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Comparison of hematological and histological analysis of "Clinomon" and "Litovit-M" in mice

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ABSTRACT: Based on earlier studies we activated Mongolian Tsagaantsav zeolite by tribo-mechanical avtivation and utilized it as a food additive, giving it the name Clinomon. This research is devoted to investigation whether the supplementations of 2 levels (0.5%and 10%) of Clinomon and Litovit-M in the concentrate feed of mice have any effects on their hematological parameters and compare parameters of Clinomon and Litovit-M.

Keywords: Tsagaantsav zeolite, Clinomon, Litovit-M, clinoptilolite, dietary supplement , hematological parameters, histo-pathology analysis.

INTRODUCTION

Mongolia is rich in many types of mineral resources; zeolite which has unique properties is deservedly one of them. Zeolite from the Mongolian Tsagaantsav deposit has certain advantages like having similar chemical composition and physico-chemical properties to zeolite from the Russian Kholinskii deposit. Based on earlier studies we activated Mongolian Tsagaantsav zeolite by tribo-mechanical avtivation and utilized it as a food additive, giving it the name Clinomon. The aim of this research was to investigate whether the supplementations of 2 levels (0.5%and 10%) of Clinomon and Litovit-M in the concentrate feed of mice have any effects on their hematological parameters and compare parameters of Clinomon and Litovit-M. Clinoptilolite were added to the standard mouse ration. Four preparations differing in particle size were tested: grilled by vibration mill (VC), natural clinoptilolite (NC), tribomechanically micronized clinoptilolite by attritor mill, named Clinomon (MC) and Russian dietary supplement Litovit-M (LM). A total of 54 CBA strain mice were divided into 9 groups (n=6) and supplied with food containing 0.5% or 10% clinoptilolite powders. Two mice were sacrificed from each group after 10, 20 and 30 days, and blood samples from these mice were collected for hematological parameters and liver and kidney were obtained for histopathological analysis. The mice fed Clinomon and Litovit-M didn't lose weight. Liver and kidney histopathology were not affected. Clinomon, made by Mongolian Tsagaantsav deposit's clinoptilolite and Litovit-M, made by Russian Kholinskii deposit's clinoptilolite at the levels of 0.5% and 10% in the concentrates doesn't have any adverse effect on RBC, WBC and HGB. From these results, we concluded that there were no significant differences between Clinomon and Litovit-M on hematological and histological analysis. In further, we should study it in long term with large variants animals.

