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Effects of Bentonite on Rat Growth and Organ Microstructure

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Abstract: In this experiment, 72 rats were equally divided into 6 groups with 12 in each group where six were male and the others were female. In order to investigate the effects of bentonite on the growth and organ development, test groups were fed with 1.5, 3, 4.5, 10 and 20% bentonite in their feed, respectively and the control group was fed with basal diet. The experimental period was 60 days. The result showed that the growth and allelotaxy of rat were promoted by adding 1.5-4.5% bentonite into feed ($p>0.05$) and the growth and allelotaxy of rat were significantly promoted by adding 10-20% bentonite into feed ($p<0.05$ or $p<0.01$). But the test groups suffered from pulmonary emphysemamore or less. Alveolar wall rupture and bronchial epithelial hyperplasia were found in the group with 3% bentonite in the feed. Rats in the group with 20% bentonite in their feed had lung hemorrhage.

Key words: Bentonite, rats, growth and development, organizational structure, India

INTRODUCTION

Bentonite, a aluminum silicate minerals is water-bearing layer structure which is composed of montmorillonite clay (Ying, 2008). It is with tiny particles, large surface area and has a strong adsorption capacity and cation exchange capacity. In the feed industry, the bentonite acts as a carrier of the premix, an anti-caking agent, an agent to improve the environment and an adhesive to absorb particles such as feed and mycotoxins. Mikolaichik and Morozova (2009) reported that diets for cows supplemented with bentonite can increase milk production and digestibility and improve milk quality. Varadyova *et al*. (2003) reported that adding bentonite in high concentrate diets of ruminants can control rumen pH and improve the rumen environment. Berthiaume *et al*. (2007) reported that adding bentonite to the silage can increase the amount of animal feed, average daily gain and utilization of silage. Khadem *et al*. (2007) and Aquilera-Soto *et al*. (2008) reported that adding 2 and 4% bentonite to the diet can improve the feed intake of sheep, feed conversion and daily gain and lower the feed costs of 2%. Ma and Guo (2008) reported that the addition of bentonite can significantly improve the growth performance of chicken at the age of 320 days and feed conversion rate and improve activities of intestinal maltase, aminopeptidase N and alkaline phosphatase. Xia *et al*. (2004) reported that Cu containing bentonite can significantly improve the growth performance, enhance the activities of broiler small intestine total protease, amylase and lipase and improve the role of intestinal barrier. Ambula *et al*. (2003) reported that adding 0.25 or 0.5% bentonite can reduce the impact of tannins on laying hen performance.

Zhao *et al*. (2010) reported that adding bentonite to the feed contaminated heavy metals as an adsorbent can significantly reduce the heavy metal residues in tissues; by doing adsorption experiments. Yan found that bentonite could absorb certain lysine and vitamins but the amount of adsorption was small And no absorption of methionine was found.

Since, the reports on the effects of adding bentonite into feed vary a lot and the security study is also relatively rare, it is necessary to conduct this study.

MATERIALS AND METHODS

Bentonite: Bentonite used in this study was collected from Fujian, Wu Ping, Zhongshan, sifted through a 200 mesh. Major and minor components of bentonite (Table 1) was analyzed using GB/T14506.28, X-ray fluorescence spectrometry and trace elements of bentonite was analyzed with plasma mass spectrometry (ICP-MS) (Table 2).

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Table 1: The major and minor component content of bentonite

Elements	SiO_2	Al_2O_3	Fe_2O_3	MgO	CaO	Na_2O	K_2O	TiO_2	P_2O_5	MnO	F	FeO
Content/%	72.82	15.22	1.19	2.73	0.18	0.43	0.97	0.2	0.007	0.056	0.042	0.04

Table 2: The trace elements of bentonite

Elements	Co	Cr	Cu	Ni	Pb	V	Zn	Hg	As
Content/mg kg^{-1}	2.9	4.6	3.7	8.2	33.2	9.8	65.3	0.003	4.36

