

Current Issues in Pharmacy and Medical Sciences

Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA, SECTIO DDD, PHARMACIA

journal homepage: <http://www.curipms.umlub.pl/>



Investigation of the hepatoprotective properties of “Phytovenol” capsules on the model of medicamentous hepatitis in rats

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ARTICLE INFO

Received 07 November 2014
Accepted 12 December 2014

Keywords:

liver,
hepatitis,
“Phytovenol” capsules,
hepatoprotective action.

ABSTRACT

This scientific paper is an investigation of the pharmacological activity of the new medical preparation “Phytovenol” (capsules in the dose of 150 mg/kg) on the pattern of hepatitis in rats induced by paracetamol administration. The authors of the article, for the first time under experimental conditions, detected a hepatoprotective effect that was brought about by “Phytovenol” capsule administration. This is realized due to the antioxidant, antitoxic and anticytolytic activity of the examined medicine. The study found that its efficacy is not lower than the classical hepatoprotector – “Silibor” tablets. The results obtained suggest that it is possible to extend the range of available hepatoprotectors and improve the quality of treatment for liver diseases, by introducing “Phytovenol” capsules into medical practice.

INTRODUCTION

In recent years, a considerably increasing number of people are found to be afflicted with liver diseases. Among these, toxic lesions of the liver constitute 9 % of all cases. The problem comes about by unfavorable ecological situations, the wide (too common) use of chemical toxins, alcohol abuse, polypragmasy in medical practical work and other stressful factors on the central metabolic organ – the liver [7].

The use of hepatoprotective medicines with antioxidant action correcting metabolic disorders and increasing antitoxic liver properties are pathologically substantiated in pharmacotherapy [7]. Considering the tendency of transformation of liver diseases into chronic pathological processes and the difficulties in choosing suitable medicines for the treatment of severe liver diseases, an adequate pharmacological regulation of pathological process can be ensured by the medicines possessing a pronounced effect that is without negative influence in case of their long-term administration. Medicines of a plant origin having a wide range of therapeutic doses, an ability to actively positively influence afflicted membranous-cellular structures and metabolic processes, a high biological availability, a mild action upon the body and an ability to bring about physiological correction of disturbed functions,

low toxicity and allergen capacity - meet all these requirements [7].

The objective of our research was to study the hepatoprotective properties of a new medicine developed at the National Pharmaceutical University (Kharkiv, Ukraine). This is “Phytovenol”. Phytenol comes in a capsule form, and is a ground mixture of the following medicinal plants: horse chestnut fruits, leaves of *Hamamelis virginiana*, seeds of *Avena sativa*, fruits of *Sophora japonica*, the *Centaurium vulgare* herb, the *Bistorta officinalis* herb, and the *Melilotus officinalis* herb [5]. The biologically active substances (polyphenol, flavonoids, saponins, lectins, polysaccharides, polyvitamins, ecdysteroids etc.) contained in these medicinal plants are known to possess an integrated anti-inflammatory, bile expelling, demineralizing, antiseptic, spasmolytic, capillary strengthening and antibacterial action. In addition, they are known to intensify the disintoxication liver function, normalize the gallbladder tonus [5], and play an important role in the treatment of the hepatic-biliary system. Hence, the effects and low cost of the medicament substantiate the reason to perform the given study.

MATERIALS AND METHODS

The research was conducted on outbred white rats with simulated acute liver affliction brought about by means of

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the administration of paracetamol (acetaminophen), known for its toxic action on the liver [6]. The animals were placed within four groups: the 1st one – negative control, the 2nd one – positive control (observed pathology – untreated hepatitis), the 3rd and 4th – experimental groups. The animals of the negative and positive control were given in subcutaneous injection, purified water in a quantity corresponding to the body weight, over the course of the experiment. The rats of the experimental groups were administered with intragastric “Phytovenol” capsules in the dose of 150 mg/kg (the 3rd group) and a comparative medicine (reference drug) – “Silibor” tablets (Pharmaceutical company “Health”, Ukraine) in the dose of 35 mg/kg (the 4th group) during 30 days, in the preventive-therapeutic regime. “Phytovenol” capsules were given in a conditionally therapeutic dose with membrane-stabilizing activity fixed in the previous studies [11]. The dose of the comparative drug was calculated by means of its specific stability coefficient, as suggested by Yu.R.Rybolovliev, on the basis of the daily human dose [8]. Five days before the end of the experiment, the rats of the positive control group and the experimental groups were given the hepatotoxin, paracetamol, intragastrically (“Stirol-biopharm”, Ukraine) in the dose of 2,5 g/kg in a 2% starch paste. The experimental groups were given paracetamol one hour before administration of the medicines being examined [9].

