Research Square

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Construction of Hyperuricemia Model Rats with Yeast Extract Combined with Oteracil Potassium

Dilidaer Xilifu
first affiliated hospital of xinjiang medical university
Alimu Kateer
first affiliated hospital of xinjiang medical university
Nijiati Rehemu
first affiliated hospital of xinjiang medical university
Zhao-yong Li
first affiliated hospital of xinjiang medical university
jie Jiang
first affiliated hospital of xinjiang medical university
Xin-rong Zhou
first affiliated hospital of xinjiang medical university
Bao-zhu Wang
first affiliated hospital of xinjiang medical university
Fa-peng Li
first affiliated hospital of xinjiang medical university
Jian Zhang
first affiliated hospital of xinjinag medical university
Yan Li
first affiliated hospital of xinjiang medical university
Jia-jun Zhu
first affiliated hospital of xinjiang medical university
Buamina Maitusong
first affiliated hospital of xinjiang medical university
Xiang-yang Zhang (zhangxy_xjmedical@163.com)
first affiliated hospital of xinjiang medical university
Hui-ping Sun
first affiliated hospital of xinjiang medical university

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Abstract

Background: Hyperuricemia is the most important risk factor for gout, hypertension, coronary artery disease and other cardiovascular diseases. The incidence of hyperuricemia gradually increased in recent years and it is very necessary to explore the medications of the prevention and treatment of hyperuricemia using hyperuricemia animal models.

Objective: The objective of present study is to explore the optimal dose of yeast extract and oteracil potassium in the establishment of hyperuricemia rat model.

Method: Sixty-four male rats were randomly divided into 8 experimental groups. Rats were treated with yeast extract by intraperitoneal injection or yeast extract by intraperitoneal injection combined with various doses of oteracil potassium by intragastric feeding or intraperitoneal injection for 28 days. The serum uric acid, urea nitrogen and creatinine levels of different groups were measured at 0th day, 7th day, 14th day, 21th day and 28th day.

Results: The serum levels of uric acid in the groups of intraperitoneal injection with yeast extract alone, yeast extract by intraperitoneal injection combined with 50-200 mg/kg oteracil potassium by intragastric feeding and yeast extract by intraperitoneal injection combined with 50-100 mg/kg oteracil potassium by intraperitoneal injection were higher than that in the control group. But we found no significant effect on rat kidney, heart or artery in the above groups. In the group of yeast extract by intraperitoneal injection combined with 200 mg/kg oteracil potassium by intraperitoneal injection, we observed the significantly high level of serum uric acid and morphological and pathological changes in rat kidney, heart and artery.

Conclusion: In the present study, we found that continuously treated with yeast extract combined with oteracil potassium is an effective method to establish rat hyperuricemia model. Intraperitoneal injection of yeast extract combined with 200 mg/kg oteracil potassium is an optimal dosage for the construction of a persistent and stable hyperuricemia animal model.

Instruction

Hyperuricemia caused by purine metabolic abnormalities or reduced excretion of uric acid (UA) in the body is the most important risk factor for gout [1-3]. With the improvement of living standards, the incidence of hyperuricemia and gout also gradually increased in recent years. Previous study has shown that the prevalence of hyperuricemia is up to 13.7% in healthy adults from northern and northeastern Chinese provinces [4]. In recent years, several studies on hyperuricemia prevalence in China have been performed and a latest meta-analysis indicated that the pooled prevalence of hyperuricemia was 13.3% in China from 2000 to 2014 [5-8]. In addition, it has been demonstrated that hyperuricemia is also significantly associated with hypertension, coronary artery disease, hyperlipidemia and other cardiovascular diseases [9-16]. Therefore, it is very necessary to explore the medications of the prevention and treatment of hyperuricemia.

Experimental animal models are the basis of disease pathogenesis and related drug research. However, there is currently no standard method of the establishment of hyperuricemia model rats [17]. We performed the present study to establish hyperuricemia rat model using yeast extract (YE) combined with different doses of oteracil potassium (OP) and to explore the optimal dose for model establishment.

Materials And Methods

Animals

Male Sprague-Dawley rats (n=64, 8 weeks, 224±37.51g) were supplied by the Center of Experimental Animal of the First Affiliated Hospital of Xinjiang Medical University and were housed in cages and maintained under standard conditions (12-h light/dark cycle, 23±1°C ambient temperature, and 55±10% relative humidity). The protocols of the animal experiments were approved by the Animal Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University.

