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Screening Research of Pharmaceutical Compositions Based on Succinic Acid, Ascorbic Acid and Rutin

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Abstract: We conduct research on the development of new medicines based on succinic acid, ascorbic acid and rutin. We studied the anti-inflammatory action (exudative inflammation, model of carrageenan-induced paw edema of rats), the hepatoprotective (injury of rats with carbon tetrachloride) and renal protectie action. In the study, the anti-inflammatory activity of our medicines (exudative inflammation, karahenin model of inflammatory edema of paws rats) is set to ability of suppression of the inflammatory response by 24.4 %. Against the background, the action of carbon tetrachloride observed a positive effect on cholesterol- and pigment- forming liver function. Reducing the activity of enzymes ALT (alanine transaminase) and AST (aspartate transaminase) in groups of animals, which treated with our medication, talks about their ability to recover morpho functional integrity of the membranes of hepatocytes and warn of changes in the liver damage with carbon tetrachloride. Renal protective action screening study found that investigated mixture capable of increasingly lower level of creatinine in the blood of rats: 27.27~39.18 mmol/L in Lespeflan. Similarly, the concentration of urea in the smaller of the studied using mixtures is 5.18 mmol/L, while the application lespeflanu urea concentration slightly higher is 6.78 mmol/L. This shows that hypoazotemic effect is traced compared with the control group, and better than the comparator Lespeflan. Study of acute toxicity showed that the investigated mixture refers to practically nontoxic drugs. It is confirmed the need for further studies on the pharmacological activity of our facility to determine the effect on capillaries and the immune system, and as a result, prevention and treatment of influenza and ARI (acute respiratory infections).

Key words: Succinic acid, ascorbic acid, rutin, pharmacological action, acute toxicity.

1. Introduction

Study of pharmacological activity is one of the most important stages of drugs research. Developing of new drug requires many years of research and a lot of costs. We conduct research on the development of new medicines based on succinic acid, ascorbic acid and rutin [1-4].

Succinic acid is an endogenous metabolite, and it has a wide range of applications in medical care. The researchers studied the pharmacological action of certain pharmaceutical compositions comprising succinic acid: antioxidant, antiradical, adaptogenic properties, act protective, cerebroprotective, cardioprotective, immunotropic and stress protective [5-12]. A study is carried out using succinic acid in paediatrics and the treatment of mitochondrial diseases [13, 14]. A mixture of ascorbic acid and rutin has capillaries firming action. In our opinion, the combination of all these components in a single dosage form has a term used in medicine, in particular for the prevention and treatment of influenza and ARI (acute respiratory infections) [15] and prevents the occurrence of complications. Often combined drugs have several types of pharmacological activity and a wide range of applications. For optimize the study of pharmacological activity in the development of new combined drug from a succinic acid, ascorbic acid and rutin, we conducted an analysis of evidence data about the pharmacological activity of succinic acid and

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famous combination therapies based on it, and according to the results conducted our pharmacological research.

Our study included 29 articles (23 RCT (randomized controlled trial) and 6 reviews). The evidence varied in terms of: scope and years of research, type of treatment, study duration, sample size (< 100 to > 500 patients). Generally, the mean age of included patients was average. For pharmacological properties and by relevance RCT are grouped as follows: antigypoxic action were 7 RCT (2003~2013), improve iron absorption in the gastrointestinal tract were 6 RCT (1966~1974), hepatoprotective were 4 RCT (2013~2014). One RCT proves the efficiency of succinic acid used in gastroenterology (a combination with omeprazole, 2012), depression (2013), in transplantation (1993) at menopause (2008), renal failure (2013), to improve body temperature during surgery (2007). Analysis of review opens the new prospects for the use of succinic acid in cancer, diabetes and hepatitis C treatmen [16-21].

2. Materials and Methods

Pharmacological methods of studying the anti-inflammatory action (exudative inflammation, model of carrageenan-induced paw edema of rats), hepatoprotective (injury of rats with carbon tetrachloride) and nefroprotektor action (determination of creatinine and urea in the blood of rats in experimental models Hydrargyrum bichloratum $HgCl_2$, nephropathy) were used. The study of acute toxicity was conducted on white mice. Investigated mixtures are given in doses of 1 g/kg, 3 g/kg and 6 g/kg (6 animals of each species per dose) once through a metal tube into the stomach of animals. Observations on mice conducted for two weeks [22-23].

