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# EFFECT OF VETOM 21.77 PREPARATION
## ON SERUM BIOCHEMICAL PATTERN IN MICE

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**SUMMARY**

It is known that predatory *Hyphomycetes* fungi play an important role in measures taken for helminthic infestation prevention. There is little evidence on properties of compounds contained in predatory fungi and their effect on animals. Results of tests of Vetom 21.77, new microbiological preparation based on *Duddingtonia flagrans,* for its effect on serum biochemical pattern of laboratory mice are presented. The preparation was administered to the animals at the dose of 2, 5, 50 and 300 µl/kg of body weight. The animals were examined for tested parameters prior and 2 and 7 days after daily administration of the preparation. The parameters remained within the normal physiological limits in all test group as well as in control group not given the preparation. The test results indicated safety of the said microbiological preparation. Significant increase in total protein and albumin contents was recorded in mice given the maximum dose of the preparation as compared with those of control group.

Key words: Vetom, biochemical pattern, mice, *Duddingtonia flagrans*.

## INTRODUCTION

Among natural regulators of helminth populations, *Hyphomycetes* fungi, which can be carnivorous towards parasites, can raise a certain interest. *Hyphomycetes* are the subject of numerous research studies, still being done by scientists from many countries. The advantages of the use of these fungi are believed to include their presence practically in all parts of the world and all climate zones, for example [5]. According to the available publications, these nematophagous fungi, if found in the common microenvironment, can produce traps [12], which are able not only to grab nematodes, but also to adhere to them and thus spread in the parasitic habitat [9]. Another advantage of these predators is safety for the host organism [7].

One of the most effective *Hyphomycetes* is carnivorous *Duddingtonia flagrans* fungus, *Orbiliaceae* family [10, 11]. As shown by the results of different studies, the fungi are effective not only against parasites; for example the use of *D. flagrans* isolate together with *Saccharomyces cerevisiae*, improved milk performance in cows, at the same time decreasing the worm burden [6].

The lipid composition of all *Arthrobotrys* species is presented by triglycerides up to 80% in content, including palmitic, oleic and linoleic acids; sterols make up about 12% of lipids in these fungi, which are, apparently, necessary for the production of latex-like viscous and adhesive substances, accumulated on the surface of predaceous mycelium trapping rings [1]. This fact suggests possible changes in lipid and protein metabolism biochemical values of mice, fed with *D. flagrans* spore mass.

Elevated levels of calcium, potassium and phosphorus, revealed by T.V. Teplyakova using x-ray microanalysis in predaceous mycelium (according to the author's hypothesis, calcium is involved into cell contractions during predation processes) [4], raised certain interest and inspired to include these electrolytes into the list of parameters under study.

In general, due to the lack of available scientific publications on the properties of compounds, found in carnivorous fungi (like sesquiterpenes and phospholipases) and influencing animal organisms, it was decided to cover the greatest possible range of biochemical parameters in this study.

The aim of the work was to study the effect of Vetom 21.77 microbiological drug, containing *D. flagrans* spore and mycelial mass, on serum biochemical profile of laboratory mice.

## MATERIALS AND METHODS

The scientific experiment was carried out in the Scientific and Research Veterinary Laboratory of the Agricultural Technological Park within the State University named after Shakarim (Semey, Kazakhstan).

The drug under study, Vetom 21.77, is a freeze-dried isolate of apathogenic nematophagous *D. flagrans* fungus formulated in a dry immobilization matrix with prebiotic, adsorbing and anti-toxic properties.

The sera biochemical parameters were analyzed in 105 19.8 ± 0.3 g nonlinear mice. Five groups of animals (one control and four test groups), 20 animals per group, were formed. Five more mice were used for biochemical analysis, performed before the trial started. The drug at the doses of 2, 5, 50 и 300 µl/kg of weight was given to test animals and was not given to the control animals. The parameters under study were checked before the test and two and seven days post everyday use of the abovementioned doses in test animals.

Mice were examined every day, data about their appearance, behavior, appetite and body weight fluctuations were registered in individual animal cards.

Blood was taken every day at the same time using common techniques [2, 8]. During the trial, blood was collected immediately from the myocardium after humane killing from 5 mice before the trial and 10 mice from each group on Days 2 and 7.

