www.ThePharmaJournal.com

The Pharma Innovation

ISSN: 2277- 7695
TPI 2015; 4(6): 87-91

www.thepharmajournal.com
Received: 24-06-2015
Accepted: 25-07-2015

Bogdan N.S
National University of Pharmacy, Kharkiv Bukovinian State Medical University, Chernivtsi, Ukraine.

Tikhonov O.I
National University of Pharmacy, Kharkiv Bukovinian State Medical University, Chernivtsi, Ukraine.

Correspondence:
Bogdan N.S
National University of Pharmacy, Kharkiv Bukovinian State Medical University, Chernivtsi, Ukraine.

Pharmacological, microbiological and derivatographic studies of an unti-ulcer pharmaceutical Drug made on the basis of bee products

Bogdan NS, Tikhonov OI

Abstract: Pharmacological, derivate and microbiological studies of bee breeding-based medicament for treatment of ulcers of stomach and duodenum were conducted.
Pronounced antiulcer effect of the drug in the form of granules was revealed in alcohol-prednisolone model with preventive mode of administration that enables its use as therapeutic and prophylactic measure to prevent recurrence of the disease and in acute phase.
Studies of thermographic analysis of substance "Plantaglucid", FHPP, honey and powdered standardized investigational product were conducted that enables to talk about the absence of chemical reactions between them and set the temperature regime of manufacture process.
Microbiological product indicators was studied and it was proved that he had only antiulcer action without antimicrobial properties what makes it safe for development of normal intestinal microflora.
The results of pharmacological, microbiological and derivate studies show the effectiveness of study product and prospects for its further research to treat ulcers of stomach and duodenum.

Keywords: *Gastric ulcer, anti-ulcer products, capsules, PPHP (propolis phenolic hydrophobic preparation), «plantoglyutsyd», natural honey powder, derivatographic studies, microbiological studies.*

1. Introduction

It is known that drugs of natural origin that show divergent effects and meet high safety standards used to prevent and cure gastric (GU) and duodenal (DU) ulcer remains top of the agenda.
First of all, it is an effective and highly safe therapy during extensive treatment of chronic diseases, especially in pediatrics and gerontology. The lowest possible toxicity level of drugs of natural origin makes it possible to prescribe a long-term course in complex or simpler combinations for anti-relapsing or rehabilitative treatment.
Secondly, a rich chemical composition of these drugs ensures the polyvalence of pharmacological properties, thus allowing to achieve a maximum therapeutic effect and to make gentle and safety impact on many systems of a body involved in a pathological process anyway. Drugs of natural origin can be used as monotherapy for an early stage of GU, during exacerbation – as an additional treatment together with classic synthetic drugs, and during the phase of anti-relapsing therapy – the treatment for effects of synthetic xenobiotics is fully justified [1, 2, 3, 8].
Studying pharmaceutical drugs made of biologically active substances (BAS) of natural origin is one of the promising trends. The investigational drug contains propolis phenolic hydrophobic preparation, natural honey powder, and plantoglyutsyd. The combination of biologically active substances (BAS) determines a wide range of its pharmacological activity (anti-inflammatory, reparative, immunostimulating, antimicrobial, etc.), microbiological properties and their possible interaction (BAS) among themselves under different temperature conditions.
That is why it is relevant to study pharmacological, microbiological and derivatographic properties of this drug as a promising anti-ulcer treatment for different pathological conditions of gastro-intestinal tract accompanied by gastric ulcer.
Thus, the research has focused on pharmacological, microbiological, and derivatographic study of the natural remedy made on the basis of bee products to treat gastric ulcer of rats. The remedy has been obtained on the basis of NUPh under the O.I. Tikhonov's guidance, the

professor of the department of Pharmacy-based Technology of Drugs.

The investigational drug is a powder of light brown color with a specific smell.

The anti-ulcer activity of the drug has been compared with the activity of ranitidine (Zdorov'ya, Pharmaceutical company, LLC, Ukraine), that has been chosen as a reference drug, since this drug, according to the literature data, is successfully being used in clinical practice for treatment of gastric (GU) and duodenal (DU) ulcer and has been included in the State Drug Formulary of Ukraine.

It is known that pharmacological study of potential anti-ulcer drugs should be started with the help of a model of "acute" gastric ulcers that can be easily reproduced, which includes the affection caused by the injection of both prednisolone and ethanol [6]. The use of such a combination is based on the influence of a steroid hormone that causes the inhibition of phospholipase synthesis resulting in negative trophic effect of gastric mucosa (GM), reduction of energy and plasty of tissues, and greater influence of aggressive factors of gastric acid. Moreover, the use of corticosteroids is accompanied by apparent stress, enhancement of gastric secretion and circulatory ischemia of GM villi, disorders of processes of peroxide lipid acidification (PLA), release of free radicals, salivary discharge and cell repair. As a result, gastro-destructive properties of ethanol increases several times, and the concentration of ethanol (80%) causes dehydration and coagulative necrosis of GM [6].