Establishment of hyperuricemic rat model

Sixty-four male rats were randomly divided into 8 experimental groups (n=8): (1) the control group (NC); (2)YE by intraperitoneal injection (i.p.) group; (3) YE i.p. +50 mg/kg OP intragastric feeding (i.g.) group; (4) YE i.p.+100 mg/kg OP i.g. group; (5) YE i.p.+200 mg/kg OP i.g. group; (6) YE i.p.+50 mg/kg OP i.p. group; (7) YE i.p.+100 mg/kg OP i.p. group; (8) YE i.p.+200 mg/kg OP i.p. group. Oteracil potassium was configured as suspension (20g/L) with sterilized distilled water and were administered to the rats once daily according to the above group. The NC group was fed with normal feed. All the treatments were continued for 28 days.

Sample collection

On the 7th day, 14th day, 21th day, 28th day and before the drug administration, blood samples were collected from the tail vein. The blood samples were centrifuged at 3000×g for 10 min and serum were collected and stored in -80 ℃ refrigerator. At the end of 28 day, all the rats were anesthetized with pentobarbital sodium (40 mg/kg, intraperitoneal injection) and kidney, heart and thoracic aorta were quickly dissected and stored. The removed femoral artery were fixed with 15% paraformaldehyde and embedded in paraffin for histological analysis. The 4 μm-thick paraffin sections were

stained with hematoxylin and eosin (H&E).

Determination of SUA

The serum uric acid, urea nitrogen and creatinine levels of different groups at 0th day, 7th day, 14th day, 21th day and 28th day after drug administration were measured to determine the successful establishment of the hyperuricemia model.

Statistical analysis

The data were expressed as mean ± SD and analyzed by SPSS17.0. Significant differences between means were evaluated by one-way analysis of variance (ANOVA). LSD test were used for post hoc evaluations, and $P < 0.05$ was considered to represent a statistically significant difference.

Result

Changes of serum uric acid, urea nitrogen and creatinine levels of different groups

The rat serum uric acid levels of different groups were shown in table 1. The serum uric acid levels of YE group and YE combined with OP group were significantly higher than that in NC group (P all 0.05). We found that not only the YE combined with OP group, but also the YE group may successfully establish animal models of hyperuricemia. In

addition, the serum uric acid levels of YE i.p.+200 mg/kg OP i.p. group were significantly higher than that in other groups (P all 0.05).

The rat serum urea nitrogen and creatinine levels of different groups were shown in table 2 and table 3. The serum urea nitrogen levels of YE i.p.+200 mg/kg OP i.p. group were significantly higher than that in NC group at the 28th day (P 0.05). However, there exists no significantly difference of serum creatinine levels between different groups.

Pathological changes of kidney in rats

The kidney tissue morphology of YE i.p.+200 mg/kg OP i.g. group, YE i.p.+100 mg/kg OP i.p. group and YE i.p.+200 mg/kg OP i.p. group showed slight pathological changes, including the thickening of the glomerular wall, proliferation of some endothelial cells in glomerular capillary loop and edema of renal tubular epithelial cells. There also exists little chronic inflammatory cell infiltration in the renal interstitial and urate crystal deposition in some rats of the above group. Furthermore, we also observed the endothelial cell proliferation of renal interstitial artery and lumen stenosis in the YE i.p.+200 mg/kg OP i.p. group (Fig.1).

Pathological changes of heart tissue in rats

As shown in Fig. 2, morphological and pathological analysis demonstrated there exists no pathological change of the rat heart tissue in all groups except the YE i.p.+200 mg/kg OP i.p. group. There exist myocardial interstitial vasodilation with congestion and bleeding, endothelial cell proliferation, neovascularization and infiltration of plasma cells and lymphocytes in myocardial interstitial in YE i.p.+200 mg/kg OP i.p. group.

Pathological changes of thoracic aorta in rats

There exists no pathological change of the rat thoracic aorta in all groups except the YE i.p.+200 mg/kg OP i.p. group. There exist exfoliation of arterial endothelial cells, proliferation of vascular smooth muscle cells and light proliferation of adventitial nutrient vessels with congestion in YE i.p.+200 mg/kg OP i.p. group (Fig.3).

