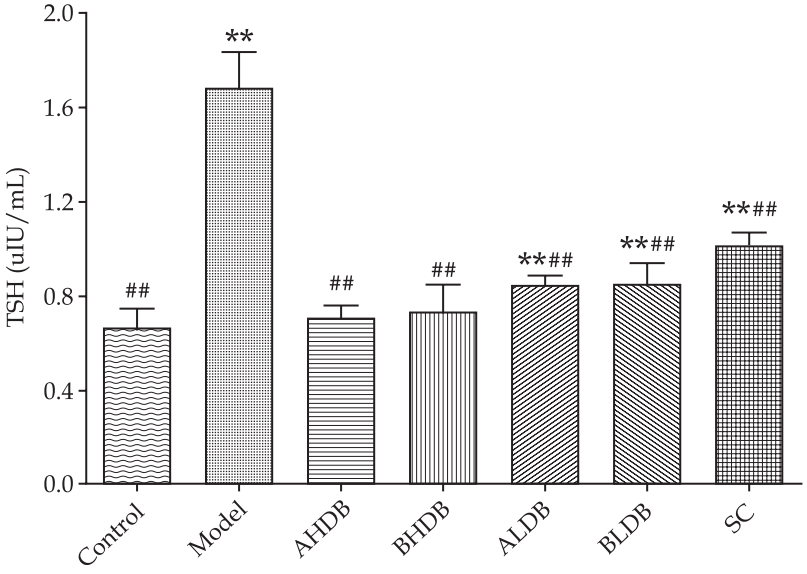

Fig 7. Effects of Bokhi on levels of the hormone, TSH, in KYDS model rats compared with control rats and rats receiving drug therapy. $^{**}P < 0.01$ and $^{*}P < 0.05$ compared with the Control group; $^{\#\#}P < 0.01$ and $^{\#}P < 0.05$ compared with the Model group.

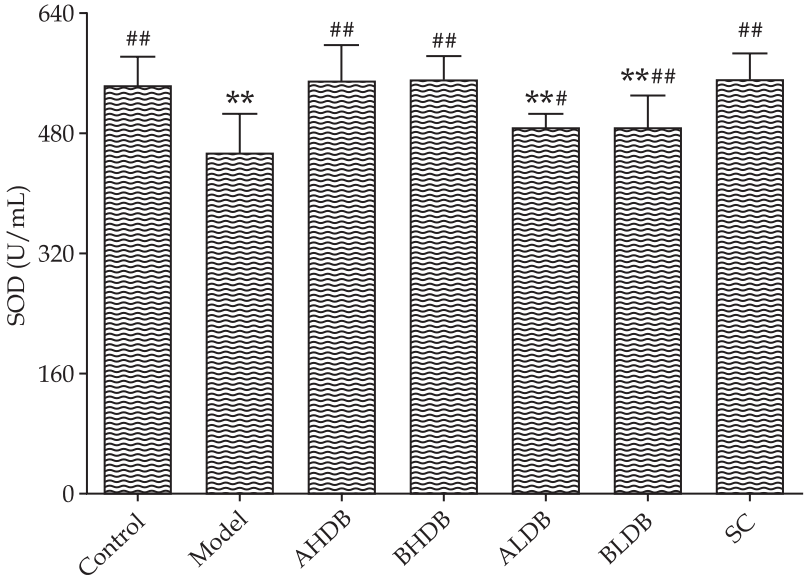

Fig 8. Effects of Bokhi on SOD in KYDS model rats compared with control rats and rats receiving drug therapy. $^{**}P < 0.01$ and $^{*}P < 0.05$ compared with the Control group; $^{\#\#}P < 0.01$ and $^{\#}P < 0.05$ compared with the Model group.

Discussion

Growth rate of model rats following 14 successive days of subcutaneous injection with hydrocortisone grew similarly and significantly more slowly than rats in the Control group. This phenomenon was consistent with the report as discussed by Xiao *et al* (2008) who used hydrocortisone injection over a short-term (7 days) to induce Kidney-Yin Deficiency and, over a longer period (10 days) to induce Kidney-Yang-Deficiency when weight increased slowly. The general condition of rats during the period of model establishment was the same as the condition observed as discussed by Liang *et al* (1999) who observed that long-term high doses of hormones resulted in slow increases in body weight, lethargy, dull hair, crowding together, polyuria and oliguria.

