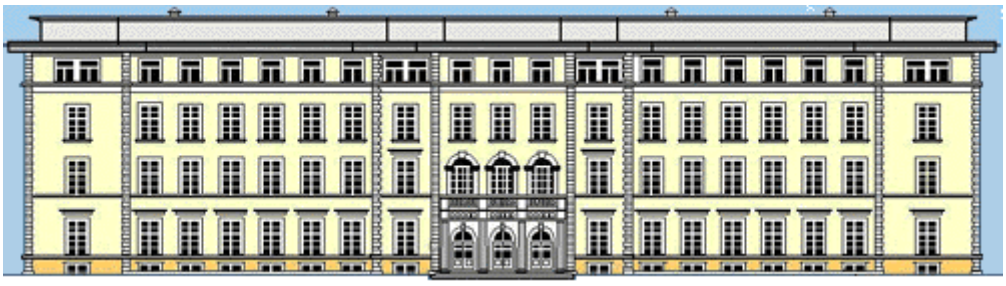
MEDICAL UNIVERSITY – SOFIA

FACULTY OF PHARMACY

DEPARTMENT OF PHARMACOLOGY, PHARMACOTHERAPY AND TOXICOLOGY



RESEARCH PROJECT REPORT

"EVALUATION OF THE HYPOLIPIDEMIC ACTIVITY OF LYZME-5® IN WISTAR RATS"

Sofia

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MATERIALS AND METHODS; EXPERIMENTAL DESIGN

Experimental animals

96 male Wistar rats were supplied from the National Breeding Centre at the Bulgarian Academy of Sciences (Slivnitsa, Bulgaria) and were housed in a controlled environment: temperature 20-22°C, free access to food (either standard food or a 1% cholesterol enriched formula)and water, 12 h alternating light and dark cycles, at the Animal Care facility of the Faculty of Pharmacy, MU-Sofia.

The animals were randomly distributed in sixteen treatment groups, as follows:

1. Untreated control (sacrificed after 4 weeks) (6 animals);
2. Untreated control (sacrificed after 5 weeks) (6 animals);
3. Untreated control (sacrificed after 6 weeks) (6 animals);
4. Untreated control (sacrificed on the 31st day) (6 animals);
5. Positive control (HCD) (sacrificed after 4 weeks) (6 animals);
6. Positive control (HCD) (sacrificed after 5 weeks) (6 animals);
7. Positive control (HCD) (sacrificed after 6 weeks) (6 animals);
8. HC D animals, treated with Lyzime (1 ml/kg/day) (sacrificed after 4 weeks) (6 animals);
9. HC D animals, treated with Lyzime (1 ml/kg/day) (sacrificed after 5 weeks) (6 animals);
10. HC D animals, treated with Lyzime (1 ml/kg/day) (sacrificed after 6 weeks) (6 animals);
11. HC D animals, treated with Lyzime (0.5 ml/kg/day) (after 4 weeks) (6 animals);
12. HC D animals, treated with Lyzime (0.5 ml/kg/day) (sacrificed after 5 weeks) (6 animals);
13. HC D animals, treated with Lyzime (0.5 ml/kg/day) ((sacrificed after 6 weeks) (6 animals);
14. HC D animals, treated with Lyzime (0.25 ml/kg/day) (sacrificed after 4 weeks) (6 animals);
15. HC D animals, treated with Lyzime (0.25 ml/kg/day) (sacrificed after 5 weeks) (6 animals);
16. HC D animals, treated with Lyzime (0.25 ml/kg/day) (sacrificed after 6 weeks) (6 animals);

Treatment

The experiments were carried out in accordance with the requirements of the European Convention for Protection of Vertebrate Animals used for Experimental and other Specific Purposes (1991). Healthy, pathogen free male Wistar rats were used in this study, whereby every experimental group consisted of 10 animals. The exposure to the 1% cholesterol-enriched diet (HCD) was commenced 2 weeks prior to the treatment with either Lyzime or purified water (in the control groups). The treatment was carried out using a gastric tube and the daily dose was divided in two administration carried out at 10.00 a.m. and 04.00 p.m. The animals were treated for 4,5 or 6 weeks and sacrificed accordingly.

Serum lipid measurements

Animals were sacrificed, blood samples were collected via cardial punction and thereafter the serum fractions were isolated. The determination of serum lipids levels was carried out in the Higher Institute of Veterinary Medicine, using standard methods. These tests included total cholesterol (TC), low-density lipoprotein holesterol, (LDL-C), high-density lipoprotein cholesterol, (HDL-C), triglycerides (TG). The TC/HDL ratio as well as the atherogenic indices ((TC - HDL-C)/HDL-C) were determined as well.

Post-mortal evaluation

After collecting the blood samples the carcasses were necropsied by a qualified vet surgeon, and the visceral organs (liver, spleen, stomach, intestines) were examined for gross signs of toxicity. Moreover the animal body mass was monitored on regular basis as a non-specific marker of general toxicity.

Data processing and statistics

The results from lipid level investigations were statistically evaluated using a paired Student's t-test and post hoc Dunnet test, using BMD P4V, BMD P3D and BMD P7D software.

EXPERIMENTAL RESULTS

As evident form the presented data (**Tables 1-3**; **Figs 1-3**), exposure to the HCD food was consistent with a significant increase in total cholesterol and LDL-C as compared to the rats fed standard diet.

Lyzime® treatment was consistent with a strong, statistically significant protection of animals against the hyperlipidemic effects of the cholesterol enriched diet. The effect generally was dose-dependent and especially prominent after 5 and 6 weeks treatment.

A characteristic feature of the biological activity of Lyzime was the striking lowering of the LDL cholesterol especially following longer treatment periods of 5 weeks or more. These favorable hypolipidemic effects were more pronounced at the higher dose levels of 1 ml/kg/day or 0.5 ml/kg/day.

Throughout the study period there was neither mortality nor alteration in the feeding behavior of treated animals as compared to the untreated controls. The post mortem examination of the visceral organs failed to reveal any signs of toxic deleterious effects in the treatment groups, as compared to the controls. Moreover the exposure of animas to Lyzime® caused no alterations in the weight gain rates of treated vs untreated animals.

Taken together these findings indicate that Lyzime® exerts prominent modulating effects on serum lipids in a model of high-cholesterol diet induced dislipidemia in the rat. At the same time the formula is virtually devoid of gastric mucosa irritating or general toxic effects within the tested dose intensity range and within the studied exposure period.