Pathological changes of femoral artery in rats

There exists no pathological change of the rat thoracic aorta in all groups except the YE i.p.+200 mg/kg OP i.p. group. There exist endothelial cell proliferation, lumen stenosis and neovascularization in peripheral adipose tissue in YE i.p.+200 mg/kg OP i.p. group (Fig.4).

Discussion

The establishment of hyperuricemia animal model is an effective way to develop and verify therapeutic drugs of hyperuricemia. However, there is currently no standard method of the establishment of hyperuricemia model rats. In the present study, we designed to establish hyperuricemia rat model using yeast extract combined with different doses of oteracil potassium and to explore the optimal dose for model establishment. Our results showed that intraperitoneal injection of yeast extract combined with 200 mg/kg oteracil potassium is an optimal dosage for the establishment of a persistent and stable hyperuricemia animal model.

Hyperuricemia is one of the increasingly common diseases, which is reported to have afflicted more than 2 million men and women in the United States alone, and is growing rapidly in China due probably to changes in dietary habits [18–19]. Hyperuricemia is associated with abnormal uric acid concentrations in the body, resulting in the deposition of urate crystals in the joints and kidneys that lead to inflammation, as well as gouty arthritis and uric acid

nephrolithiasis. In addition to an increased risk of hyperuricemia and gout, excess uric acid is also related to cardiovascular disorders, nephrolithiasis and diabetes [20–26].

Two major mechanisms have been proposed for hyperucicemia in man, excess production and insufficient metabolisation of uric acid. Yeast extract paste and oteracil potassium were used to mimic both mechanisms: yeast represents excess production of UA, probably the main mechanism in man, and oteracil potassium impairs metabolisation [21, 27]. Potassium oxonate is most frequently employed to develop an animal model of hyperuricemia by inhibiting uricase that converts uric acid to allantoin. However, there exist great differences among different researchers in the type of drug compatibility, the way of drug use, the dosage and the method of animal selection and there is currently no standard method of the establishment of hyperuricemia model rats.

In the present study, serum uric acid levels were significantly increased on 7th, 14th, 21th and 28th day in the groups of intraperitoneal injection with yeast extract alone, yeast extract by intraperitoneal injection combined with 50–200 mg/kg oteracil potassium by intraperitoneal injection and yeast extract by intraperitoneal injection combined with 50–200 mg/kg oteracil potassium by intragastric feeding.

Hyperuricemia has been closely associated with renal dysfunction. High level of uric acid could increase the burden of the kidney and cause renal damage [28]. In the present study, YE i.p. +200 mg/kg OP i.p. group developed the elevated levels of serum urea nitrogen and creatinine levels compared with NC group, and showed pathological changes of kidney, including the thickening of the glomerular wall, proliferation of some endothelial cells in glomerular capillary loop and edema of renal tubular epithelial cells, chronic inflammatory cell infiltration in the renal interstitial and urate crystal deposition and endothelial cell proliferation of renal interstitial artery and lumen stenosis (Fig. 1), which is consistent with the results of various studies, but the mechanism involved is not clear.

Purine metabolism in the circulatory system yields uric acid as its final oxidation product, which is believed to belinked to the development of gout and kidney stones. In addition, hyperuricemia is closely correlated with cardiovascular disease too. A number of epidemiologic studies have confrmed an association between hyperuricemia and CVD [16]. A study in Japan has reported that hyperuricemia is positively associated with obesity, hypertension and dyslipidemia, and hyperuricemic subjects tend to have a clustering of these cardiovascular risk factors. In the present study, we found morphological and pathological changes in heart, thoracic aorta and femoral artery in the YE i.p.+200 mg/kg OP i.p. group, indicating that intraperitoneal injection of yeast extract combined with 200 mg/kg oteracil potassium is an optimal dosage for the construction of a hyperuricemia animal model with pathological damages related to secondary cardiovascular diseases.

Conclusion

In the present study, we found that continuously treated with yeast extract combined with oteracil potassium is an effective method to establish rat hyperuricemia model. Intraperitoneal injection of yeast extract combined with 200 mg/kg oteracil potassium is an optimal dosage for the construction of a persistent and stable hyperuricemia animal model; it is a favorable model for the study of pathological damages related to secondary cardiovascular diseases caused by hyperuricemia.