Data were analyzed by variation statistics using Student's *t*-test and η (M.O. Plohinsky correlation ratio). All values are expressed as means ± SEM. Differences were considered significant when $*p \leq 0.05$.

3. Results and Discussion

At the beginning, we have studied the anti-inflammatory activity of the studied powder mix and its individual components. Exudative inflammation is a classic example of the acute inflammation. Studying the impact of these substances on the course of exudative phase of inflammation was based on model of carrageenan-induced paw edema of rats [22].

The experiment was performed on nonlinear white rats of both sexes weighing 180~220 g. Total edema caused by injection under aseptic conditions 0.1 mL of 2% solution carrageenan under the sole aponeurosis rat hind limb. The presence of inflammatory reaction set by onkometrycs limb volume method at the beginning of the experiment and 4 h after action of flohohen agent. For 40 min before the introduction of the solution of carrageenan animals were injected intraperitoneally or intragastric of the substance. For comparison, in similar conditions, we studied the antiexudative effect of known anti-inflammatory drugs: diclofenac, ketanov and quercetin in the medium therapeutic doses. For the study, we used a mixture of No. 1 (succinic acid), a mixture of No. 2 (ascorbic acid + rutin), a mixture of No. 3 (succinic acid + ascorbic acid + rutin).

The percent (%) of inhibition of inflammatory response was calculated by using the formula:

$$\text{Percent of inhibition of inflammatory response (\%)} = (Vc - Ve)/Vc \times 100$$

where, *Vc*—paws average volume control, and *Ve*—paws average volume experiment.

Under these conditions, the experiment (carrgeenan model of inflammation) found that the substance in No. 1 and No. 3 found the ability to inhibit the inflammatory response in 19.6% and 24.4%, respectively. As shown in Table 1, the highest inhibition of inflammation observed in diclofenac sodium is 43.4% (increase volume paws 4 night—72.1%). On the second level is located ketanov: the rate of inflammatory is inhibition 37.8% (an increase

Table 1 Study of anti-inflammatory activity (M ± m; *n* = 6).

Test substances	Dose (mg / kg)	The percentage of growth of paw volume at 4 o'clock	Index of inhibition of inflammatory response (%)
Control	-	127.7	-
Diclofenac a / per	8	72.1	43.3
Ketanov a / per	10	79.1	37.8
Quercetin in / h.	50	123.1	-
Mix No. 1 (succinic acid)	100	102.0	19.6
Mix No. 2 (ascorbic acid + rutin).	100	121.1	-
Mix No. 3 (succinic acid + ascorbic acid + rutin)	100	96.6	24.4

n: the number of animals in the group.

of volume of paw after 4 h—79.1%). This is followed we examined powder mixture No. 3: inhibition of inflammation indicator is 24.4% (an increase of volume of paw after 4 h—96.6%). Next on the ability of a substance to inhibit inflammation is succinic acid: inhibition rate of inflammation is 19.6% (an increase of volume of paw after 4 h—102.0%). There were no anti-inflammatory effect of quercetin and the mix No. 2 (ascorbic acid + rutin).

The next step was to study the hepatoprotective activity of the studied powder mixtures in comparison with succinic acid. The study of our medication detected a hepatoprotective effect. Against the background, the action of carbon tetrachloride observed a positive effect on cholesterol- and pigment-forming liver function. Reducing the activity of enzymes ALT (alanine transaminase) and AST (aspartate transaminase) in groups of animals, which treated with our medication, talks about their ability to recover morpho functional integrity of the membranes of hepatocytes and warn of changes in the liver damage with carbon tetrachloride. In the study, the anti-inflammatory activity of our medicines (exudative inflammation, karahenin model of inflammatory edema of paws rats) is set to ability of suppression of the inflammatory response by 19.6. We conducted the study hepatoprotective activity investigated powder mixtures (No. 3). As the comparator used succinic acid (No. 1).