Table 3: The formulations and nutrient levels of experimental diet

Groups	Basal diet */%	Bentonite /%	DM/%	CP/%	EE/%	NDF/%	ADF/%	Ash/%	Ca/mg g^{-1}
Test group I	100	1.5	88.90	22.00	6.0	55.90	20.50	7.50	1.0
Test group II	100	3.0	88.00	21.80	5.6	54.80	20.00	8.30	1.0
Test group III	100	4.5	87.30	20.10	5.1	53.60	19.70	9.90	1.1
Test group IV	100	10.0	82.60	16.90	4.2	52.10	18.00	13.78	1.1
Test group V	100	20.0	79.45	14.40	3.4	50.00	16.00	19.45	1.3
Control group	100	0.0	89.26	22.94	6.0	56.40	21.00	7.23	1.0

*Corn 30%, soybean meal 21%, wheat bran 10%, sub-powder 28%, 9% fish meal, yeast powder 1.5%, 1.7% dicalcium phosphate, salt 0.3%, 1% cod liver oil, multi-dimensional 200 g t^{-1}

Test animals and grouping: Seventy two Conventional Sprague-Dawley rats (purchased from Fujian Institute of Medical Sciences) with weight 100±5 g were keeping with the basic diet adaptation. After 1 week, they were randomly divided into control group and experimental groups named I, II, III, IV and V with 12 rats in each group, half male and half female.

Experimental program: The 1.5, 3, 4.5, 10 and 20% bentonite were added into the basal diet for the test groups (I-V) and control group was fed with the basal diet. Pre-feeding and experiment periods are 5 and 6 days, respectively. Experimental diet formulations and nutrient levels are shown in Table 3.

Rats were watered with distilled water and fed twice at 8:00 and 18:00 every day. Rats daily activities and diet were observed every day. They were weighed before feeding in the morning every 10 days. Feed consumption was detected as well. In the experiment was carried out at ambient temperature 24~35 with humidity 50-65% and under light for 12 h.

Index detection

Detection of organ quality: After the experiment, wiped the blood on the surfaces of heart, liver, spleen, lung, kidney and other organs with filter study and then measured their qualities.

Body length, Lee's index, organ index:

- Length refers to the length from nose to anus
- Lee's index = [Weight (g)×10^{-3}/Body length (cm)]×10^3
- Organ index = Organ mass (g)/Body weight (g)×100%

Microscopic observation of organs and tissues: Heart, liver, spleen, lung and kidney were dissected and fixed in 10% neutral formalin. Get tissue and put under running water overnight and then dehydrate with gradient ethanol dehydration. They were embedded in paraffin, sliced and HE stained. Microscopic examination and pictures were taken in the end.

Data processing: Statistical Software SPSS13.0 were used to carried out the ANOVA single factor analysis of variance. Experimental data were represented as mean±Standard Deviation ($\overline{X}$ ±SD).

RESULTS AND DISCUSSION

The influence of bentonite on the growth and development in rats: The whole story of the rats body weight and Average Daily Gain (ADG) of each stage during the experimental period are shown in Table 4 and 5. As can be seen in Table 4, body weights increased by 22.37% ($p<0.01$) and 17.74% ($p<0.05$) in the test groups IV and V compared with the control group. Final weights in test groups I-III don't show significant difference ($p>0.05$) compared with control group.

Table 5 shows 1-10 days: the ADG in each group was not significantly different from others ($p>0.05$); 11~20 days: the ADG of test groups IV and V were increased 86.58% ($p<0.01$) and 99.66% ($p<0.01$) compared to the control group; 21-30 days: the ADG of test group II was increased 49.60% ($p<0.05$) compared with the control group; 31-40 days: the ADG of test groups III-V were increased 68.60% ($p<0.05$), 168.60% ($p<0.01$) and 65.12% ($p<0.05$) compared with the control group. 41-50 days: the ADG of test groups III-V were increased 84.21% ($p<0.01$), 58.95% ($p<0.05$) and 128.42% ($p<0.01$) compared with the control group; 1-50 days: the ADG of test groups IV and V were increased 39.03% ($p<0.01$) and 35.69% ($p<0.01$) compared with the control group. The ADG of the most test groups has been increased compared to the control group but the difference was not significant ($p>0.05$).

