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Effect of Konjac Glucomannan on Hyperuricemic Rats

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Research Article

ABSTRACT

Objective: The effect of konjac glucomannan (KGM) on hyperuricemic rats was studied.

Method: To establish a hyperuricemic model, rats in experiment groups were orally administrated with adenine and potassium oxonate every morning and continuously fed diets containing 20% yeast. Rats in positive drug group and KGM groups were orally administrated with benzbromarone and KGM respectively every afternoon. Blood was collected weekly and rats were sacrificed at 4th week, the biochemical indexes and histological analysis were measured.

Results: During the experiment, the level of uric acid (UA), Creatinine (Cr) and blood urea nitrogen (BUN)in model group increased significantly, indicating a hyperuricemic model was established successfully. But the level of the three indexes in positive drug group and KGM groups were lower to different degrees. UA in middle group exhibited the lowest level and had no significant with that of control group in the end. Besides, the activities of serum xanthine oxidase (XOD) and serum adenosine deaminase (ADA) in model group gradually increased and had significant difference with that of control group. KGM could significantly decrease the activities of XOD and ADA in serum and in liver, especially at middle dose. However, the activity of guanine deaminase (GD) did not have a significant fluctuation either in serum or liver during the experiment. Pathological analysis of kidneys showed KGM caused a strong reduction in the number of urate crystal, rats in high dose group had the lightest renal histological lesion.

INTRODUCTION

Uric acid (UA) is the poorly soluble circulating end product of purine nucleotide metabolism in human beings [1]. Hyperuricemia, caused by purine metabolism disorder or reduction in uric acid excretion is the most important biochemical basis of gout [2]. It is generally accepted that hyperuricemia has also been shown to correlate with the cluster of metabolic disorders [3], closely associate with the features of metabolic syndrome [4] and chronic renal lesion [5]. Besides, hyperuricemic patients are also at greater risk for evolving into many conditions such as metabolic [4,6,7], cardiovascular [8-13], renal [4,5] and many other comorbidities [14-16], which collectively result in an increased mortality [17,18]. Drugs such as benzbromarone, probenecid, and allopurinol are usually used in the treatment of hyperuricemia, However, these drugs can only be used to relieve the illness, long-term use of the drugs would lead to side effects on liver and kidney [19,20]. Epidemiological evidence has indicated that diet may reduce the risk of chronic disease [21-24]. Therefore, it is an important aspect and an inevitable trend to make use of bioactive food components to mitigate hyperuricemia.

Konjac glucomannan (KGM), a kind of excellent dietary fiber polysaccharide, has been broadly used as food, food additive and traditional Chinese medicine for a long history [25, 26]. It has been reported that KGM has several valuable functions of healthcare

and pharmacology, such as obesity-suppression, tumor-suppression, as well as the treatment of cough, hernia, and skin disorders [27]. However, what worth mentioning is that no one studied the effect of KGM on hyperuricemia before. In the preset study, rats were used as model animals to investigate the effect and potential mechanisms of KGM on hyperuricemia, and the effect of KGM on renal histological lesion was also analyzed.

MATERIALS AND METHODS

Reagents

Konjac glucomannan offered by Chongqing Konjac Co., Ltd (China) was used in this study. Benzbromarone and yeast were purchased from HEUMANN PHARMA (German) and Jiangshan Biological Co., Ltd (China) respectively. Adenine and potassium oxonate was offered by Hefei Bomei Biotechnology Co., Ltd (China).

Animals

SD male Rats (SK Yu 2012-0005) were purchased from Chongqing Tengxin Biotechnology Co. (China). After a week of acclimation, animals were randomly distributed into control and experiment groups. All animals were housed in a controlled atmosphere (25 ± 1°C at 50% relative humanity) and with a 12-h light/12-h dark cycle. Animals had free access to food and water.

Experimental procedure

48 SD male rats with body weight around 200 ± 10 g were divided into 6 groups of 8 each. Except control group, all rats in experiment groups were orally administrated with adenine and potassium oxonate every morning at the dose of 250 mg/kg and 50 mg/kg respectively and continuously fed diets containing 20% yeast [28]. Rats in positive drug group and KGM groups were orally administrated with benzbromarone at 60 mg/kg and KGM at 210 mg/kg, 168 mg/kg and 126 mg/kg respectively every afternoon. Blood was collected weekly. Rats were then sacrificed at 4th week, liver and kidneys were collected. The entire left kidneys were fixed with 10% neutral formalin solution for histopathology study.

