American Journal of Medicine and Medical Sciences 2021, 11(6): 471-475
DOI: 10.5923/j.ajmms.20211106.03

Evaluation of the Pharmacological and Therapeutic Effects of Drugs of Natural Origin in the Management of Biliary Insufficiency in Tetracycline Induced Hepatitis

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Abstract The effectiveness of the medicine- Convaren for restoration of bile-forming function of liver was studied in comparison with Celagrip, Lesbokhol, Liv.52, and Silibor on tetracycline induced acute hepatitis model. Experimental studies were conducted on white male rats with an initial body weight of 170-200 g. The drug lesion was reproduced by daily oral administration of tetracycline at a dose of 500 mg/kg into the stomach using a metal probe for 5 days. The animals were treated with Celagrip (25 mg/kg), Lesbokhol (25 mg/kg), Convaren (50 mg/kg), Liv.52 (100 mg/kg), and Silibor (80 mg/kg) for 6 days. Under the influence of Convaren, the excretion of bile increased by 48,2%, bile acids excretion increased by 89,6%, cholesterol by 94,8%, and bilirubin by 32,6% in tetracycline-induced hepatitis. Convaren showed a more significant impact on the improvement of the bile-secretion activity of the liver in drug-induced hepatitis compared with Silibor, Liv.52, Celagrip and Lesbokhol.

Keywords Drug-induced hepatitis, Hepatoprotective activity, Bile formation, Lipid peroxidation, Antioxidants

1. Introduction

The consumption of various drugs are increasing yearly, and the problem of toxic damage of internal organs, in particular the liver, has been rising. The drug – induced hepatitis is still a huge problem due to consumption of drugs without prescription [1].

More than one thousand drugs are known that can cause a toxic damage of liver, among which antibacterial medications occupy a special place [2,3]. According to numerous researches, one of the most common side effects associated with drugs is hepatotoxicity. Drug – induced liver damage accounts for about 10% of all adverse reactions due to the use of pharmacological therapy. The high prevalence, a wide range of clinical manifestations, the lack of unambiguous diagnostic methods, and often a poor diagnosis make drug-induced liver damage one of the most difficult problems in clinical practice [3]. World practice shows that hepatoprotective agents are usually used in the treatment of the hepatobiliary system diseases. However, despite the large range of these types of drugs obtained from various sources, they do not completely solve the problem of the effective treatment of drug-induced liver damage [4].

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Received: Apr. 19, 2021; Accepted: May 26, 2021; Published: Jun. 15, 2021
Published online at http://journal.sapub.org/ajmms

It should be noted that the liver is the main organ of biochemical homeostasis, and the pathology of the liver in case of bacterial infection may not be only associated with the infective agents but also because of the toxicity of antibacterial drugs. At the same time, almost all drugs used in phthisiology have hepatotoxic effects. It has been reported that among all anti-infectious agents, antibiotics occupy a leading position in terms of their hepatotoxicity [5,6,7]. It is known that the liver takes a leading place in the biotransformation of xenobiotics and endogenous toxic substances. By neutralizing toxins, the liver itself is exposed to its negative effects. Therefore, the use of antibacterial agents, especially in very-high doses to cure the infectious pathologies, may lead to the development of toxic liver damage. In this regard, there is a fairly large amount of research on tetracycline antibiotics [6,7]. These circumstances make the search for new drugs for the treatment of drug-induced liver disorders.

We have previously studied the effectiveness of a number of pharmacological agents in restoring the functional activity of the liver in acute lesions of various etiologies [8,9,10]. The aim of this work was to evaluate effectiveness of herbal preparation Convaren -extract of *Convolvulus arvensis L*. in comparison with Celagrip, Lesbokhol, Liv.52, and Silibor in experimental drug-induced hepatitis.

2. Material and Methods

2.1. Experiments

Since the bile excretion function of the liver fully reflects the functional state of hepatocytes, in order to objectively assess the degree of liver damage, we studied the state of the exocrine function of the liver and the chemical composition of bile in tetracycline-induced acute hepatitis model.