Table 4: Effect of bentonite on the body mass of rats (unit: g)

Items	Control group	Test group I	Test group II	Test group III	Test group IV	Test group V
Initial weight	121.81 ± 6.550	121.85 ± 5.570	121.77 ± 5.790	121.31 ± 8.510	121.02 ± 8.620	122.04 ± 6.920
Final weight	260.75 ± 41.41^{abA}	270.75 ± 41.02^{ac}	288.67 ± 45.29	282.17 ± 41.90	319.08 ± 64.86^{bdB}	307.00 ± 47.77^{cd}

Table 5: Effect of bentonite on the ADG of rats (unit: g)

ADG (days)	Control group	Test group I	Test group II	Test group III	Test group IV	Test group V
1-10	7.41 ± 1.01	7.62 ± 0.86	7.35 ± 1.27	7.31 ± 1.16	7.61 ± 1.45	7.50 ± 1.03
11-20	2.98 ± 1.15^{B}	4.48 ± 1.22^{AB}	3.11 ± 0.84^{B}	2.93 ± 1.12^{B}	5.56 ± 3.80^{A}	5.95 ± 2.01^{A}
21-30	1.27 ± 0.54^{ad}	1.58 ± 0.40	1.90 ± 0.49^{bcA}	1.57 ± 0.80	1.69 ± 0.69^{ac}	1.20 ± 0.48^{bdB}
31-40	0.86 ± 0.36^{aB}	1.14 ± 0.40^{abB}	1.14 ± 0.32^{abB}	1.45 ± 0.53^{bB}	2.31 ± 0.95^{abA}	1.42 ± 0.83^{bB}
41-50	0.95 ± 0.29^{adeB}	1.04 ± 0.34^{cdBC}	1.46 ± 0.69^{bceAB}	1.75 ± 0.59^{abAC}	1.51 ± 0.40^{bcAB}	2.17 ± 1.24^{adA}
1-50	2.69 ± 0.53^{abA}	3.17 ± 0.47^{ab}	2.99 ± 0.44^{a}	3.00 ± 0.51^{a}	3.74 ± 1.20^{bB}	3.65 ± 0.77^{bB}

Table 6: Effect of bentonite on feed intake and feed conversion of rats

Items	Control group	Test group I	Test group II	Test group III	Test group IV	Test group V
ADFI	19.04 ± 2.6300^{cd}	19.86 ± 5.8800^{cd}	16.45 ± 3.0200^{acB}	17.71 ± 5.3700^{bcB}	22.03 ± 7.8100^{bd}	24.72 ± 6.0100^{abA}
F/G	12.16 ± 3.1761	14.34 ± 4.4661	14.53 ± 3.7361	10.83 ± 2.4161	10.35 ± 2.2161	13.79 ± 3.5961

Table 7: Effect of bentonite on body length and lee's indexes of rats

Items	Control group	Test group I	Test group II	Test group III	Test group IV	Test group V
Body length	20.71 ± 0.910^{acBD}	20.31 ± 0.950^{bcBD}	19.69 ± 0.750^{bdB}	19.79 ± 1.37^{abcdB}	21.36 ± 2.210^{adACD}	22.21 ± 1.190^{abcdA}
Lee's indexes	313.93 ± 12.02^{b}	321.03 ± 15.33^{abB}	317.32 ± 13.22^{abB}	322.40 ± 8.15^{abB}	314.03 ± 12.16^{b}	302.90 ± 12.95^{aA}

Table 8: Effeet of bentonite on the organs mass of rats (unit: g)