This paper was based on the animal welfare application of zeolite. We focused more on previous studies about biomedicine and the animal nutrition of zeolite. Various biomedical applications of natural zeolite. Clinoptilolite is non-toxic and safe for use in human and veterinary medicine. Animal fodder containing zeolites has been shown to increase biomass production in fisheries [1], to promote weight gain of chicken, swine and sheep, to improve the quality of animal products such as eggs or wool , to reduce bacterial contamination of the gut and to counteract harmful effects of ingested toxic substances [2]. In human medicine zeolites have been applied as anti-diarrheal remedies , for the external treatment of wounds and athlete foot, and for the removal of ammonia ions from kidney dialysates.[3] Recent experiments in vitro and in vivo have suggested that clinoptilolite could be used as an adjuvant in anticancer therapy. Croatian researchers and scientists have made an antioxidant dietary supplement, named Megamin, based on their long years of study and experiments with zeolite. In the 1970s, Japanese scientists had success in applying natural zeolite (clinoptilolite-containing tuff) in veterinary medicine for the prophylaxis and treatment of various diseases. Also Zeomic which contains silver ions with antimicrobial power into the three-dimensional alumino-silicate mineral structure of zeolite was recently approved in Japan as an antimicrobial agent for dental treatments . Some Russian publications deal with the utilization of zeolites for medical purposes in humans, particularly in the capacity of food additives. It was pointed out that biostimulant food additives which were created on the basis of natural minerals (zeolite clinoptilolite) with a proper ratio of vegetable constituents like rye, wheat, oats, devil's apron etc. and were represented by variable forms like powder, granules and pressed forms (drag), alleviate the activity of a number of diseases of the gastrointestinal tract, immune, endocrinological, nervous and cardiovascular systems . The majority of patients with various forms of gastrointestinal diseases (ulcer disease, chronic colitis due to disbacteriosis, some chronic persistent hepatitis), encephalomyelitis, neurosis, chronic fatigue syndrome and dystrophic arthritis, which had been treated with the zeolite-containing food additive "Litovit" presented with the general amelioration of the status as judged by subjective self-assessment . In particular, the patients had a better mood, their stool normalized, edema dropped, life activity rose, sleep and weight normalized as well. The patients considered all this to be a result of certain "purgation of the organism." "Litovit M" exerts a positive effect in the case of ulcerative stomatitis and streptoderma, as well as in the case of insect bites (arresting the edema, pruritus and pain) and posttraumatic haematomas, when applied topically. In Cuba inexpensive indigenous natural zeolites have been studied as buffers to reduce stomach acidity and to treat stomach ulcers [4], and made a new anti-diarrheal drug based on purified natural clinoptilolite – Enterex. [5] Zeolites can be used as drug carriers, and as adjuvants in anticancer therapy, dietetic supplements or antimicrobial agents as well for only medical field. For blood chemistry analysis, Maryin Kleiner reported that ingestion of powdered clinoptilolite-rich tuff, a natural ion-exchanger, caused a modest (20%) elevation of serum potassium in normal laboratory mice, without changes in other serum electrolytes or in indicators of kidney and liver function. P.D. Katsoulos, N.Roubles and N.Panousis have established that the long term supplementation of clinoptilolite at the levels of 1.25% and 2.5% in concentrates of dairy cows feeding doesn't have any adverse effect on packed cell volume (PCV), hemoglobin (HGB) and white blood cell (WBC) values. [6]

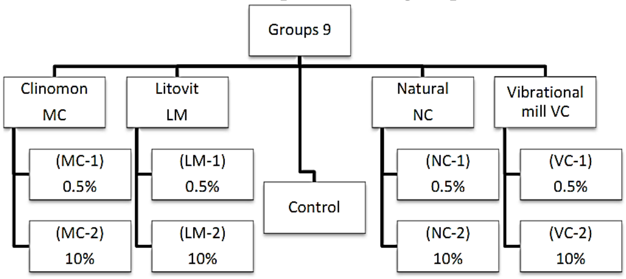
EXPERIMENTAL

Natural zeolite (clinoptilolite) is harvested from the countryside province deposit of Mongolian East and South-east sections (Tsagaantsav deposits, Dornogovi, Mongolia).

A fraction of clinoptilolite sample, sieved by diameter=0.1mm sieve was chosen for the investigations. About 5 kg homogenous sample was obtained. Four preparations differing in particle size (1-5μ) were tested: activated clinoptilolite with 1μ by attitor mill (MC) or (Clinomon), activated clinoptilolite with 3μ by vibrational mill (VC), natural clinoptilolite with 5μ (NC), and Russian clinoptilolite food additive with 1μ Litovit (LM).

CBA strain mice were 3-4 months old at the beginning of the experiment. A total of 54 normal mice were divided into 9 groups (Table 1) of six, assigned to nine food regimens: normal food (controls), food supplemented with 0.5% or 10% micronized clinoptilolite by attritor mill, MC-1 and MC-2 respectively, food supplemented with 0.5 or 10% micronized by vibrational mill, VC-1 and VC-2 respectively, food supplemented with 0.5 or 10% natural clinoptilolite, NC-1 and NC-2 respectively, and food supplemented with 0.5 or 10% Litovit-M LM-1 and LM-2 respectively. The mice were housed six per cage, with free access to food and water. All mice were obtained from the Public Health Institute.