Experimental studies were conducted on 42 outbred white male rats with an initial body weight of 170-200 g. Laboratory animals were kept under standard vivarium conditions with free access to food and water, natural light and dark changes. The animals were divided into 7 groups, each of which consisted of 6-8 individuals. The drug-induced hepatitis was reproduced by daily oral administration of tetracycline at a dose of 500 mg/kg once a day for 5 days into the stomach using a metal probe [11,12]. In 24 hours after repeated administration of the tetracycline, the animals were divided into 7 groups.

The rats of the 2nd group received an aliquot of water (untreated group). After the last administration of tetracycline, animals of 3, 4, 5, 6 and 7th groups received Celagrip (25 mg/kg), Lesbokhol (25 mg/kg), Convaren (50 mg/kg), Liv.52 (100 mg/kg), Silibor (80 mg/kg), respectively. The duration of the treatment was 6 days. The functional state of the liver- the intensity of bile secretion and the chemical composition of bile was studied 24 hours after the final administration of the preparations [8,13]. In parallel, studies were carried out in 6 intact rats (group 1), which did not differ significantly in age and body weight from animals of other groups. They received drinking water in a volume of 0.5 ml per 200 g of body weight intragastrically.

Bile was collected using a polyethylene tube inserted into the common bile duct for 4 hours under anaesthesia (aethaminalum sodium 40 mg/kg, intraperitoneally). The concentration of the total content of bilirubin, bile acids, and cholesterol was determined in hourly portions of bile, data presented in the mg [4]. To eliminate the differences of the obtained data due to the different weight of the animals, the results were adjusted per 100 g of body weight of rats.

The hexenal test was carried out by intraperitoneal injection of a freshly prepared hexenal solution at a dose of 100 mg/kg. The duration of sleep time were determined [8,13].

The intensity of lipid peroxidation processes was assessed by determining the level of acylhydroperoxides (ACHP) and malondialdehyde (MDA) in the blood serum. The concentration of ACHP was determined by the method of V.B. Gavrilov, M.I. Mishkarudny [12], and MDA by the method of L.I. Andreeva et al. [11].

Biochemical blood tests were performed on a semiautomatic biochemical analyzer Mindray (China, 2014), by using test kits (Human, Germany and Cypress diagnostics Belgium).

Experimental studies were conducted in accordance with the "Rules and requirements for work using experimental animals", as well as the rules adopted by the European Convention for the Protection of Vertebrate Animals used for Experimental Research or for Other Scientific Purposes (ETS No. 123), Strasbourg, 18.03.1986.

2.2. Statistical Analysis

The obtained research results were processed by the method of variation statistics using the standard StatPlus 2009 software package with an assessment of the significance of indicators (M±m) and differences in the samples under consideration by the Student's t-test. The difference was considered significant at a probability level of 95% and more ($p < 0.05$) [11].

3. Results and Discussion

The results of the experiments showed that repeated administration of tetracycline led to a decrease secretion of bile by 32%, while the content of bile acids in bile decreased by 38,5%, cholesterol by 35,5%, and bilirubin by 33,3%. Consequently, liver pathology develops under the influence of tetracycline, which is accompanied not only by decreasing of bile secretion, but also decreasing of bile acids, cholesterol, and bilirubin in excreted bile. The formation of bile and its secretion is an energy consumable process. It is known that tetracycline forms a chelate compound with divalent ions, as a result of which, the activating effect of magnesium and calcium ions is suppressed, which leads to suppression of biosynthetic processes. All these are manifested in a decrease of the excretion of bile and its components.

Table 1. Influence of herbal substances to the secretion of bile and its chemical composition in tetracycline-induced hepatitis (the bile was collected for 4 hours, the data is presented per 100 g of animal body weight)

№	Groups	Bile, ml	Bile acids, mg	Cholesterol, mg	Bilirubin, μg
1	Intact	1,34 ± 0,100	6,91 ± 0,49	0,268 ± 0,036	105,7 ± 3,86
2	Hepatitis	0,911 ± 0,04*	4,25 ± 0,24*	0,173 ± 0,015	70,5 ± 3,67
3	Hepatitis + Celagrip	1,20 ± 0,015[a]	6,21 ± 0,40	0,253 ± 0,021	86,3 ± 4,51
4	Hepatitis + Lesbokhol	1,21 ± 0,050[a]	6,55 ± 0,35	0,273 ± 0,020	93,0 ± 5,10
5	Hepatitis + Convaren	1,35 ± 0,030[a]	8,06 ± 0,54	0,337 ± 0,035	93,5 ± 3,96
6	Hepatitis + Liv.52	1,10 ± 0,070	6,43 ± 0,73	0,251 ± 0,030	78,8 ± 5,50
7	Hepatitis + Silibor	1,02 ± 0,022	4,89 ± 0,23	0,230 ± 0,028	103,7 ± 6,69