Organs	Control group	Test group I	Test group II	Test group III	Test group IV	Test group V
Heart	0.89 ± 0.13^{bc}	0.90 ± 0.17^{bc}	0.82 ± 0.11^{cdB}	0.85 ± 0.14^{cdB}	0.99 ± 0.30^{ab}	1.06 ± 0.20^{adA}
Liver	8.53 ± 1.20^{ab}	8.10 ± 1.33^{abc}	7.20 ± 1.16^{cdB}	7.98 ± 1.42^{bd}	9.30 ± 2.91^{acA}	9.25 ± 1.47^{abcA}
Spleen	0.52 ± 0.08	0.53 ± 0.08	0.45 ± 0.09	0.50 ± 0.10^{b}	0.55 ± 0.15	0.60 ± 0.09^{a}
Lung	1.33 ± 0.17^{bd}	1.37 ± 0.20^{abc}	1.33 ± 0.51^{abB}	1.31 ± 0.28^{adB}	1.48 ± 0.44^{cd}	1.57 ± 0.30^{acA}
Kidney	1.66 ± 0.26^{ab}	1.62 ± 0.32^{b}	1.53 ± 0.27^{abB}	1.65 ± 0.28^{ab}	1.76 ± 0.53^{ab}	1.91 ± 0.39^{aA}

Table 9: Effect of bentonite on the organ indexes of rats (unit: g)

Organs	Control group	Test group I	Test group II	Test group III	Test group IV	Test group V
Heart index ($\times10^{-2}$)	0.32 ± 0.04	0.32 ± 0.05	0.34 ± 0.03	0.33 ± 0.03	0.32 ± 0.03	0.34 ± 0.03
Liver index ($\times10^{-2}$)	3.11 ± 0.34	2.94 ± 0.48	2.93 ± 0.23	3.09 ± 0.55	3.00 ± 0.26	3.03 ± 0.27
Spleen index ($\times10^{-2}$)	0.19 ± 0.04	0.19 ± 0.02	0.18 ± 0.03	0.19 ± 0.04	0.18 ± 0.03	0.20 ± 0.00
Lung index ($\times10^{-2}$)	0.49 ± 0.08	0.50 ± 0.07	0.54 ± 0.18	0.51 ± 0.09	0.49 ± 0.10	0.52 ± 0.12
Kidney index ($\times10^{-2}$)	0.60 ± 0.06^{ABD}	0.58 ± 0.04^{aBD}	0.62 ± 0.05^{bBC}	0.64 ± 0.04^{AC}	0.57 ± 0.04^{D}	0.62 ± 0.06^{bBC}

In the same row, values with different capital letter superscripts mean extremely significant difference ($p<0.01$) and with different small letter superscripts mean significant difference ($p<0.05$) and with the same letter or not letter means no significant difference ($p>0.05$)

Effect of bentonite on feed intake and feed conversion of rats: According to Table 6, the ADFI (Average Daily Feed Intake) of test groups was increased 29.83% ($p<0.05$) more than the control group; there is a tendency increase in test groups I-IV compared with the control group but the difference was not significant ($p>0.05$). The Feed Gain ratio (F/G) of test groups III and IV were decreased by 14.88 and 10.94% compared with the control group. The Feed Gain ratio (F/G) of test groups I, II and V were increased by 17.93, 19.49 and 13.40% more than the control group.

Table 7 shows that the body length of test group V increased 7.24% ($p<0.01$) more than the control group at the end of the test and the body length of test group II decreased 4.93% ($p<0.05$) compared to the control group, differences in the test groups I, III and IV were not significant ($p>0.05$) compared to the control group. Lee's index of test group V decreased 3.51% ($p<0.05$) compared to the control group, the differences of Lee's index in test groups I-IV were not significant ($p>0.05$) compared to the control group.

Effect of bentonite on the organs mass and organ indexes of rats

Effeet of bentonite on the organs mass of rats: Table 8 shows that the organs mass in test groups were not significantly different ($p>0.05$) from the control group except for the heart and lung weight in test group V were increased 19.10% ($p<0.05$) and 18.05% ($p<0.05$) compared to the control group and the liver weight in group II decreased 15.59% ($p<0.05$) compared to the control group.

Effect of bentonite on the organ indexes of rats: As can be seen from Table 9 the indexes of heart, liver, spleen, lung and renal were not significantly different ($p>0.05$) from the control group.