Abbreviations

l.g.

intragastric feeding; i.p.:intraperitoneal injection; OP:oteracil potassium; NC:control group; UA:uric acid; YE:yeast extract.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

Conceived and designed the experiments: Dilidaer·Xilifu, Xiang-yang Zhang, Hui-ping Sun and Alimu Kateer; Performed the experiments: Dilidaer·Xilifu and Alimu Kateer; Analyzed the data: Nijiati·Rehemu, Zhao-yong Li and jie Jiang; Contributed reagents/materials/analysis tools: Xin-rong Zhou, Bao-zhu Wang, Fa-peng Li, Jian Zhang, Yan Li, Jia-jun Zhu and Buamina Maitusong; Wrote the paper: Dilidaer·Xilifu. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (Xinjiang, China). It was conducted according to the standards of the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 Rat serum UA levels of different groups

Group	Serum UA level (μmol/L)				
	0 day	7 days	14 days	21 days	28 days
Blank control	87.50±16.29	78.00±16.27^{bb}	67.50±11.17^{bb}	97.00±4.78^{bb}	92.25±30.94^{bb}
YE i.p.	81.75±13.04	137.00±6.55^{aabb}	215.00±45.28^{aa}	175.25±10.57^{aabb}	115.00±7.30^{bb}
YE i.p.+50 mg/kg OP i.g	86.50±20.87	189.25±12.15^{aab}	181.25±3.65^{aabb}	316.75±18.52^{aa}	297.75±13.40^{aabb}
YE i.p.+100 mg/kg OP i.g	86.00±6.48	111.50±5.68^{abb}	174.75±9.27^{aabb}	189.75±45.38^{aabb}	241.00±64.79^{aabb}
YE i.p.+200 mg/kg OP i.g	90.25±11.35	175.25±48.67^{aabb}	188.00±17.78^{aabb}	191.75±18.54^{aabb}	276.75±41.85^{aabb}
YE i.p.+50 mg/kg OP i.p	77.75±6.70	148.00±25.49^{aabb}	173.25±5.71^{aabb}	196.25±15.66^{aabb}	339.50±35.29^{aabb}
YE i.p.+100 mg/kg OP i.p	89.25±10.44	249.00±54.02^{aa}	277.00±12.14^{aa}	320.50±46.97^{aa}	295.50±50.49^{aabb}
YE i.p.+200 mg/kg OP i.p	94.00±7.16	231.50±21.98^{aa}	271.50±27.71^{aa}	331.75±7.07^{aa}	450.50±31.59^{aa}

$^{a}P < 0.05$, $^{b}P < 0.01$, *vs.* blank control group; $^{c}P < 0.05$, $^{d}P < 0.01$, *vs.* YE i.p.+200 mg/kg OP i.p. group; YE: yest extract; OP: oteracil potassium; i.g.: intragastric feeding; i.p.: intraperitoneal injection.

Table 2 Rat serum urea nitrogen levels of different groups

Group	Serum urea nitrogen levels (mmol/L)				
	0 day	7 days	14 days	21 days	28 days
Blank control	6.25±1.29	7.87±0.79	6.90±0.78	8.13±0.55	7.55±0.63
YE i.p.	6.125±1.06	6.50±0.29	6.375±1.32	8.25±0.34	8.35±0.44
YE i.p.+50 mg/kg OP i.g	6.48±0.70	6.30±1.25	6.25±0.57	7.83±0.81	8.35±0.80
YE i.p.+100 mg/kg OP i.g	6.20±1.20	6.10±0.78	6.85±0.64	7.15±1.02	7.40±0.81
YE i.p.+200 mg/kg OP i.g	6.025±0.91	5.90±1.05	7.03±0.45	6.95±0.59	8.00±0.59
YE i.p.+50 mg/kg OP i.p	5.88±0.76	5.68±0.85	6.80±0.89	7.07±0.93	8.65±0.27
YE i.p.+100 mg/kg OP i.p	6.78±0.67	6.70±0.45	6.73±0.91	8.05±1.44	8.80±0.70
YE i.p.+200 mg/kg OP i.p	7.05±0.93	7.03±0.63	7.43±0.47	8.77±0.71	10.10±0.70[a]

[a]$P < 0.05$, [b]$P < 0.01$, *vs.* blank control group; [c]$P < 0.05$, [d]$P < 0.01$, *vs.* YE i.p.+200 mg/kg OP i.p. group; YE: yest extract; OP: oteracil potassium; i.g.: intragastric feeding; i.p.: intraperitoneal injection.