It is also known that the mechanism of cumulative ulcerogenic effect of glucocorticosteroid (GC) and ethanol is based on the ability of 3-isoenzyme of alcohol dehydrogenase to oxidize both ethanol and steroids. The common way of their metabolism is a factor which contributes to ulcerogenesis. As a result, prednisolone and ethyl alcohol potentiate each other's action that would also allow reducing the steroid dose [7].

Reproducibility of a model is 100%. Ulcers develop in 24 hours after injection of damaging agents.

The agar diffusion method ("well diffusion" method) has been used to determine the antimicrobial activity.

The derivatographic analysis has been used to study chemical and physiochemical processes of the substance that occur under changing temperature conditions.

2. Experimental procedure

2.1 Pharmacological study. Studies have been conducted in white non-pedigree rats weighing 200-250 g that have previously passed 24 hours of absolute diet with no limitation to drink water. The mixture of alcohol and prednisolone (Prednisolone-Darnitsa, tablets of 5 mg each No. 40, CJSC Pharmaceutical Firm Darnitsa), at the dose of 20 mg/kg and ethyl alcohol (80% at the rate of 0.6 ml per 100 g of rat mass), have been injected intragastrically at the same time on a one-off basis.

In total there were 4 groups of rats used in the research, 6 in each group: 1st group – intact control, 2nd group – confirmatory pathology, 3rd group – animals that have been injected the drug at the dose of 100 mg/kg, 4th group – 20 mg/kg of ranitidine for prophylactic purposes [6].

The investigational drugs have been injected intragastrically on a one-off basis for prophylactic purposes during three days; the ulcerogenic agent has been injected on the third day. Animals have been euthanized on the fourth day of the research in compliance with the principles laid down in the European Convention for the Protection of Vertebrate Animals kept for Experimental and other Scientific Purposes (Strasbourg, 1986) and in the 6th National Bioethics Congress (Kyiv, 2010).

Stomachs have been extracted, cut along its greater curvature, and washed with normal saline solution. Then the GM research has been conducted. Upon completion of the research, the anti-ulcer activity has been estimated.

Evaluation of peptic ulcer depth and intensity of anti-ulcer activity has been made using macroscopic indices of intensity of ulcer formation in GM: percentage of animals with ulcers in the group (A_u), average area of ulcers in the group (S_{Bcep}), mm^2, ulcer index (UI), anti-ulcer activity (AU A, %).

Ulcer index and anti-ulcer activity have been calculated by formulas 1.1; 1.2:

$$UI = (Звсср \times A_u) / 100 \quad (1.1)$$

$$AUA, \% = 100\% - ((UI_{ЛІК} \times 100\%) / UI_K) \quad (1.2)$$

where $UI_{ЛІК}$ – UI in the group of animals with confirmatory pathology that have been treated;

UI_K —UI in the group of animals with confirmatory pathology (have not been treated).

In addition, the appearance and overall well-being of animals (behavior, reflexes, in particular food reflex, state of hair, etc.) and other indicators of the status of gastro-intestinal tract (GIT) (signs of distension of the stomach and intestinal tract) and GM, i.e. signs of hyperemia, hemorrhage, edema, and mucosal folds have also been estimated. The estimation of the indicators have been made on the basis of their significance: 0 points (b) –no signs; 1,2,3 –faintest, moderate, and strong signs, respectively [6].

The analysis of the research results shows that all animals of the intact control group have good appetite, good reaction to sound and photic stimulus; uropoiesis and defecation processes are satisfactory; symptoms of respiratory disturbance and spasms have not been detected. The reflex irritability is satisfactory. Animal hair is also satisfactory. All the animals have remained unchanged until the end of the experiment. The death of animals has not been recorded. GM of intact animals is also satisfactory: its color and folds are normal, there is no hemorrhage and edemas, GIT distension is not found (table 1, 2). Macroscopic examination of GM of the group of intact animals revealed no ulcers (table 1, 2).

The experiment shows that animals of the confirmatory pathology group are low active, don't want to eat their food, have a poor reaction to irritators and their reflex irritability has increased straight after injection of ulcerogenic agent with its further complete loss.

The GM research of animals with simulative pathology (table 1) shows that the injection of alcohol and prednisolone mixture causes formation of multiple deep ulcers; there are signs of edema (2 b), significant hyperemia (2,3 b), GM bleeding (2,8 b), and fold mucosal disorders (2,5 b). All the animals have distension of the whole GIT (2,6 b). All animals of the group have signs of ulcer defects, both pitting and mass ones; an average area of ulcers is 53,25±3,7mm^2, the ulcer index is 53,25.

Rats with simulative pathology that have been treated with the investigational drug are almost similar to animals of the intact control group in their appearance and behavior. The rats of this group have GIT distension (1.3 b.), and the indicators of fold mucosal disorders (0.3 b.), signs of hemorrhage (1 b.), hyperemia (0.5 b.), and edema (1.3 b.) are lower than that of the simulative pathology group. An average ulcer area is 36.08±5mm^2 (confirmatory pathology group has 53.25±3.7mm^2). An integral indicator of ulcer activity (ulcer index) of this group is 36.08 (confirmatory pathology group has 53.25). As a result, the anti-ulcer activity of the drug is

32.24% (table 1,2).