A characteristic pattern of liver disease are at tetrachloride intoxication (CCl_4), because CCl_4 xenobiotics with the most highly selective hepatotoxicity. Poisoning by carbon tetrachloride experimental animals picture morphological and biochemical changes is close to acute liver disease of various etiologies in humans and animals.

The material of the study were white males rats weighing 200~220 g, which are kept in a vivarium of Danylo Glytsky Lviv National Medical University in standard terms of diet and in accordance with sanitary standards. The studied animals were divided into four groups of six rats each. The first group is intact, the second is control, while the third and the fourth group was administered drugs in a dose of 100 mg per 1 kg of body weight. Chronic toxic liver disease caused by the introduction of carbon tetrachloride as a 20% oil solution at a dose of 0.2 mL/100 g weight intragastric twice a week for 45 days. The second group was administered the study medication. The biological material (blood and liver) were taken on Day 3 after the last administration of intoxicants by decapitation by ether anesthesia. Serum samples were tested bilirubin, cholesterol, ALT and AST (Table 2).

Research of serum of rat, which enter CCl_4 pointed to a significant increase of total bilirubin. Hyperbilirubinemia constitutes a violation of absorption, conjugation and excretion of bilirubin in the bile, because for toxic liver damage occurs parenchymal jaundice. Reducing the concentration of total cholesterol is a sign of dysfunction of hepatocytes that perform a leading role in the synthesis and regulation of its level in the blood. A high

Table 2 Biochemical parameters of blood by the action CCl_4 (M ± m; *n* = 6).

Groups	Cholesterol (mmol/L)	Bilirubin microns (mmol/L)	ALT	AST
Intact	1.71 ± 0.12	8.71 ± 0.67	93.12 ± 7.81	232 ± 16.44
Control	0.91 ± 0.07	20.6 ± 1.55	690.6 ± 38.1	804.3 ± 41.7
Mix No. 1 (succinic acid)	1.19 ± 0.08*	14.05 ± 1.87*	181.2 ± 10.8*	212.4 ± 12.7*
Mix No. 3 (succinic acid + ascorbic acid + rutin)	1.24 ± 0.1*	12.84 ± 2.11*	168.4 ± 14.7*	453.3 ± 24.0*

*: significant difference compared with those of control grou ($p \leq 0.05$);
n: the number of animals in the group.

Table 3 Study of hypoazotemic action (M ± m; *n* = 6).

Indicators	Intact control	Control pathology	The investigated mix (mg/kg)	Lespeflan (2 mL/kg)
Creatinine (mmol/L)	41.6 ± 5.8	89.05 ± 7.64	27.78 ± 4.14*	39.18 ± 4.78*
Urea (mmol/L)	4.30 ± 0.88	7.12 ± 2.14	5.18 ± 1.22	6.78 ± 2.45

*: significant difference compared with those of control grou ($p \leq 0.05$);
n: the number of animals in the group.

level of activity of marker enzymes ALT and AST suggests the depth of organ damage.

At the macroscopic study of livers of rats in the control group found that they were increased in volume, with jagged edges, clay-colored. Most affected was the right and left inner fate and left lateral. In the group of rats who were administered the substance and liver were darker color compared to the control group. The substance discovered a hepatoprotective effect. Against CCl_4 observed a positive effect on the pigment- and cholesterol forming function of liver.

Reducing the activity of enzymes ALT and AST in groups of animals treated with study medication, says about their ability to recover membrane of hepatocyte morphofunctional integrity, prevent morpho-physiological changes in the liver, damaged CCl_4.