Table 3 Rat serum creatinine levels of different groups

Group	Serum creatinine level (μmol/L)				
	0 day	7 days	14 days	21 days	28 days
Blank control	31.00±5.23	33.00±3.12	36.75±3.58	34.50±2.88	32.25±4.79
YE i.p.	29.25±6.65	30.75±6.43	33.75±5.92	34.25±3.33	38.75±14.31
YE i.p.+50 mg/kg OP i.g	28.25±7.34	26.25±5.74	34.50±3.42	33.75±5.82	43.25±4.19
YE i.p.+100 mg/kg OP i.g	27.50±8.35	29.75±4.23	28.25±6.11	35.00±3.46	41.75±5.73
YE i.p.+200 mg/kg OP i.g	29.75±3.30	31.25±4.74	38.25±8.89	38.50±7.35	42.00±7.39
YE i.p.+50 mg/kg OP i.p	30.25±1.50	33.00±2.00	32.25±3.01	38.00±3.78	40.50±7.85
YE i.p.+100 mg/kg OP i.p	29.00±6.16	29.50±7.03	36.25±1.75	35.75±3.15	40.00±6.27
YE i.p.+200 mg/kg OP i.p	26.75±8.34	30.75±2.87	38.00±3.46	41.50±4.44	43.50±2.65

[a]$P < 0.05$, [b]$P < 0.01$, *vs.* blank control group; [c]$P < 0.05$, [d]$P < 0.01$, *vs.* YE i.p.+200 mg/kg OP i.p. group; YE: yest extract; OP: oteracil potassium; i.g.: intragastric feeding; i.p.: intraperitoneal injection.

Figures

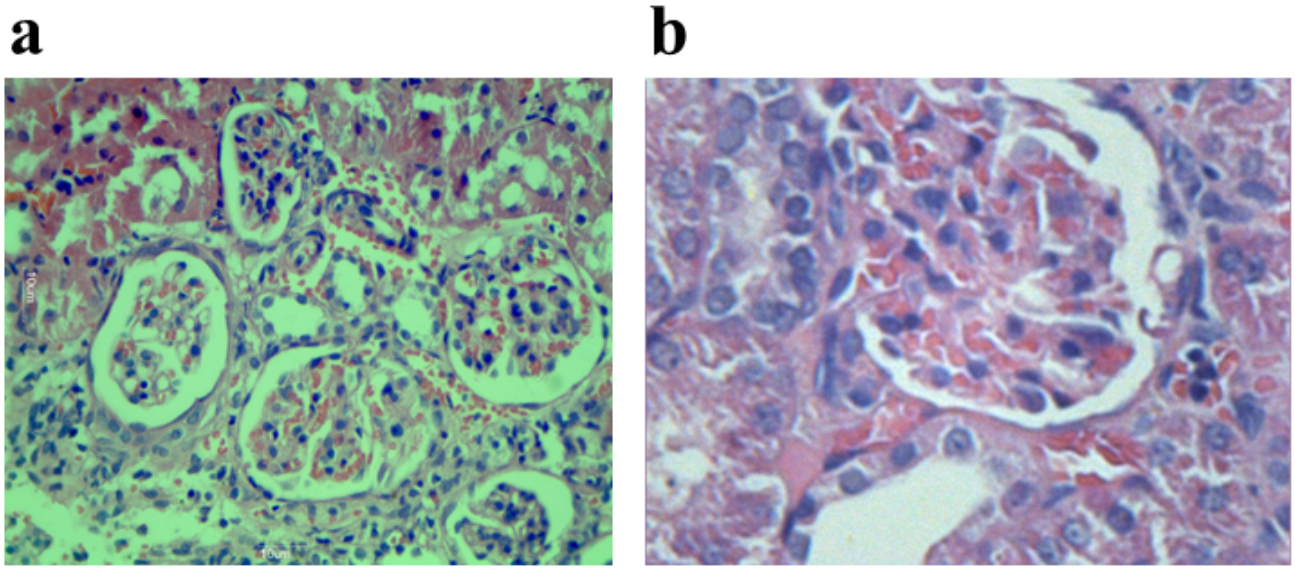

Figure 1

Histopathological changes of rat renal tissues (Hematoxylin-eosin staining, ×400). a: Blank control group; b: YE i.p.+200 mg/kg OP i.p. group.