SCR and BUN, respectively, are the end products of the metabolism of nitrogen-containing organic compounds and proteins and, to a certain extent, reflect renal function. Treatment with Bokhi significantly improved the permeability of the glomerular filtration membrane. The excretion of SCR and BUN in metabolic products improved renal function and reduced the symptoms of KYDS. The high dose of Bokhi regulated SCR and BUN most effectively as they returned to close to the normal value.

KYDS does not only cause a functional disorder of the hypothalamus-pituitary-adrenal-cortex axis, but

also dysfunction to varying degrees in different target gland axes (such as the thyroid axis and the gonadal axis) and the neuroendocrine system. These can cause declines in reproductive function in patients with KYDS due to decreases in plasma androgen in males and plasma oestrogen levels in females (Si, 1994). The experimental results showed that following establishment of KYDS in rats, subsequent drug therapy was unable to return T levels to the level of the Control group. Serum T content decreased, which showed that the changes in sex hormones resulted in different levels of gonadal axis dysfunction (Qiu *et al,* 1999; Hu *et al,* 2014). The level of TSH in rats with KYDS was higher than that in control rats, which showed that there was a certain degree of functional disorder in the thyroid axis (Wang *et al,* 2015). High doses of Bokhi was the best treatment to improve levels of T in KYDS rats and reduce the levels of TSH, thereby indirectly regulating KYDS caused by rat gonad axis and thyroid axis disorders.

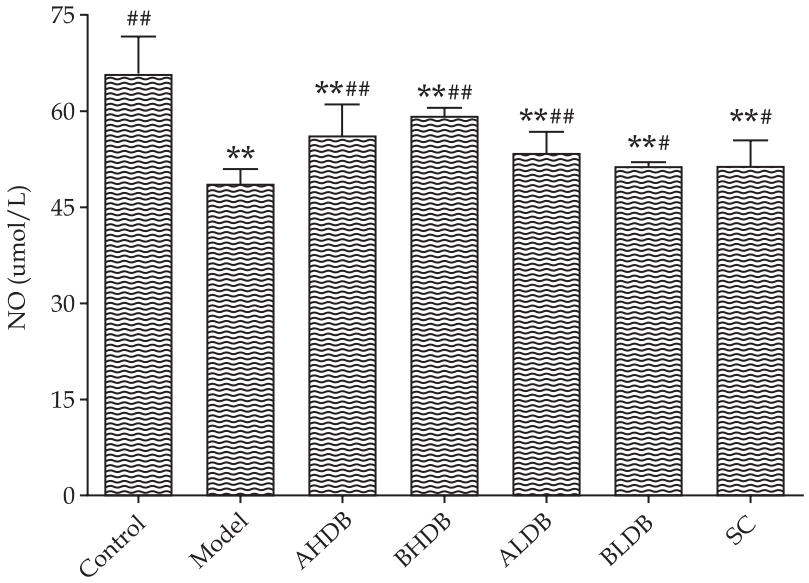

Fig 9. Effects of Bokhi on NO in KYDS model rats compared with control rats and rats receiving drug therapy. $^{**}P < 0.01$ and $^{*}P < 0.05$ compared with the Control group; $^{\#\#}P < 0.01$ and $^{\#}P < 0.05$ compared with the Model group.

KYDS can lead to decreased SOD activity, i.e. the body's ability to eliminate free radicals is weakened (Rong *et al,* 2016). High doses of Bokhi were most effective at increasing levels of SOD, improving scavenging of free radicals and reducing symptoms of premature senility. In KYDS rats the serum NO content decreased significantly. This reduced secretion was probably caused by insufficient cell damage, leading to cell proliferation, which causes glomerular sclerosis and may be important for the symptoms of KYDS caused by hydrocortisone. High doses of Bokhi increased the content of NO. The mechanism for this may be due to increased activity of NOS, which can directly relax blood vessels, increase renal blood flow and glomerular filtration rate. This would reduce kidney damage, causing smooth muscle relaxation, increased intracavernosal blood flow and increased erectile potential (Chancellor *et al,* 2003).

Injection with high doses of an exogenous glucocorticoid, such as hydrocortisone, is a classical method of establishing KYDS in a model animal and induces adrenocortical insufficiency after abrupt withdrawal of the glucorticoid (Tan *et al,* 2014). To a certain extent this animal model mimicks the pathological state of suppression of the hypothalamic–pituitary-adrenal (HPA) in humans with KYDS and can contribute greatly to important advances in the current understanding of the underlying mechanisms of KYDS as well as treatments (Yang *et al,* 2008; Wang *et al,* 2012). In this study, Bokhi collected from different regions had similar effects as a treatment for KYDS; the only difference occurred between the high and low doses. High doses of Bokhi significantly improved symptoms of KYDS in rats and the treatment effect was better than treatment with SC, a drug commonly used for treatment of KYDS. The research as discussed by Guo *et al* (2013) has reported that acute toxicity of camel Bokhi and showed that it produced no toxic effects within 24 h in mice at a dose of 5,000 mg/(kg bw). We therefore propose that it is safe to use Bokhi in the short term without obvious side effects.