Table 1. Experimental groups

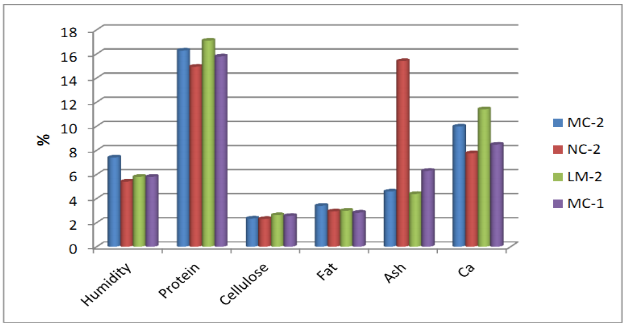


Clinoptilolite-containing preparations were added to the mouse ration which was tested in the laboratory of feed quality. See Table 2. The ration was powdered to enable addition of clinoptilolite powders. The mixture was made into dough by the addition of tap water. The daily allowance was 10g of pure ration per mouse which contains wheat, bran, bread, bone flour and fish flour (plus 0.5% and 10% clinoptilolite powder). See the compositions of per mouse ration in Table 2.

Table 2. Compositions and Ingredients of the daily ration per mouse

Compositions	*Wheat*	*Bran*	*Bread*	*Bone flour*	*Fish flour*	*Clinoptilolite*
Control	3g	2g	5g	0.2g	0.2g	-
0.5%	3g	2g	4.95g	0.2g	0.2g	0.05g
10%	3g	2g	3.96g	0.2g	0.2g	1.04g

Thus, the average daily supply of clinoptilolite was 0.5g and 1.04g per mouse. Rations were tested in laboratory of Nutrition Estimation and shown in picture 1.



Picture 1. shows that experimental mice were fed food which had necessary nutrients.

Peripheral blood samples and hematology. Two mice from each food were sacrificed 10, 20 and 30 days after the experiment started. Blood samples of 0.1mL (10-15 drops) were individually collected from the heart of mice into glass vials containing K3-EDTA as an anti-coagulant (Vacuum tube 1ml).

Collected blood samples were processed within 2 hours by means of an automated hematological analyzer (Sysmex XS-1000i and MINDRAY BC-2800, SYSMEX Corporation, Kobe, Japan) in the Laboratory Diagnosis of Gyals center. The following blood parameters were determined: total number of white blood cells (WBC), total number of red blood cells (RBC), and hemoglobin concentration (HGB).

Histopathology analysis. After collecting blood samples, kidneys and livers of experimental and control mice were placed into plastic containers containing 10% neutral buffered formalin, a fixative used to preserve tissue from degradation. As shown in Table 3, after overnight 24 hours washing by tap water, kidney and liver samples were dehydrated through a graded ethanol series, paraffin wax-embedded, cut in sections (7 μm) and mounted on slides with paraffin Canada balsam (LEICA

Table 3. Procedure of Histopathology

<table>
<tr><td colspan="6">Processing</td><td colspan="6">Embedding</td><td colspan="3">Sectioning</td><td>Staining</td></tr>
<tr><td colspan="2">24 hr</td><td colspan="4">Ethanol 18-24 hr</td><td colspan="3">Absolute 18-24 hr</td><td colspan="3">Xylene 15-30 min</td><td colspan="3">Paraffin 15-30min</td><td>HE</td></tr>
<tr><td>Procedures</td><td>Washing</td><td>70%</td><td>80%</td><td>90%</td><td>96%</td><td>ABS I</td><td>ABS II</td><td>ABSIII</td><td>Xylene I</td><td>Xylene II</td><td>Xylene III</td><td>Paraffin I</td><td>Paraffin II</td><td>Paraffin III</td><td>Microscope</td></tr>
</table>

EC-1160) (a) for histological assessment by light microscopy (Nikon eclipse E-600, Japan) (b) in the histopathology laboratory of the Veterinary Institute of the Mongolian Academy of Science.

