

Prolonged HUMET_e-R feeding in the rat

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The aim of study was to test the effect of a long-term feeding with 2.5, 7.5, 25, 75 and 250-fold (calculated for kg b.w.) of the human daily dose (about 2 mg/kg b.w.) of the syrup-free HUMET_e-R on a) rat mortality; b) weight changes; c) organ weights; d) haematopoiesis; and e) chemical data in test groups of 10 rats each.

Major methods: Standard rat fodder (Code 624, LATI, Gödöllő) was ground and added to syrup-free HUMET_e-R (HORIZON-MULTIPLAN Ltd.) in quantities calculated for the humic acid content of the preparation. After homogenisation the mixture was dried, press granulated, then dried again at room temperature. Taking a daily food consumption of 20 g/day per rat, the daily doses corresponded to 5, 15, 50, 150 and 500 mg/kg b.w. calculated for the humic acid content. After a one-week preliminary period this fodder was administered for 4 weeks.

Of blood sampled under ether anaesthesia, blood cell counts (Medicor, Hungary, automatic models PHA-1 and PHA-2) and chemical properties were determined (Hitachi, Japan, automatic model 717). Body mass was measured weekly, organ masses at the end of the study.

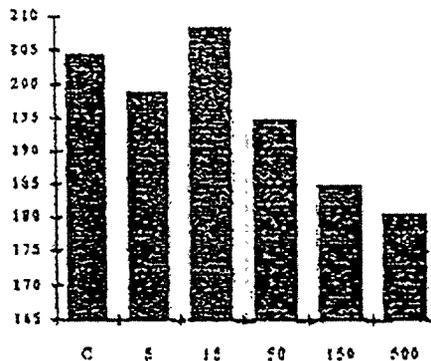


Fig. 6.1: Effect of a 4-week feeding of syrup-free HUMET_e-R on the body mass of the rat

(ten animals each per group, C = Control).

Ordinate: Body mass means in gram, which did not differ at the beginning of the study. The abscissa shows the quantity of trace-element-enriched and syrup-free humic acid product consumed daily (mg/kg b.w.). The tested daily doses corresponded to 2.5 - 7.5 - 25 - 75 - 250-fold human doses (about 2 mg/kg) calculated for mass unit. By the end of the 4-week HUMET_e-R feeding with the doses corresponding to 75 and 250-fold of the human dose, weight gain was less ($P < 0.05$) than in the untreated controls.

Results:

- a) All animals survived.
- b) By the end of the third week the animals treated with 150 and 500 mg/kg b.w. (75x and 250x calculated human dose) had lost appetite and
- c) at the end of week 4 their body and organ weights were lower than those of the untreated controls (Fig. 6.1).
- d) Haematological or chemical data (erythrocyte, platelet and white blood cell counts, Hgb, MCV, MCHC, serum iron, transferrin, glucose, creatinine, total protein, albumin, GOT, GPT, HBDH, LDH, CK, alkaline phosphatase, amylase, Ca, P, cholesterol, triglycerides) showed no difference between the treated and untreated groups.

Conclusion:

Prolonged administration of the trace element enriched humic acid preparation HUMET_®R had no toxic effects even in a dosage containing 25 times the amount of the recommended human daily dose. Weight gain was stopped by exceptionally high pharmacological doses only.

HUMET-R: EFFECT OF PROLONGED ORAL DOSE ON RATS

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We studied the effect of the prolonged feeding of HUMET-R containing food to rats; its effect on whole body and organ weights, and on hematological and clinical-chemical parameters.

Material and Method**Test animals**

Random-grouped, 150 ± 17 g female Wistar rats were used for the experiment. The animals were kept in a room of constant temperature and humidity, and alternating illumination. Normal or HUMET-R enriched granuled food and tap water was administered ad libitum. The rats were housed in Type II plastic cages (5 animals/cage). General physical condition was monitored on daily basis, and eventual mortalities were recorded.

Compounds

HUMET-R (HR) was manufactured and supplied to us by the Client. Humic acid enriched food was prepared from HR supplied to us, as a function of the active substance content. Standard rat food (Code 624, LATI, Gödöllő) was ground, and HR was added. After homogenization, it was granulated, and dried at room temperature. In consideration of the feed applied (20 g/day), the daily HR consumption by the animals was determined as 1, 3, 10, 30 and 100 mg HR/200 g body weight/day.

Hematological studies

At the end of the experiment, the animals were ether anesthetized, the abdominal aorta was penetrated, and blood samples were taken into plastic syringes. Blood samples were distributed into chemical test tubes.

From the blood samples, complete blood counts were tested, and quantitative assays were made by MEDICOR-PHA-1 and PHA-2 automatic devices, and by HITACHI 717 analyser.

Determination of whole body and organ weights

Weight changes were recorded 1 week before starting the experiment, then weekly for 4 weeks.

At the end of the study, after blood sample taking, the lungs, heart, spleen, liver and kidneys were removed, resultant macroscopic alterations were recorded, and organs were weighed.

Groups were acclimatized to laboratory conditions 1 week before starting the treatments. Following treatments were performed:

Control	10 animals
Continuous feeding for 4 weeks, with 1 mg/200g b.w. HR enriched food	10 animals
Continuous feeding for 4 weeks, with 3 mg/200g b.w. HR enriched food	10 animals
Continuous feeding for 4 weeks, with 10 mg/200g b.w. HR enriched food	10 animals
Continuous feeding for 4 weeks, with 30 mg/200g b.w. HR enriched food	10 animals
Continuous feeding for 4 weeks, with 100 mg/200g b.w. HR enriched food	10 animals

Control and test groups were arranged one week before the start of the study. All animals received normal food for one week, then Groups 2-6 consumed different doses of HUMET-R.

Control (Group 1) showed continuous weight gain during the 5 weeks of the experiment. In the HR-groups (Groups 2-6), a trend similar to the controls was observed until Day 21 of the study. In Groups 5 and 6 (30 and 100 g/200mg b.w. HUMET-R, resp.) weight loss was seen from Week 3 on. A possible cause of this change is that the quantity of certain trace elements in HUMET-R (e.g. copper) may influence the health of the animals, which leads to decreased food consumption and thereby weight

The effect of 4-week-long feeding on weight gain is shown in Fig. 1.

no significant changes were found between white blood cell, red blood cell and platelet counts of controls (Group 1), and HR groups (Groups 2-6). Practically the same applies to hematological and chemical parameters as well. We underline that no changes were seen even in the serum calcium level in the highest dose group (100 mg/200g b.w./day).

Most tested chemical parameters and enzymes were similar to the controls in each test group.

and conclusions

Results with test animals fed with HR-enriched food permanently for 4 weeks are summarized as follows:

HR humic acid product did not influence the general physical condition (motility, behavior, etc.) and some other biological parameters (whole body and organ weights, hematological and chemical values) of animals even with prolonged exposition to oral HR-food.

Significant differences were observed in the 30 and 100 mg/200g b.w./day groups, where the animals lost appetite 3-4 days after the start of consuming HR-food, and this resulted in marked weight loss. The experimental data of test animals obviously proved that permanent exposition to HR-food caused mortality during the study period.

The comparative study of hematological and chemical parameters showed that there was no significant difference between the controls and the different test groups.

March 30, 1994

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