Biochemical indexes measurement

Serum levels of UA, Cr, BUN, XOD, and ADA were measured using commercial kits (Nanjing Jiancheng Biotechnology Institute, China) according to the manufacturer-provided standards and protocols. Serum GD levels were determined using commercially available ELISA kits (Shanghai Shifeng Biological Technology Co., Ltd., China). The liver tissue homogenate in appropriate dilutions was used to determine levels of liver XOD, ADA and GD.

Histological analysis of kidney [29]

Left kidneys were fixed in 10% neutral formalin solution overnight and were cut into small cubes, then embedded in paraffin. Tissues were cut into slices of 3-6 μm thickness and stained with hematoxylin-eosin (H&E). The renal pathologic changes were observed under the microscope.

Statistical analysis

All data were presented as mean ± SD. Analysis of variance (ANOVA) model, performed with SPSS software, was used for the comparison of differences among groups. $P < 0.05$ was considered to be statistically significant. Microsoft Excel was used for figures.

RESULTS

Effect of KGM on serum UA level

There was no significant difference among groups at first, whereas the UA level in model group increased continuously then, and was significantly higher than control group, which indicating a successful hyperuricemia model was established. UA values of the rest experiment groups rise at first, and then declined, and were lower than that of model group, this result showed that benzbromarone and KGM could reduce serum UA level. The UA values of KGM groups were significantly smaller than model group and positive drug group from the third week. Rats treated with KGM middle dose had the lowest level of UA which was not significantly different with control group at the end of the experiment **(Figure 1).**

Effect of KGM on serum Cr level

Serum Cr is usually used to estimate renal function [30]. As was shown in **Figure 2**, no significant differences among Cr values of six groups at first, then the Cr levels in experiment groups increased continuously and were significantly higher than that of control group. But Cr values in KGM groups were smaller than model group during the whole experiment, and were also smaller than positive group in the end. This result suggested that KGM could reduce Cr values and mitigate abnormal renal function of hyperuricemic rats effectively especially at high dose.
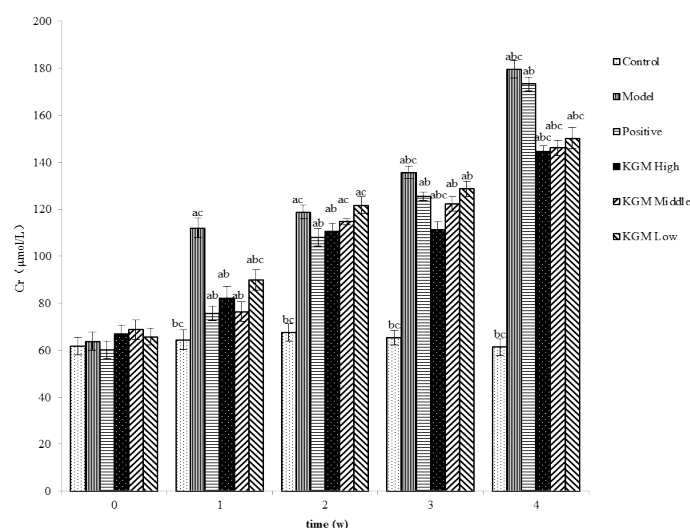
Effect of KGM on serum BUN level

BUN is the indicator commonly used to reflect renal function. An increment in BUN means kidney dysfunction and a decreased

metabolic ability of some harmful toxins in the body [31]. As was shown in **Figure 3**, no significant differences can be found among the initial BUN values. With the development of hyperuricemic model, BUN in experiment groups kept rising, but BUN levels in positive group and KGM groups were lower than that of model group. Rats in KGM middle dose group owned the smallest BUN values among the experiment groups at each time which implies that KGM at this dose played a better role in reducing BUN values.
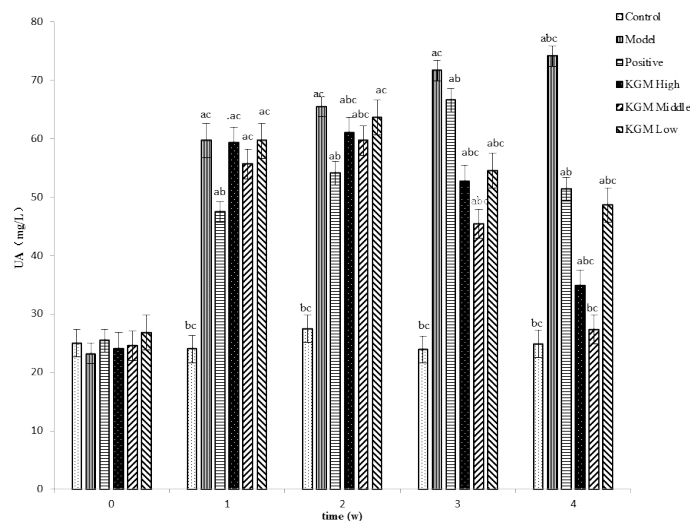
Figure 1. Serum UA level of rats. Values were expressed as mean±SD. a=p<0.05 vs. control; b=p<0.05 vs. model; c=p<0.05 vs. positive.