*Note: * - statistically significant compared with the intact rats; a - in comparison with the untreated group of animals.*

In contrast, the treatment with herbal substances led to restoration of the exocrine function of the liver, and the chemical composition of bile. Even then, the use of Silibor led to an increase of the excretory function of the liver by 12%, and the excretion of bile acids, cholesterol, and bilirubin, by 15%, 33%, and 47.1%, respectively. Although the drug is not effective enough in improving bile secretion, however, it rather strongly stimulates the excretion of cholesterol and especially bilirubin.

It can be seen from the data in the table 1, the multi-component compound Liv.52, in comparison with Silibor, has a more pronounced effect on the studied parameters, especially on the excretion of cholates (increased by 51.3%) and cholesterol (increased by 51.3%). However, the drug was less effective on the excretion of bilirubin in animals with tetracycline-induced liver damage.

Celagrip is a drug containing polyphenolic compound gossypol, which has antioxidant and hepatoprotective properties [8]. Therefore, investigation of its effectiveness was of great interest in tetracycline-induced hepatitis. The results of study of its impact on the liver biliary function showed that the drug increased the amount of excreted bile by 32% compared to the untreated group. It is noteworthy that the drug increases the excretion of bile acids, cholesterol, and bilirubin by 46.1%, 46.2%, and 22.4%, respectively. At the same time, the values of the studied parameters were not statistically significant compared to the corresponding parameters of healthy rats.

Lesbokhol is a mixture of extracts from medicinal plants: *Hypericum scabrum L.*, *Ziziphora pedicellata*, *Mediasia macrophylla* and *Glycyrrhiza glabra L.*. The drug has a distinct choleretic property [14]. Thereby, we investigated its effectiveness for restoration of the exocrine function of the liver in drug-induced damage. As can be seen from the data in the table 1, under the influence of lesbokhol in tetracycline-induced hepatitis, bile secretion increased by 33%. There was an increase of bile acids by 54,1%, cholesterol by 57,8%, and bilirubin by 32,6% under the influence of Lesbokhol. It can be seen that Lesbokhol did not significantly differ from Celagrip in its impact on the excretory function of the liver. However, Lesbokhol stimulates the secretion of bile acids, cholesterol, and bilirubin more strongly than Celagrip.

Our further experiments showed that an even more significant effect was noted when used Convaren, which is a sum of biologically active substances from the aerial part of *Convolvulus arvensis L*., which had a distinct choleretic effect. Under the influence of Convaren, the excretion of bile increased by 48,2%, bile acids excretion was increased by 89,6%, cholesterol by 94,8%, and bilirubin by 32,6% in animals with tetracycline-induced hepatitis. The above data clearly show the high efficiency of this preparation in comparison with other medicines in the treatment of the impaired liver functions. The hepatoprotective property of the multi-component drug Liv.52 and the monocomponent drug Silibor was also observed in tetracycline-induced hepatitis in our study. From the data in the table 1, it can be concluded that both medicines have a positive effect on the liver function, but its activity is inferior in comparison to the other medicines. It should be noted that Liv.52 was equivalent to Celagrip by its action on the excretion of bile acids and cholesterol, but Silibor had a superior effect on excretion on bilirubin.

Thus, the analysis of our studies showed that the investigated medicines had a beneficial effect on the specific function of the liver, but differs in their activity. In this regard, preference should be given to Convaren. Further, we were interested in the explanation of the possible mechanism of the positive effect of Convaren which was the most effective preparation among the studied herbal preparations to improve the liver function in tetracycline-induced damage of liver. Since the Convaren had a distinct antioxidant effect [8,9,15], it can be assumed that the investigated preparation may prevent the damaging of the membranes of intracellular organelles and improve the normal function of membrane-bound enzyme complexes by suppressing the intensity of free radical processes. In this aspect, it is important to improve the activity of the monooxygenase enzyme system localized in the endoplasmic reticulum of hepatocytes, in which the synthesis of bile acids from cholesterol, conjugation of bilirubin takes place.