The development of pathology and the pharmacological activity of the medicines was estimated by the detection of mass coefficient of the liver (MCL), as well as by biochemical bile indices (rate of bile secretion, cholesterol level, bile acids level), liver homogenate (the level of TBA-reactive compounds, diene conjugates (DC), reduced glutathione (GSH)) and the blood serum (the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), the level of general lipids and cholesterol) by means of the common methods [1,2,9].

All the experiments were conducted according to the requirements of the EC Directions 86/609/EEC November 24, 1986 concerning care and use of laboratory animals.

While applying the method of mathematical statistics, the significance level of $p < 0,05$ was accepted. To draw statistical conclusions during the comparison of statistical sampling of relative variables after single-factor disperse analysis (Kruskal-Wallis criterion for the data that do not correspond to the regular distribution law) of the detected differences between the experimental groups, Neumann-Keils and Mann-Whitney criteria were used. To conduct mathematical calculations, a standard set of statistical programs, Statistica 6.0, was employed [10].

RESULTS

The conducted experiments detected (Table 1) that paracetamol administration to the animals of the positive control group resulted in a 1,5 times increase of MCL, as compared with the corresponding index in the group of negative control. ALT activity in the blood serum of the positive control group was twice as in the negative control group, AST was 1,2 times higher, ALP was 1,4 times higher, the level of general lipids was 1,6 times higher,

and cholesterol was 1,5 times higher. The same group, in comparison with the group of negative control, demonstrated a 1,8 times decrease of bile secretion rate, and a 1,5 times decrease of cholesterol and bile acids content. In the liver homogenate, the content of DC and TBA-reactive compounds were found to be in 2,5 and 1,8 times increase, respectively, against the ground of a 1,2 times GSH decrease, as compared with the animals of the negative control group.

Table 1. The effect of “Phytovenol” capsule administration on functional-biochemical indices of induced acute hepatitis in rats engendered by paracetamol ($M \pm m$, $n=6$)

| Indices | Conditions of the experiment | | | |
|-----------------------------------|------------------------------|------------------------------|-----------------------------------|-------------------------------|
| | Negative control | Positive control (hepatitis) | Hepatitis + “Phytovenol” capsules | Hepatitis + “Silibor” tablets |
| MCL | 3,40±0,15 | 5,17±0,16 ¹ | 4,17±0,33 ² | 3,93±0,37 ² |
| Blood serum | | | | |
| ALT, mmol/(L×hour) | 0,49±0,02 | 1,00±0,06 ¹ | 0,63±0,07 ² | 0,62±0,07 ² |
| AST, mmol/(L×hour) | 0,60±0,01 | 0,72±0,04 ¹ | 0,66±0,07 ² | 0,66±0,06 ² |
| ALP, mcmol/(s×L) | 1,10±0,08 | 1,52±0,03 ¹ | 1,11±0,03 ² | 1,08±0,03 ² |
| General lipids, g/L | 1,85±0,10 | 2,99±0,17 ¹ | 1,98±0,10 ² | 2,06±0,11 ² |
| Cholesterol, mmol/L | 1,42±0,06 | 2,12±0,15 ¹ | 1,59±0,14 ² | 1,66±0,04 ² |
| Bile | | | | |
| Bile secretion rate, mg/min/100 g | 6,74±0,36 | 3,84±0,55 ¹ | 6,37±0,41 ² | 6,60±0,41 ² |
| Bile acids, mg/100 g | 772,73±16,14 | 514,52±14,08 ¹ | 750,69±18,86 ² | 760,95±40,55 ² |
| Cholesterol, mg/100 g | 35,27±2,76 | 23,47±1,54 ¹ | 30,75±1,47 ² | 31,08±1,60 ² |
| Liver homogenate | | | | |
| GSH, mcmol/g | 5,04±0,05 | 4,20±0,06 ¹ | 5,88±0,51 ² | 5,39±0,54 ² |
| TBA-reactive compounds, mcmol/g | 43,37±3,24 | 76,56±3,32 ¹ | 38,25±2,80 ² | 43,80±4,32 ² |
| DC, mcmol/g | 58,18±2,83 | 147,00±4,62 ¹ | 80,30±8,22 ² | 88,26±2,71 ² |

Significance level of $p < 0,05$ concerning ¹ – negative control; ² – positive control; n – number of animals in the group.

Under the influence of “Phytovenol” capsules and “Silibor” tablets, normalization of the examined indices was found to be approximately similar: a decrease of MCL in 1,3 times in regard to the positive control, ALT activity (1,6 times), AST (1,1 times), ALP (1,4 times), lipid level (1,5 times) and cholesterol (1,3 times). “Phytovenol” capsules and “Silibor” tablets increase bile secretion in 1,7 times, the content of bile acids and cholesterol in bile in 1,5 and 1,3 times respectively. Under the influence of both the studied medicine and the comparative drug, GSH level increased in 1,4 and 1,3 times, respectively, in the liver homogenate, against the decrease of TBA-reactive compounds (in 2 and 1,8 times respectively) and DC (1,8 and 1,7 times, respectively).