Figure 2. Serum Cr level of rats. Values were expressed as mean±SD. a=p<0.05 vs. control; b=p<0.05 vs. model; c=p<0.05 vs. positive.
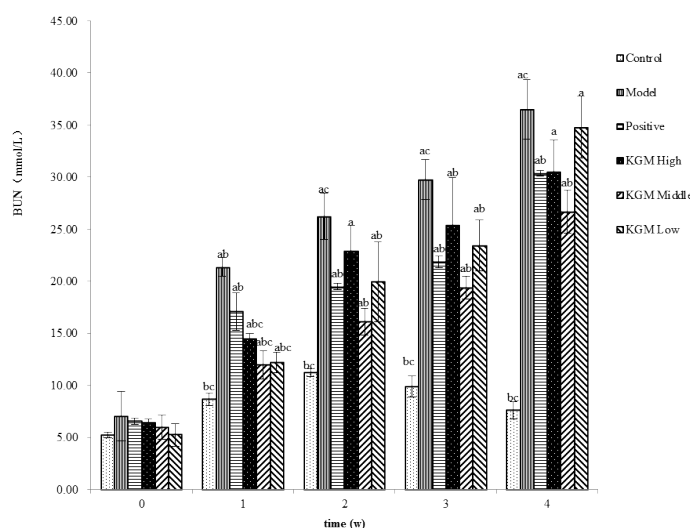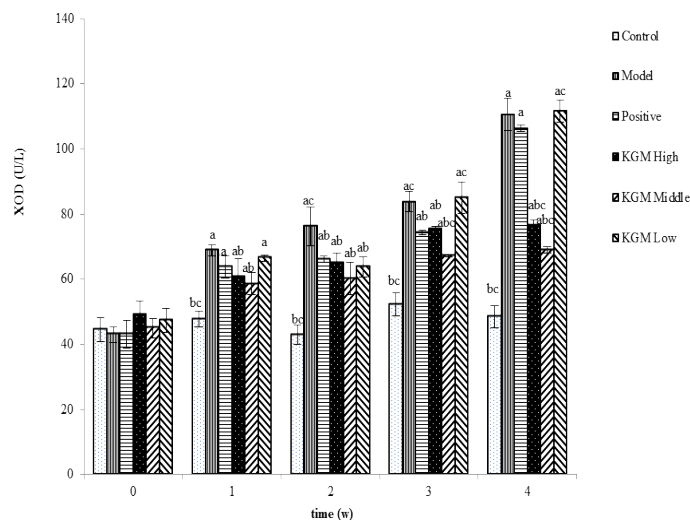

Figure 3. Serum BUN level of rats. Values were expressed as mean±SD. a=p<0.05 vs. control; b=p<0.05 vs. model; c=p<0.05 vs. positive.

Effect of KGM on serum XOD activity

XOD is the key enzyme to catalyze uric acid production [32]. As was shown in **Figure 4**, no significant differences among XOD

activity in six groups can be found at the beginning of experiment. With the development of hyperuricemic model, XOD activities of rats in experiment groups kept rising and were significantly higher than that of control group at each time. However, compared with model group, KGM at high and middle dose exhibited significant lower XOD activities during the experiment and the XOD activity in KGM middle group was closest to that of control group at the end of experiment. These results suggested that KGM, especially KGM at middle dose, played critical role in serum XOD activity reduction.

Figure 4. Serum XOD activity of rats. Values were expressed as mean±SD. a=p<0.05 vs. control; b=p<0.05 vs. model; c=p<0.05 vs. positive.