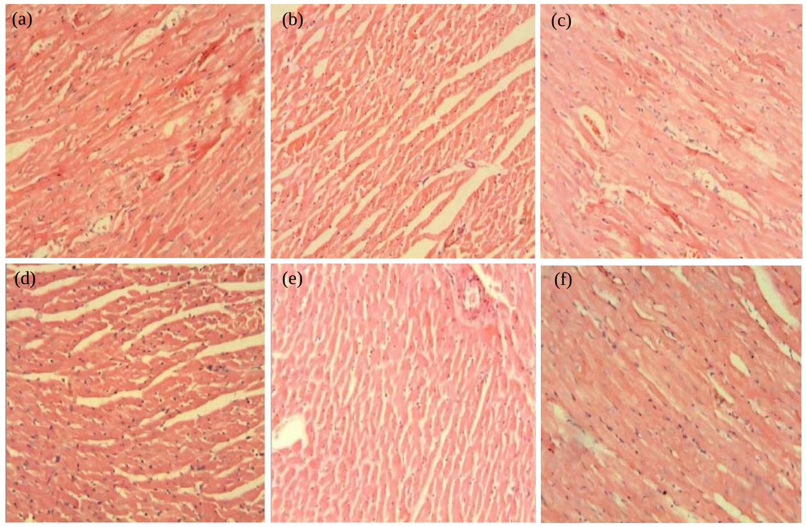

Fig. 1: Eeffect of bentonite on microstructure of heart in rats (HE stain 200x). a) control group; b) test group I; c) test group II; d) test group III; e) test group IV; f) test group V

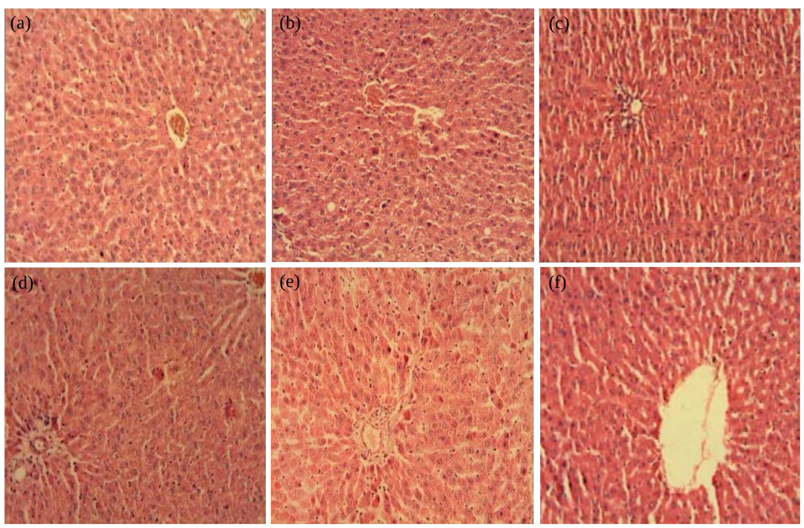

Fig. 2: Effect of bentonite on microstructure of livers of rats (HE stain 200x). a) control group. b) test group I; c) test group II; d) test group III; e) test group IV; f) test group V

Effect of bentonite on microstructure of organs of rats

Effect of bentonite on microstructure of heart of rats: It can be seen from Fig. 1 that the structure of cardiac tissue and stripes were clearly, fibers were arranged in order in each test group. There is no necrosis and fracture, no interstitial hemorrhage, edema and infiltration of inflammatory cells and no inflammatory cells in epicardial.

Effect of bentonite on microstructure of livers of rats: As can be seen from Fig. 2, lobule structure is clear in each experimental group. The size and shape of liver cell are normal and the sinus is clear. No infiltration of inflammatory cells, liver cell degeneration, necrosis and proliferation of fibrous connective tissue and other pathological changes are observed. There was no significant difference between the control group and test groups.

Effect of bentonite on microstructure of spleens of rats: It can be seen from Fig. 3 that there was no infiltration of inflammatory cells, liver cell degeneration, necrosis and proliferation of fibrous connective tissue and other pathological changes. There was no significant difference between the control group and test groups.