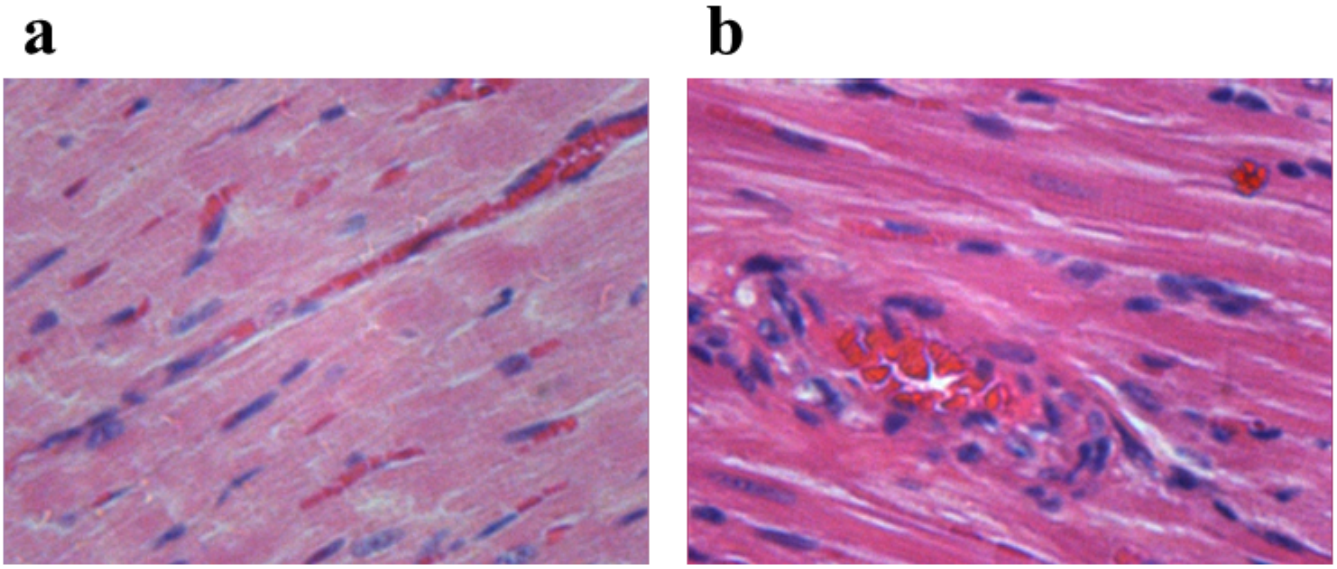

Figure 2

Histopathological changes of rat cardiac tissues (Hematoxylin-eosin staining, ×400). a: Blank control group; b: YE i.p.+200 mg/kg OP i.p. group.