CRF (chronic renal failure) is one of the most important problems of modern nephrology and usually the end result of progressive course of chronic nephros disease. In 2012 in Ukraine, there were 490,234 patients with chronic renal failure, which was 1,078.5 cases per 100,000 of population. For the treatment of chronic renal failure among others also used drugs that have the ability to display products of protein metabolism from the blood and urine (hypoazotemic drugs). The range of nephro tread (hypoazotemic) drugs in the modern pharmaceutical market is limited. Acute renal failure in rats at screening study called single intraperitoneal administration of mercury dichloride solution at a dose of 2 mg/kg (experimental model sulemovoyi nephropathy). The experiment was performed on 24 white outbred rats weighing 180~210 g Investigated substance and drug comparison Lespeflan (2 mL/kg) was administered internally through a tube daily for 10 days. Rats of the control group were injected appropriate amount of water. For control was taken six healthy rats. At the end of the experiment, 11 days, conducted sampling of biological material (blood, kidneys) following decapitation of animals on the background of ether anesthesia [23].

To install hypoazotemic activity of these substances decapitation of the rats were determined in blood urea and creatinine concentrations, conducted pathological study of the structure of rat kidney for installation of renal protective activity investigated substance.

As you can see from Table 3, investigated mixture capable of increasingly lower level of creatinine in the blood of rats: 27.27~39.18 mmol/L in Lespeflan. Similarly, the concentration of urea in the smaller of the studied using mixtures is 5.18 mmol/L, while the application lespeflanu urea concentration slightly

Table 4 Results of study of acute toxicity of mixture No. 3, mixture No. 2 and succinic acid.

Tested object	The dose of the studied object (g/kg)	The observed effect, the number of dead animals / total animals in group
Mix No. 3 (succinic acid + ascorbic acid + rutin)	1	0/6
	3	0/6
	6	0/6
Mix No. 2 (ascorbic acid + rutin).	1	0/6
	3	0/6
	6	0/6
Mix No. 1 (succinic acid)	1	0/6
	3	6/6 (immobile, convulsions, and death within 24 h)
	6	6/6 (immobile, convulsions, and death within 24 h)

Table 5 The results of determine the degree of toxicity of the studied of mixture No. 3, mixture No. 2 and succinic acid.

No.	Tested object	These acute toxicity LD_{50}
1	Mix No. 3 (succinic acid + ascorbic acid + rutin)	> 6,000 mg/kg
2	Mix No. 2 (ascorbic acid + rutin).	> 6,000 mg/kg
3	Mix No. 1 (succinic acid)	1,900 mg/kg

higher—6.78 mmol/L. This shows that hypoazotemic effect is traced compared with the control group, and better than the comparator Lespeflan.

The study of acute toxicity (LD_{50}) with injected into the stomach conducted by conventional method, in accordance with International principles of the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (Strasbourg, 1986) and the *Law of Ukraine* "On Protection of Animals from Cruelty" from February 26, 2006.

The study was conducted on white mice which were kept under standard vivarium conditions. Investigated mixtures are prepared in doses of 1 g/kg, 3 g/kg and 6 g/kg (6 animals of each species per dose) once through a metal tube into the stomach of animals. Observations on mice conducted for two weeks (Table 4).

Thus, according to the classification [22], based on the maximum dose used in toxicological experiment, examined objects can be attributed to a practically nontoxic drug (V class of toxicity, LD_{50} 5,000~15,000 mg/kg).

So adding succinic acid to the mixture of ascorbic acid and rutin not lead to increasing toxicity (Table 5).

4. Conclusions

A systematic review was carried out to perform a qualitative assessment of succinic acid adding in the various medicines and to determine of their pharmacological action.

A comprehencive overview of these studies estimated the evidence of succinic acid addition of the drugs. Succinic acid, as endogenous metabolite, is a part of drugs of different pharmacological actions. These clinical trial results have been retrieved and give the possibility to develop of new drugs with succinic acid also in Ukraine.

According to results of evidence data we confirmed pharmacological activity of investigational combination product based on succinic acid. At this stage revealed that the studied powder mixture manifests anti-inflammatory, hepatoprotective and nefroprotektor action that corresponds to the data based medicine feasibility of the introduction of succinic acid of the combined drugs.

Study of acute toxicity showed that the investigated mixture refers to practically nontoxic drugs.

Thanks hypoazotemic and anti-inflammatory action of the studied powder mixture can be used to prevent the complications of influenza. It is confirmed the need for further studies on the pharmacological activity of our facility will determine the effects on capillaries and the immune system and, as a result, prevention and treatment of influenza and ARI.

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