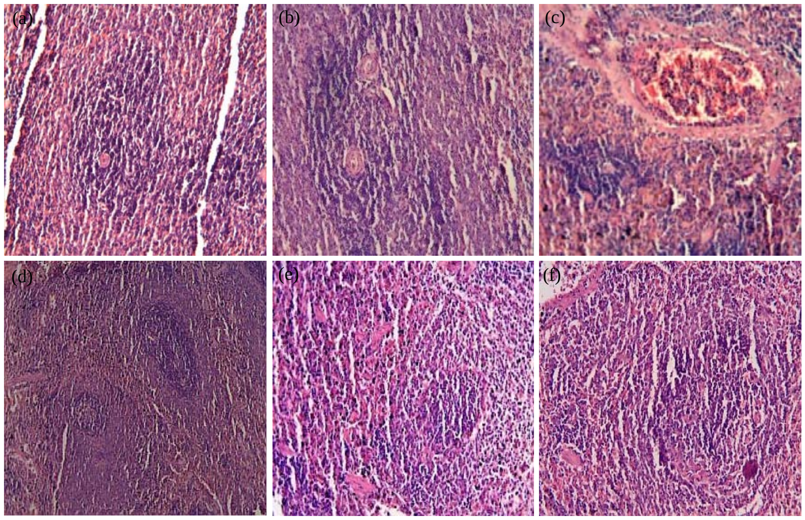

Fig. 3: Effect of bentonite on microstructure of spleens of rats (HE stain 200x). a) control group; b) test group I; c) test group II; d) test group III; e) test group IV; f) test group V

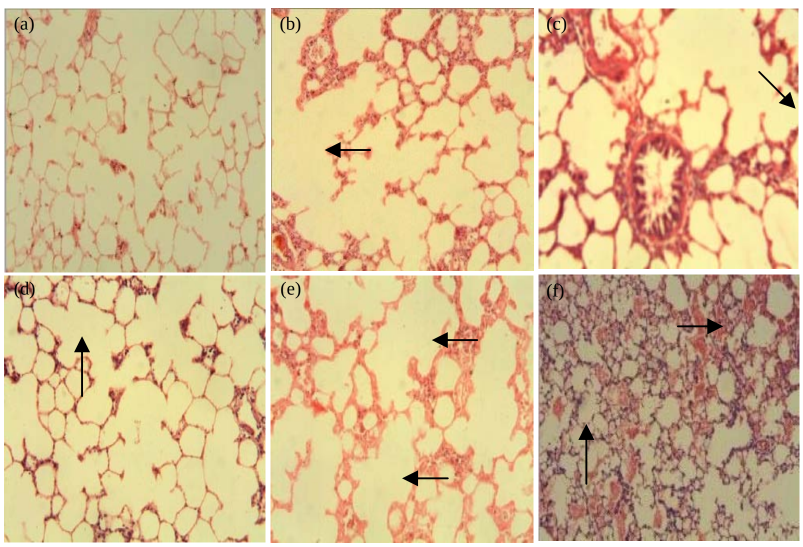

Fig. 4: Effect of bentonite on microstructure of lungs of rats (HE stain 200x). a) control group; b) test group I (Emphysema↑); c) test group II (Alveolar wall rupture↑); d) test group III (Emphysema↑); e) test group IV (Emphysema↑); f) test group V (pulmonary congestion, hemorrhage↑)

Effect of bentonite on microstructure of lungs of rats: It can be seen from Fig. 4 that the alveolar was visible in control group, the structure of alveolar wall was normal. Layers of the bronchial wall, alveolar septum, alveolar cavity were all normal. Layers of the bronchial wall, alveolar septum, alveolar cavity were normal too. There was no edema, hemorrhage, inflammation and other changes (Fig. 4a). Test group I-IV: There are different degrees of emphysema (Fig. 4b-e). Test group II: Emphysema, alveolar wall rupture, bronchial epithelial hyperplasia (Fig. 4c); Test group III: pulmonary congestion, hemorrhage (Fig. 4f).

Effect of bentonite on microstructure of kidney in rats: As can be seen from Fig. 5, the organizational structure and boundaries of kidney cortex and medulla in each experimental were clear, morphology was normal too. Glomeruli was visible and glomerular capillary was normal. Renal capsule and tubular structure were normal. There was a clear outline of proximal tubule and no swelling and tube. Interstitial has no congestion, no hyperplasia and no inflammatory cell infiltration. And there was no abnormal renal capsule and no significant difference between the control group and test groups.