Bokhi, which has a dark brown colour, watery consistency and heavy, somewhat sweet aroma saturates the long nape hair of sexually mature animals and is used to scent mark the hump and objects in the environment. The research as discussed by Ayorinde *et al* (1982) has reported that the male Bactrian camel's occipital scent gland produces a series of steroids including 5a-androst-16-en-3-one in addition to a series of fatty acids and γ-dodecalactone. 3-Methylbutanoic acid is the most volatile of the acids which include hexanoic, a decenoic and the saturated acids from C15 to C25 with the exception of C24. Mass spectrometry results showed that their molecular ions of these compounds have high indices of hydrogen deficiency. The constitution of this secretion changes appreciably with the season.

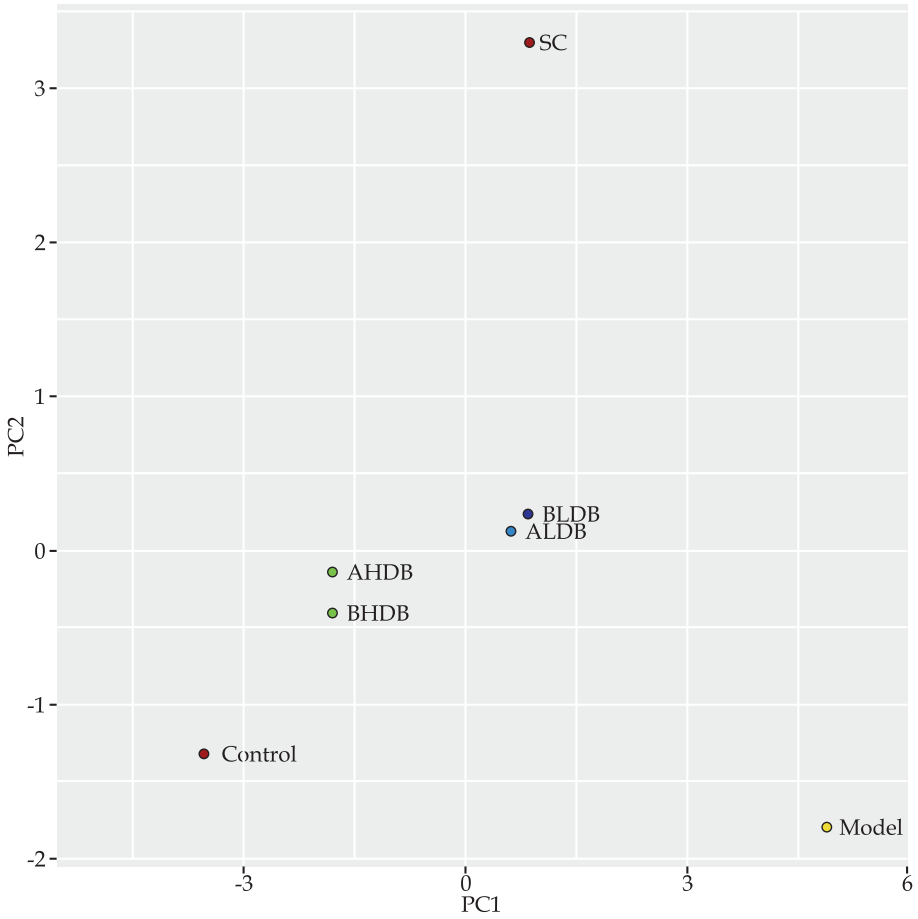

Fig 10. Effects of Bokhi on PCA in KYDS model rats compared with Control rats and rats receiving drug therapy.

To date, the underlying mechanisms of KYDS have been unclear, especially as there are no effective drug treatments for KYDS. What the components of Bokhi are that improve the symptoms of KYDS also needs further research. Bokhi has naturally high levels of medicinal ingredients and would have considerable potential as a new drug.

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Ethical Approval

All animal procedures were approved by Animal Care and Use Committee at Inner Mongolia Agricultural University.

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