Effect of KGM on serum ADA activity

Adenosine deaminase (ADA) is one of the important enzymes in the catabolism of adenosine, it is also involved in the decomposition of nucleic acid, purine base and ATP, therefore it plays an important role in uric acid production [33]. As was shown in **Figure 5**, there was no significant difference among the initial ADA activity of rats. ADA activity of model group gradually increased with the development of hyperuricemic model and was significant higher than that of control group. ADA activities of KGM groups were at relatively low levels during the experiment, which indicated that KGM could cause reduction in ADA activity. Moreover, it can be observed that ADA activity in KGM middle dose group was lower than any other experiment group in the end, therefore, KGM at middle dose showed the strongest ability to reduce the activity of ADA.
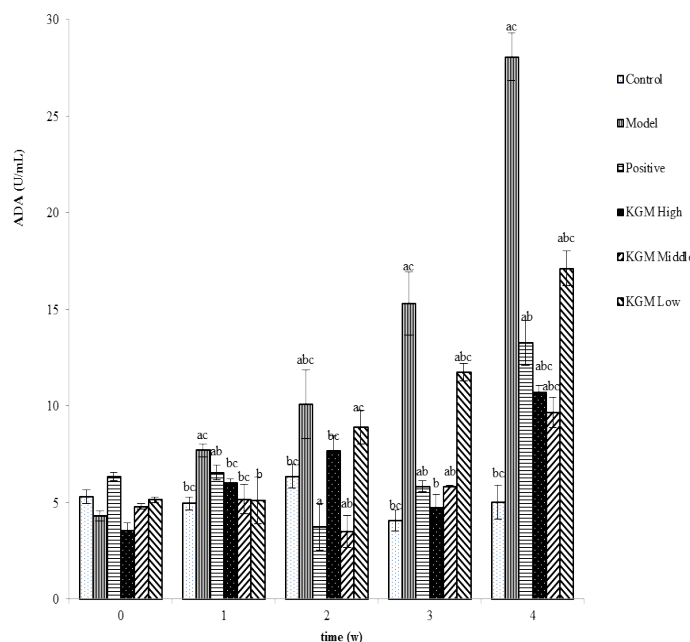
Figure 5. Serum ADA activity of rats. Values were expressed as mean±SD. a=p<0.05 vs. control; b=p<0.05 vs. model; c=p<0.05 vs. positive.

Effect of KGM on serum GD activity

GD activities of six groups fluctuated within the normal range during the experiment. This result **Table 1** suggested that GD did not play an important role like XOD and ADA in uric acid production and the reduction of UA caused by KGM in this hyperuricemic model had no close relationship with GD activities.

Table 1. serum GD activity Values were expressed as mean ± SD. a=p<0.05 vs. control; b=p<0.05 vs. model; c=p<0.05 vs. positive.

	0 week	1 week	2 week	3 week	4 week
Control	11.43 ± 0.50	12.88 ± 0.90	11.69 ± 0.61	10.24 ± 0.30	7.74 ± 1.17
Model	12.40 ± 2.13	11.17 ± .95	11.30 ± 1.82	9.98 ± 1.09	8.14 ± 1.08
Positive	11.82 ± 1.45	12.48 ± 0.89	11.30 ± 0.54	8.53 ± 0.24	7.48 ± 1.81
KGM High	11.69 ± 0.60	10.24 ± 0.85[ac]	10.11 ± 1.34	12.35 ± 2.34[abc]	8.66 ± 1.55
KGM Middle	13.10 ± 0.90	9.98 ± 0.14[ac]	11.29 ± 0.97	8.92 ± 0.30	11.17 ± 0.80[abc]
KGM Low	11.25 ± 0.53	11.69 ± 0.80	10.90 ± 0.36	9.98 ± 0.33	12.61 ± 0.75[abc]

## Effect of KGM on the activity of XOD, ADA and GD in live

After the consecutive treatment for 4 weeks, the activities of XOD and ADA in experiment groups increased significantly, compared with those of control group. It can be also observed in **Table 2** that benzbromarone and KGM could decrease the activity of XOD and ADA to certain degrees. Moreover, in KGM middle dose group, the activity of XOD and ADA which were lower than that of other experiment group, were closest to the levels in control group. These results showed that KGM at middle dose has the strongest inhibitory effect on the activities of XOD and ADA. However, similar to the result of serum GD level, GD activities in liver did not change significantly among the six groups.

Table 2. activity of XOD, ADA and GD in liver Values were expressed as mean ± SD. a=p<0.05 vs. control; b=p<0.05 vs. model; c=p<0.05 vs. positive.