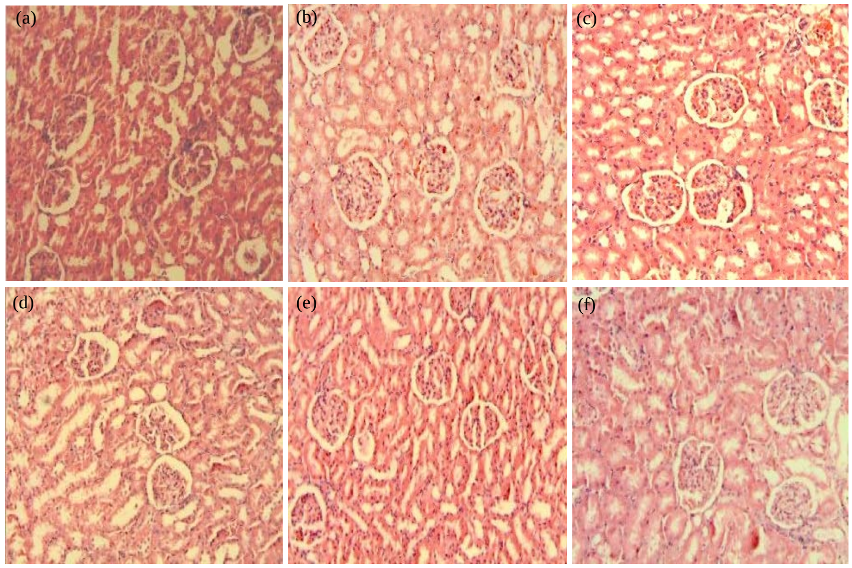

Fig. 5: Effect of bentonite on microstructure of kidney in rats (HE stain 200x). a) control group; b) test group I; c) test group II; d) test group III; e) test group IV; f) test group V

Influence of bentonite on the growth performance of rats: Weight coefficient of main organs animals, body weight, body size and other indicators are not only the basic features of species but the basic data necessary for the study of physiology, pathology and toxicology (Li, 1994).

Bentonite is a material with physical activity. The results showed that low dose supplementation of bentonite (1.5~4.5%) plays a role in promoting rat growth trend. And significant growth promoting effect ($p<0.01$) was observed by adding a large dose (10~20%).

The influence of bentonite on the development of rat organs: In order to meet the needs of growth and development, reproduction and environmental change and other needs, the ratio of organ weight to body weight of animals is always maintained at a certain relationship so that they can meet the needs of the physiological changes (Liao *et al*., 2002). Meanwhile, the organ coefficient of variation can usually reflect the toxicity of chemical poison to the consolidation of the organs; it can be the circumstantial evidence of the possibility of pathological changes and also an important clue to to find the target organs of toxic effects (Huang *et al*., 2003). This experiment showed that the additions of 1.5-20% bentonite had no significant effect on rat organ index.

The influence of bentonite on the organ microstructure of rat organs: Bentonite is aluminosilicate compounds, mainly composed of SiO_2. Zhang *et al*. (2007) reported that SiO_2 dust showed obvious toxic effects on rat macrophages. Yu *et al*. (2008) reported that a small dose of SiO_2 dust have damage to the Chinese Hamster Lung fibroblasts (CHL) DNA. Li *et al*. (2009) also pointed out that the SiO_2 is harmful to the lungs of rats.

CONCLUSION

This study showed that the tissues of heart, liver, spleen and kidney organs had no significant pathological structural changes in the test groups. But the test groups had pulmonary emphysema at different degrees. In the groups adding with 3% bentonite, mphysema, alveolar wall rupture, bronchial epithelial hyperplasia were observed. Rats fed with 20% bentonite in their feed had lung hemorrhage. Therefore, there is a certain influence on the microstructure of rat lungs in the diet supplemented with bentonite, its safety needs to be further studied.

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