**Table 1**
**Laboratory Animal Blood Biochemical Parameter Values Before Experiment**
**($n = 5, x \pm \mu$)**

| Parameter | Result | Norm |
|---|---|---|
| Total protein (g/l) | 48.60 ± 0.24 | 43–64 |
| Albumen (g/l) | 22.93 ± 0.18 | 20–47 |
| BUN (mmol/l) | 5.04 ± 0.08 | 4.3–10.0 |
| Total bilirubin (mg/dl) | 0.21 ± 0.03 | 0.1–0.9 |
| ALT (IU/l) | 49.20 ± 0.66 | 26–120 |
| AST (IU/l) | 120.0 ± 1.3 | 69–191 |
| Glucose (mmol/l) | 7.98 ± 0.09 | 5.9–15.4 |
| Phosphorus (mmol/l) | 2.20 ± 0.03 | 2–4 |
| Calcium (mmol/l) | 2.48 ± 0.03 | 2.3–3.0 |
| Potassium (mmol/l) | 6.98 ± 0.05 | 5–9 |
| Sodium (mmol/l) | 152.06 ± 1.30 | 147–167 |
| Total cholesterol (mmol/l) | 3.31 ± 0.03 | 1.6–4.5 |
| Creatinine (mmol/l) | 0.05 ± 0 | 0.04–0.07 |

Serum biochemical test was performed using Minitecno semi-automated analyzer (I.S.E. S.r.l., Italy) and Stat Fax 3300 semi-automated biochemical analyzer (Awareness Technology, USA). The following biochemical blood parameters were tested: total protein (g/l), albumen (g/l), BUN (mmol/l), total bilirubin (mg/dl), ALT (IU/l), AST (IU/l), glucose (mmol/l), phosphorus (mmol/l), calcium (mmol/l), potassium (mmol/l), sodium (mmol/l), total cholesterol (mmol/l), creatinine (mmol/l).

The test results obtained were processed using StatsDirect 3.1.15 (StatsDirect Ltd, UK) software. The data sets were compared using Mann — Whitney U-test. The differences were statistically significant at $p < 0.05$level of significance.

## RESULTS AND DISCUSSION

In the course of the experiment, 100%-livability of test animals was registered. The physiological conditions of mice in control and test groups were within the normal ranges.

The values of basic biochemical blood parameters obtained before the trial started are shown in Table 1. It was established that all parameter values were within acceptable normal limits, set for laboratory mice.

Values of test animal blood biochemical parameters after two days of the drug use also remained within physiological normal limits. The statistical significance level of differences between non-exposed control and test groups was not achieved (Table 2).

On Day 7 total protein values in animal blood from Groups 1 and 2 were 0.4% higher than the ones in the Control Group; the same value was 2% higher in Group 3; and in Group 4, in which animals received the drug at the dose of 300 µl/kg, was 2.8% higher ($p < 0,05$), that is more than a statistically significant control value (Table 3).

The increase of this value suggests intensification of protein metabolism with predominant anabolic processes in test mice [3].

Albumen fraction was also significantly higher (1.9%) than a control one in Group 4 ($p < 0,05$). Group 1 demonstrated values, which were 0.2% higher, for Groups 2 and 3 they were 0.4 and 1.7% higher, respectively.

A consistent increase in BUN content in sera of all test animals was observed at this stage of the trial, 0.4 – 2.7% compared to control animals, depending on the amount of the drug delivered. It is known that BUN and creatinine are the products of amino acid metabolism [13].

**Table 2**
**Laboratory Animal Blood Biochemical Parameter Values on Experimental Day 2**
**($n = 50, x \pm \mu$)**

| Parameter | Test result | | | | | Norm |
|---|---|---|---|---|---|---|
| | Control | Test Group 1 (2 µl/kg) | Test Group 2 (5 µl/kg) | Test Group 3 (50 µl/kg) | Test Group 4 (300 µl/kg) | |
| Total protein (g/l) | 48.80 ± 0.2 | 48.80 ± 0.37 | 48.80 ± 0.37 | 49.20 ± 0.37 | 49.20 ± 0.37 | 43–64 |
| Albumen (g/l) | 23.16 ± 0.11 | 23.18 ± 0.11 | 23.17 ± 0.13 | 23.29 ± 0.07 | 23.43 ± 0.09 | 20–47 |
| BUN(mmol/l) | 5.09 ± 0.07 | 5.12 ± 0.05 | 5.10 ± 0.08 | 5.11 ± 0.05 | 5.12 ± 0.07 | 4.3–10 |
| Total bilirubin (mg/dl) | 0.22 ± 0.02 | 0.21 ± 0.01 | 0.20 ± 0.02 | 0.21 ± 0.02 | 0.21 ±0.02 | 0.1–0.9 |
| ALT (IU/l) | 49.60 ± 0.51 | 49.60 ± 0.68 | 49.80 ± 0.49 | 49.60 ± 0.75 | 49.80 ± 0.58 | 26–120 |
| AST (IU/l) | 120.80 ± 1.43 | 121.2 ± 1.5 | 121.60 ± 1.81 | 122.00 ± 1.87 | 122.60 ± 1.44 | 69–191 |
| Glucose (mmol/l) | 8.02 ± 0.05 | 8.00 ± 0.05 | 7.97 ± 0.06 | 7.99 ± 0.09 | 7.98 ± 0.07 | 5.9–15.4 |
| Phosphorus (mmol/l) | 2.21 ± 0.02 | 2.19 ± 0.03 | 2.21 ± 0.04 | 2.20 ± 0.04 | 2.21 ± 0.03 | 2–4 |
| Calcium (mmol/l) | 2.50 ± 0.04 | 2.47 ± 0.06 | 2.49 ± 0.03 | 2.52 ± 0.03 | 2.51 ± 0.05 | 2.3–3.0 |
| Potassium (mmol/l) | 7.00 ± 0.06 | 6.99 ± 0.03 | 7.00 ± 0.05 | 7.00 ± 0.04 | 7.02 ± 0.04 | 5–9 |
| Sodium (mmol/l) | 151.67 ± 0.71 | 151.45 ± 0.84 | 152.48 ± 1.01 | 151.46 ± 0.97 | 152.38 ± 0.75 | 147–167 |
| Total cholesterol (mmol/l) | 3.34 ± 0.03 | 3.33 ± 0.04 | 3.30 ± 0.04 | 3.31 ± 0.05 | 3.32 ± 0.03 | 1.6–4.5 |
| Creatinine (mmol/l) | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.04–0.07 |