In order to answer the research question, we examined the functional activity of the monooxygenase system using a hexenal test. As the results showed that in intact rats the duration of hexenal sleep was 43,4 ± 2,11 minutes, it increased by 90,3% in rats with tetracycline-induced hepatitis (82.6 ± 7,61 minutes, $P<0,05$). At the same time, in animals treated with Convaren, the duration of sleep shortened by 37%, compared with the untreated group's animals, and did not statistically differ from the corresponding values of healthy rats. Since the biotransformation of this barbiturate occurs exclusively in the liver with the formation of pharmacologically inactive metabolites [16,17] and the duration of its hypnotic effect depends on the intensity of the metabolic process occurring in the cytoplasmic network of hepatocytes, it can be assumed that Convaren has a stimulating effect on the regeneration of biological membranes of subcellular organelles of liver cells including, and the cytoplasmic reticulum of hepatocytes.

Potentially, the preparation may stimulate the process of reparative regeneration of membrane phospholipids. This assumption is in good agreement with the results of studies of lipid peroxidation. Thus, after the end of tetracycline administration, the level of the initial products of lipid peroxidation such as acyl hydroperoxides in experimental rats increased by 80% compared to the control. The concentration of the intermediate product of lipid peroxidation as malondialdehyde rose by 39%. Tetracycline significantly enhanced the intensity of the process of free radical oxidation of phospholipids of biological membranes.

It is noteworthy that in rats treated with Convaren, there was a decrease of the free radical oxidation of

lipids, manifested in a statistically significant decrease in the concentration of acylhydroperoxide by 30% and malondialdehyde by 14.4%. In this aspect, decreasing of the intensity of lipid peroxidation might lead to the elimination of alterations of the permeability of cell membranes, and decreasing of the release of enzyme molecules into the extracellular space, thereby eliminating the phenomenon of hyperenzymemia as a characteristic of damage of liver cells.

It is known that the increasing of ALT in blood serum is a common sign of the pathology of the hepatobiliary system. Indeed, the study showed that the activity of this enzyme in blood under the influence of toxic doses of tetracycline increased by 238%, and after treatment with Convaren, it decreased 2 times in comparison with untreated group.

Since the regeneration process is an energy-dependent process, and tetracycline caused the dissociation of oxidative phosphorylation processes and led to a low-energy state. It seems that Convaren improved the oxidative-phosphorylation process in mitochondria, and might improve energy storage. It is known that this condition is accompanied by an increase of the amount of glycogen in hepatocytes, since glucose can be stored in the form of glycogen only under conditions of normal functioning of enzyme systems and a high-energy state [18].

As shown by the results of individual biochemical studies in animals with tetracycline-induced hepatitis, the glycogen content in liver decreased by 71% and by 0,69±0,17 g% against 2,38 ± 0,21 g% in the control group. After treatment with Convaren, the glycogen content in liver increased and reached to 1,93 ± 0,26 g%, i.e. in treated animals, the concentration of glycogen was significantly higher in comparison with untreated animals.

Summarizing the results of the studies, Convaren showed the membrane stabilizing activity. Possibly, due to the antioxidant effect, it improves the regeneration properties of biological membrane's functions in acute tetracycline-induced damage of liver.

4. Conclusions

1. There was a distinct suppression of the exocrine function of the liver and a decrease of the content of the main components of bile in tetracycline-induced liver damage.
2. The known herbal medicines with a choleretic effect showed a beneficial effect on the functional state of the liver in drug-induced damage.
3. Convaren showed a more significant impact on the improvement of the bile-secretion activity of the liver drug-induced hepatitis compared with Silibor, Liv.52, Celagrip and Lesbokhol.
4. The mechanism of the pharmacological and therapeutic effect of Convaren is associated with its high antioxidant, membrane-stabilizing action, and it improves energy storage.

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