RESULTS AND DISCUSSION

Body weight and food tolerance. Table 4 shows that the body weights of experimental and control groups measured 4 times during the experiment: before starting the experiment, 10, 20 days later, and at the end of the experiment (30 days). There were 3 pregnant female mice in MC-1 group.

Peripheral hematology parameters. Mean values and their standard deviations for RBC, WBC and HGB are presented in Table 5. Parameters of peripheral blood counts.

After 10,20 and 30 days, RBC and WBC values were unaffected by 0.5% or 10% supplementation of both Mongolian and Russian food additives, as there were not statistically significant differences between 5 groups in each individual samples as well ($p>0.5$) by student's test.

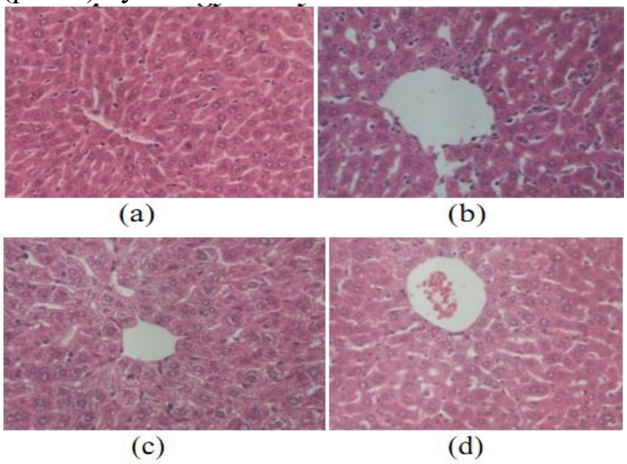


Figure 2. Liver histopahology of mice.

Table 4 Variation of body weights

	n	Control	MC-1	LM-1	MC-2	LM-2
		M±SD	M±SD	M±SD	M±SD	M±SD
Before (Mean)(g)	6	24.5±1.69	**26.0± 1.4**	26.4± 1.8	29.6± 1.6	29.8± 1.5
10 days (mean)(g)	6	26.5±1.1	**27.6± 1.3**	28.9± 1.6	31.8±1.9	31.8± 1.7
20 days (mean)(g)	4	29.1±1.2	**30.4±0.7**	31.2± 1.5	34.1± 0.8	33.8± 1.6
30 days (mean)(g)	2	32.1±0.9	**33.7± 1.33**	33± 0.2	37.2± 0.5	36.2± 0.63
Absolute wt gain (g)		7.6	**7.7**	6.6	7.6	6.4

Means and SD of mean RBC counts ($x10^{12}$/L), WBC counts ($x10^9$/L) and HGB concentrations (1g/dL) of blood samples of the five groups of mice that were evaluated 10, 20 and 30 days after starting the experiment. Mice were offered concentrates that were supplemented with either 0.5% Clinomon and Litovit-M (MC-1 and LM-1, respectively) or 10% Clinomon and Litovit-M (MC-2 and LM-2, respectively), where as those in Control were group fed normal rations.

Haematoxylin and eosin-stained paraffin-embedded liver sections from mice (a) in control group, (b) in MC-1, (c) in LM-1, (d) in MC-2, and (e) in LM-2 groups, respectively showed normal liver histology. He x400