**Table 3**
**Laboratory Animal Blood Biochemical Parameter Values on Experimental Day 7**
**($n = 50, x \pm \mu$)**

| Parameter | Test result | | | | | Norm |
|---|---|---|---|---|---|---|
| | Control | Test Group 1 (2 µl/kg) | Test Group 2 (5 µl/kg) | Test Group 3 (50 µl/kg) | Test Group 4 (300 µl/kg) | |
| Total protein (g/l) | 49.2 ± 0.2 | 49.4 ± 0.24 | 49.40 ± 0.24 | 50.20 ± 0.58 | 50.60 ± 0.51* | 43–64 |
| Albumen (g/l) | 23.5 ± 0.1 | 23.54 ± 0.15 | 23.64 ± 0.10 | 23.90 ± 0.17 | 23.95 ± 0.10* | 20–47 |
| BUN (mmol/l) | 5.16 ± 0.11 | 5.18 ± 0.07 | 5.19 ± 0.06 | 5.22 ± 0.08 | 5.30 ± 0.09 | 4.3–10 |
| Total bilirubin (mg/dl) | 0.21 ± 0.02 | 0.22 ± 0.03 | 0.22 ± 0.03 | 0.21 ± 0.02 | 0.22 ± 0.02 | 0.1–0.9 |
| ALT (IU/l) | 50.20 ± 0.37 | 50.60 ± 0.51 | 50.60 ± 0.51 | 51.00 ± 0.45 | 51.00 ± 0.55 | 26–120 |
| AST (IU/l) | 123.00 ± 1.26 | 123.20 ± 1.07 | 123.60 ± 1.25 | 124.40 ± 1.21 | 124.60 ± 1.17 | 69–191 |
| Glucose (mmol/l) | 8.00 ± 0.11 | 7.99 ± 0.08 | 7.97 ± 0.07 | 7.88 ± 0.07 | 7.82 ± 0.08 | 5.9–15.4 |
| Phosphorus (mmol/l) | 2.21 ± 0.04 | 2.20 ± 0.04 | 2.24 ± 0.03 | 2.22 ± 0.03 | 2.24 ± 0.05 | 2–4 |
| Calcium (mmol/l) | 2.52 ± 0.03 | 2.53 ± 0.03 | 2.55 ± 0.03 | 2.57 ± 0.03 | 2.58 ± 0.03 | 2.3–3.0 |
| Potassium(mmol/l) | 7.06 ± 0.05 | 7.01 ± 0.03 | 7.04 ± 0.04 | 7.10 ± 0.07 | 7.08 ± 0.05 | 5–9 |
| Sodium (mmol/l) | 151.74 ± 0.72 | 151.67 ± 1.18 | 152.30 ± 1.33 | 151.83 ± 0.87 | 152.27 ± 0.92 | 147–167 |
| Total cholesterol (mmol/l) | 3.31 ± 0.03 | 3.29 ± 0.04 | 3.30 ± 0.05 | 3.25 ± 0.04 | 3.23 ± 0.07 | 1.6–4.5 |
| Creatinine (mmol/l) | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.04–0.07 |

* $p < 0,05$.

As for electrolytes, a relatively stable picture was observed.

## COCNCLUSION

Based on the results of the trial, it was established that when using Vetom 21.77 at the doses of 2, 5, 50 and 300 µl/kg, biochemical parameters of mice sera remained within physiological normal limits both in test and control groups. At the end of the study statistically significant ($p < 0,05$) increase in total protein and albumen concentrations in blood were registered in Group 4 animals. These biochemical parameters were higher, compared to analogous values in control animal sera. Clinical significance of such results may be doubted, but it is still possible to suggest the tendency in biochemical profile improving if appropriate doses of this microbiological drug are used.

Blood parameters of all animals changed following the same pattern within physiological normal ranges adequately to their physiological status. In the course of the scientific experiment, no inflammatory or allergic reactions, associated with the drug use, were reported.

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