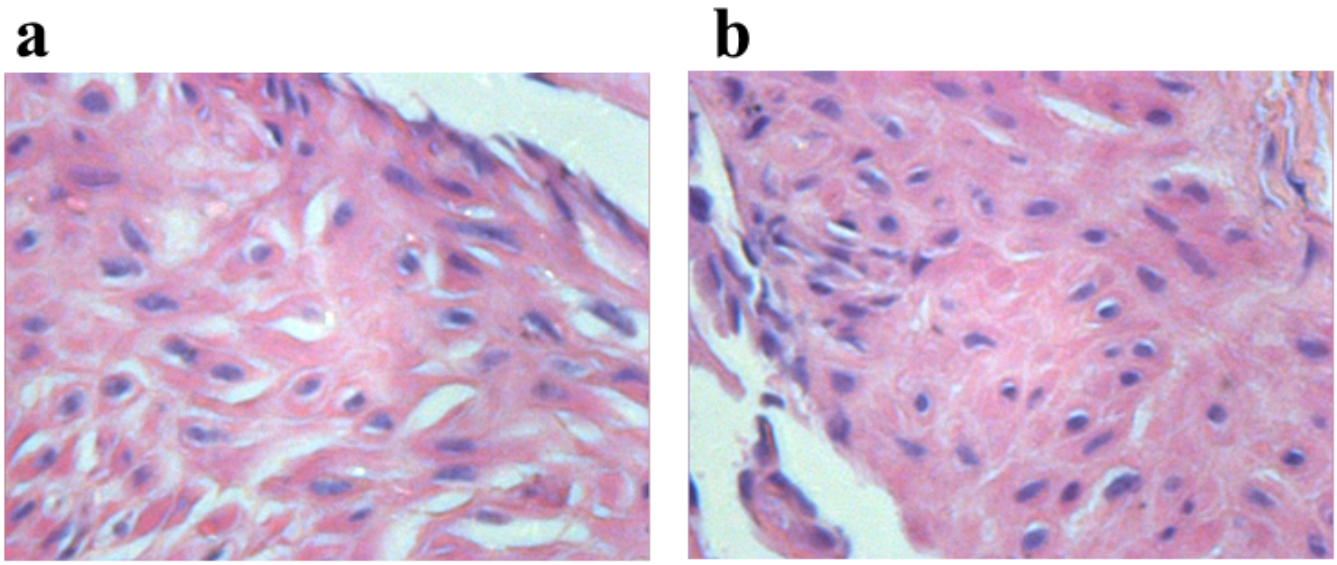

Figure 3

Histopathological changes of rat aortic tissues (Hematoxylin-eosin staining, ×400). a: Blank control group; b: YE i.p.+200 mg/kg OP i.p. group.

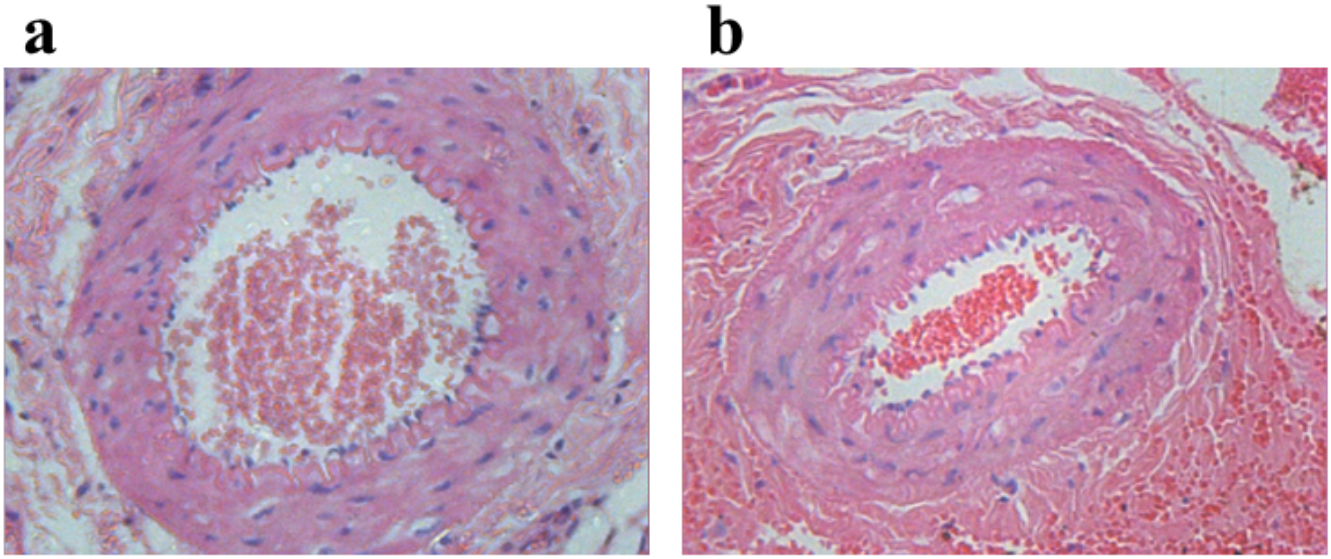

Figure 4

Histopathological changes of rat femoral arteries (Hematoxylin-eosin staining, ×400). a: Blank control group; b: YE i.p.+200 mg/kg OP i.p. group.