	XOD (gprot/L)	ADA (gprot/L)	GD (U/prot)
Control	129.85 ± 2.30[bc]	49.96 ± 5.58[b]	9.75 ± 2.70
Model	196.78 ± 1.84[a]	117.93 ± 2.81[ac]	10.96 ± 1.58
Positive	183.79 ± 2.57[a]	57.18 ± 3.23[b]	12.61 ± 0.87
KGM High	169.24 ± 1.74[ab]	59.54 ± 1.33[b]	11.39 ± 1.01
KGM Middle	157.31 ± 6.37[abc]	52.45 ± 4.20[b]	11.37 ± 1.91
KGM Low	175.78 ± 1.2[ab]	53.29 ± 1.88[b]	9.88 ± 1.78

Histological analysis of kidney

It has been reported that hyperuricemia is a risk factor for kidney disease [34]. The morphological changes of kidney and the deposition of urate crystal can be observed in **Figure 6**. The results showed that compared with control group, the glomerular atrophy, irregular cell arrangement, and large number of urate crystals appeared in the model group. Compared to model group, it can be seen that there were fewer urate crystals in positive drug group and KGM groups. Besides benzbromarone, KGM at high dose caused an obvious reduction in urate crystals. In addition, the phenomenon of glomerular atrophy and irregular cell arrangement were also appeared in positive drug group, the reason may be the side effect of benzbromarone on kidney. However, these phenomena were improved in KGM groups, particularly in KGM high dose group.

Control (A) Model (B) Positive (C) KGM High (D) KGM Middle (E) KGM Low (F)
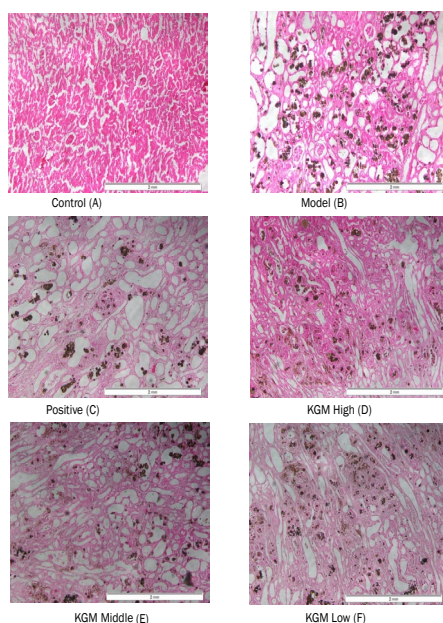
Figure 6. Histological characterization of kidney and urate crystals of rats.

DISCUSSION

Hyperuricemia has been associated with poor health-related outcomes including myocardial infarction (MI), stroke, obesity and diabetes [35, 36]. Previous research also found that asymptomatic hyperuricemia is the first course of gout, about 10% of the

patients with hyperuriceia will naturally evolve into gout, the most common inflammatory arthritis in adult males [37-39]. The overall disease burden of hyperuricemia remains substantial and may be growing [38]. However, although the drugs for hyperuricemia are clearly classified, the side effects are still serious in the process of clinical use [40]. So more effective and safer treatment for hyperuuricemia is really worth studying. KGM has been used as food material for thousand years in East Asia and approved as a generally regarded as safe (GRAS) food additive by the Food and Drug Administration (FDA) in the USA, by Health Canada in Canada and has been approved by the European Union (EU) as food additives. Moreover, evidences of the functional, nutritional and physiological properties of konjac glucomannan have appeared in the literature in recent years [41]. For example, KGM hydrolysates may inhibit certain bacteria (especially *E. coli*), may protect against oxidative stress in the human colon [42], and has the ability to improve insulin sensitivity in subjects with type 2 diabetes [43]. Since KGM has so many benefits to human beings, we therefore try to find out whether it has certain effect on hyperuricemia.

In the present study, we found that KGM, especially KGM at the dose of 168mg/kg, could cause reduction of serum UA, XOD activity and ADA activity in hyperuricemic model induced by adenine, potassium oxonate and yeast. Previous studies suggested XOD and ADA had important effect on the synthesis of uric acid [44]. ADA catalyzes the irreversible hydrolytic deamination of adenosine to produce inosine which is then cleaved to hypoxanthine and oxidized to xanthine and uric acid by xanthine-oxidase [45]. Many researches stated that some natural food resources showed expressive effect on uric acid level of hyperuricemic patients. For example, sparattosperm leucanthum crude extracts and the polyphenols in Green tea showed expressive results on urate-lowering activity in blood due to their XOD inhibition ability [46, 47]. Thus we speculated that KGM decreased serum uric acid by influencing the activity of XOD and ADA. But the mechanism still needs further study.

Besides, we also found that KGM can reduce the increment of serum Cr and BUN, which suggested that KGM can alleviate the abnormal renal function caused by hyperuricemia. This result was confirmed in the subsequent pathological analysis. Although it is still not clear that whether KGM could reverse the renal lesion caused by hyperuricemia, yet KGM, as a kind of food ingredients which has no side effect to human body, still can be a very good means of prevention and treatment of hyperuricemia.

CONCLUSION

These results suggested that KGM could lower the level of UA, Cr and BUN, decrease the activities of XOD and ADA, and alleviate renal histological lesion.

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