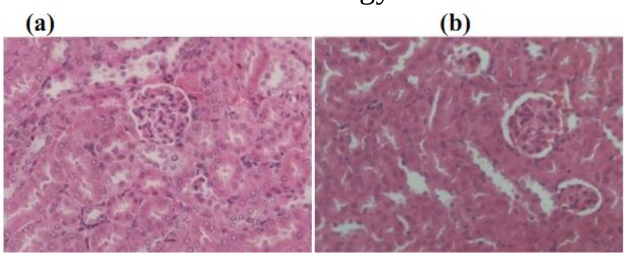


Figure 3. Kidney histopathology of mice

Table 5. Parameters of peripheral blood counts

Parameter	*Days of sacrifice*	*N*	*Control*	*LM-1 0.5%*	*MC-1 0.5%*	*LM-2 10%*	*MC-2 10%*	*References ranges*
RBC ($x10^{12}$/L)	10 20 30	2 2 2	9.39±1.2 8.94± 0.6 8.29±0.5	7.92±1.1 7.18±0.8 6.7±0.5	9.53±0.7 7.26±.5 8.75±0.7	9.96±0.6 9.19±0.5 9.67±0.7	9.67±0.4 8.57±0.5 9.46±0.6	7-11.5
WBC ($x10^{9}$/L)	10 20 30	2 2 2	3.87±0.7 6.96±0.4 11.5±0.6	11.0±0.5 6.65±0.6 5.45±0.3	10.55±0.5 4.45±0.7 2.1±0.5	6.15±0.2 6.4±1.1 6.5±0.8	4.44±0.8 5.1±0.6 5.5±0.6	2-11
HGB (x g/dL)	10 20 30	2 2 2	13.7±0.6 13.2±0.5 14.0±1.3	13.6±1.1 12.65±0.8 11.8±0.9	14.2±0.8 11.0±0.6 16.5±0.7	13.75±0.8 15.5±0.6 18.0±0.7	14.2±0.8 14.7±0.5 16.1±0.6	13-17

Kidney sections (hematoxylin and eosin staining) from mice in control (a) and experimental groups (b) showed normal kidney histology. HE x400. In the present research, a total of experimental and control mice weights were relatively different around 30% to 50% variability. The results show that all mice didn't gain much weight but they also didn't lose weights too. Therefore both zeolite clinoptilolite might be non-toxic. Clinoptilolite traps toxins and be excreted by feces. Clinoptilolite could act as its adsorbents in present experiment .Some authors used lower amounts of clinoptilolite in different durations. In studies on pigs, for example, Vrzgula *et al.* [7] used 5%, Pond and Yen [8] 3% and Ward *et al.* [9] 0.5% and Kyriakis et al., [10] 2%, and in chickens, Fethiere *et al*.[11] up to 0.16%, Dwyer *et al*. 1%, Kececi *et al*. 3 – 5 g/kg body weight and Olver [12] 50 g/kg body weight. None of the studies showed significant effects of clinoptilolite, either in the long term or during the monthly samplings on hematological parameters WBC, RBC and HGB.

In the present research, after 10,20 and 30 days, RBC and WBC values were unaffected by 0.5% or 10% supplementation of both Mongolian and Russian food additives, as there were not statistically significant differences between 5 groups in each individual samples as well ($p>0.5$). The present study provides the first evidence concerning the effect of the use of clinoptilolite Clinomon as a feed additive on the hematological parameters of mice.

Main conclusion the supplementation of Clinomon, made by Mongolian Tsagaantsav deposit's clinoptilolite and Litovit-M, made by Russian Kholinskii deposit's clinoptilolite at the levels of 0.5% and 10% in the concentrates doesn't have any adverse effect on RBC,WBC and HGB.

Liver and kidney histopathology were not affected. These data were corrected but require further experimental studies because no significant statistics sometimes notice small variants of experiments. In this studies, we should do experiment in long term it may be give us significant changes for doses. There were no significant differences between Clinomon and Litovit-M on WBC, RBC and HGB.

CONCLUSIONS

1. Have been investigated hematological parameters and other compare parameters of Clinomon and Litovit-M supplementations of 2 levels (0.5% and 10%) on experimental animal's blood.
2. Clinomon, made by Mongolian Tsagaantsav deposit's clinoptilolite and Litovit-M, made

by Russian Kholinskii deposit's clinoptilolite at the levels of 0.5% and 10% in the concentrates doesn't have any adverse effect on RBC, WBC and HGB.

3. It is proved that no significant differences between Clinomon and Litovit-M on hematological and histological analysis.

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