DISCUSSION

The changes of functional-biochemical indices in the animals of the positive control group (untreated animals) receiving paracetamol, demonstrated the development of acute hepatitis. This result corresponds with the results obtained by other researchers [9]. The increased MCL index in this group of animals was indicative of hemodynamic disorders in the organ. Moreover, the increase of activity of the cytolytic marker enzymes – ALT and AST, which

accompany inflammatory processes and characterize the hepatocyte membrane condition (lesion or destruction of cells), was indicative of the development of inflammatory-destructive changes in the liver. Furthermore, the increased level of lipids and cholesterol in the blood serum reflected disorders of the metabolic processes in the liver.

This pathological development was accompanied by disorders with regard to the bile expelling and synthetic functions of the liver, as the animals of the positive control group demonstrated decrease of bile secretion, as well as the content of cholesterol and bile acids in it.

Against the ground of decreased intensity of bile secretion and disorders of lipid metabolism, the increase of ALP activity in the blood serum is indicative of the development of cholestatic changes in the liver in the animals of the positive control group. This is because acute necrotic changes in hepatocytes may not be accompanied by increased ALP activity until the bile tubules are not involved in the pathological process, and until bile secretion is not interrupted [6,7].

Paracetamol administration to the rats was also found to disturb the balance of the oxidative-antioxidative system. The accumulation of DC and TBA-reactive compounds in the liver homogenate was indicative of the intensification of LPO processes under the hepatotoxic influence, while the decreased GSH level was indicative of the exhaustion and reduction of the activity of the antioxidant protective system.

Administration of "Phytovenol" capsules prevented the development of destructive and metabolic disorders in the liver, on, approximately, the same level as with the reference drug "Silibor". The administered "Phytovenol" capsules and "Silibor" tablets both decreased the activity of ALT, AST and MCL. This is indicative of decreased cytolytic and inflammatory reactions, as well as improvement of general trophic processes in the liver. It should be noted that in addition to their anti-cytolytic action, "Phytovenol" capsules and "Silibor" tablets possess antioxidant properties: these medicines inhibit the processes of lipid peroxide oxidation, and the increased antioxidant protection of cells, that their administration brought about, was seen in our work, by the inhibition of TBA-reactive compounds and DC accumulation, as well as the increased GSH level in the liver homogenate within the experimental groups. Both "Phytovenol" capsules and "Silibor" tablets improve lipid exchange, and in our study, the level of lipids and cholesterol in the blood serum decreased in the experimental groups. Moreover, the processes of bile formation and bile secretion were also renewed, and the indices of bile secretion, the content of bile acids and cholesterol in the bile, achieved the level of the negative control group. What is more, the decreased level of ALP seen is indicative of the renewal of the functional state of the hepatocytes and bile tubules under the influence of the examined medicines.

The pharmacological action of "Phytovenol" capsules is explained by the availability of flavonoids and tannins in horse chestnut fruits, the leaves of *Hamamelis virginiana*, the seeds of *Avena sativa*, the fruits of *Sophora japonica*, the *Centaurium vulgare* herb, the *Bistorta officinalis* herb and the *Melilotus officinalis* herb. The complex of flavonoids contained in the plants of "Phytovenol" capsules possesses

antioxidant, membrane stabilizing and anti-inflammatory properties. Of note, tannins, in the body, mobilize self-mechanisms of homeostasis, and stimulate the function of the adrenal cortex. This, to some extent, explains the anti-inflammatory activity of the examined capsules. In addition, the plants of "Phytovenol" capsules contain vitamins, macro- and trace elements, fatty oils and protein substances. Hence, ensuring the antioxidant, hypolipid and hepatoprotective activity of the examined medicine [3,4].

Thus, the results obtained are indicative of the fact that "Phytovenol" capsules influence practically upon all the links of the liver functions affected by paracetamol. The detected hepatoprotective properties of "Phytovenol" capsules are of a comprehensive character that is likely to be ensured by the combined total action of the biologically-active substances of the medicinal plants contained in the examined medicine [2,5].

CONCLUSION

Our study involving rats revealed that "Phytovenol" capsules, with regard to liver affliction induced by paracetamol, had a hepatoprotective action that is of the level of that of the classical hepatoprotector "Silibor", with regard to the antioxidant, antitoxic and anticytolytic activity of the examined medicine. The results of the hepatoprotective action of the capsules are a renewal of the hemodynamic, metabolic and synthetic processes in the liver. Moreover, the results obtained are the basis for recommending the administering of "Phytovenol" capsules in the comprehensive treatment of patients suffering from diseases accompanied by disorders of the hepatic-biliary system.

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