The injection of ranitidine also has a positive effect on the well-being of animals: hair, activity and attitude towards food intake are similar to that of the of the intact control group. But GM of animals is unsatisfactory: there are slight signs of hyperemia (0.2 b.), edema (0.8 b.), hemorrhage (0.3 b.), and fold mucosal disorders (1 b.). The signs of ulcer defects have been found in all animals of this group, and an average ulcer area is 22.08±4.5мм^2. The ulcer index and anti-ulcer activity is 22.08 and 58.5%, respectively (table 1,2).

Table 1: Effect of the investigational drug on macroscopic indices of GM and models of alcohol-prednisolone ulcer, Me (LQ; UQ)

Experimental groups, (n=6)	Intact control	Confirmatory pathology	Investigational drug	Ranitidine
Distension	0 (0; 0)	2.6(2;3)	1.3(0;3)	1.3 (0;3)
Hemorrhage	0 (0; 0)	2.8(2;3)	1(0;2)*	0.3 (0;1)*
Hyperemia	0 (0; 0)	2.3(1;3)	0.5(0;1)*	0.2 (0;1)*
Edema	0 (0; 0)	2.6(2;3)	1.3(1;2)*	0.8(0;1)*
Folding	0 (0; 0)	2.5(2;3)	0.3(0;1)*	1(0;2)*

Notes:

1. n – number of animals in the group.
2. * – statistically significant differences of the confirmatory pathology group with the significance level of $p<0.025$ (according to the Mann-Whitney criterion and Bonferroni correction);
3. ** – statistically significant differences of ranitidine with the significance level of $p<0.025$ (according to the Mann-Whitney criterion and Bonferroni correction).

Table 2: Indices of anti-ulcer activity of the investigational drug applied to the model of acute alcohol-prednisolone gastric ulcer of rats

Experimental groups, (n=6)	Number of animals with ulcers in the group,%	Average area of ulcers, mm^2	Ulcer index	Anti-ulcer activity, %
Intact control	–	–	–	–
Confirmatory pathology	100	53.25±3.7	53.25	–
Investigational drug, 100 mg/kg	100	36.08±5*	36.08	32.24
Ranitidine, 20 mg/kg	100	22.08±4.5*	22.08	58.5

Notes:

1. n – number of animals in the group.
2. * – statistically significant differences of the confirmatory pathology group with the significance level of $p<0.05$ (according to the Neumann-Keuls criterion).

Thus, the significant effect of the investigational drug has been discovered and proven with the help of an alcohol-prednisolone model.

2.2 Derivatographic research is the next stage of studies. When we develop a pharmaceutical drug, there is always a possibility of chemical interaction between active and auxiliary substances of a multi-component drug. Moreover, we need to study the influence of a thermal factor on stability of the drug in the process of its manufacturing.

Data (fig. 1) of the TG curve received during the research show that "Plantaglyutsyd" is characterized by high thermal stability – at 108ºC, there is a mass loss under drying up to 75% with maximum disintegration rate at 220ºC. The end of the disintegration process can be observed at 340ºC, the mass loss under drying constitutes 40% of batch.

The research of the PPHP substance (fig. 2) has revealed that there is no loss of mass at the temperature up to 38ºC; a maximum disintegration rate takes place at 202ºC, and 84% of the batch mass is lost at 243ºC.

Data of the Fig. 3 show that 7% of the batch mass is lost at the temperature up to 116 °C. The maximum disintegration rate of the substance starts at 68 °C, where the mass loss is 3.5%. If we fry the substance at the temperature up to 500 °C, the exothermic reaction can already be observed at 270 °C. A maximum disintegration of a standardized honey powder has taken place at the temperature of 320 °C. At the temperature of 380 °C, 68% of the batch mass is lost.

When we look at the data of Fig. 4 showing a thermogram of granules of the investigational drug, we can see that it starts losing its mass at the temperature of 38ºC. This could be attributed to the properties of the PPHP substance. According to DTA data, a calorigenic action takes place when the temperature reaches 108 ºC, which allows us to confirm that the destruction processes of the substance occur at the given temperature.

These calorigenic actions made by derivators of the substance are identical to the calorigenic actions shown in thermal gravimetric curves of each individual substance included in the preparation formula. That testifies to the absence of physical and physicochemical interaction between substances.

On the basis of the data of thermograms, the temperature of destroying the components of the drug is about 108ºC. The granules of the drug should be manufactured at the temperature of 20-30ºC. We can therefore say that a manufacturing procedure of making granules under the conditions of commercial production takes place without destroying its components.

2.3 Determination of antimicrobial activity of the investigational drug. A set of reference bacterial strains: S. aureus ATCC 6538, E. coli ATCC 25922, P. aeruginosa ATCC 9027, B. subtilis ATCC 6633, C. albicans 885633. The results are shown in Table 5.

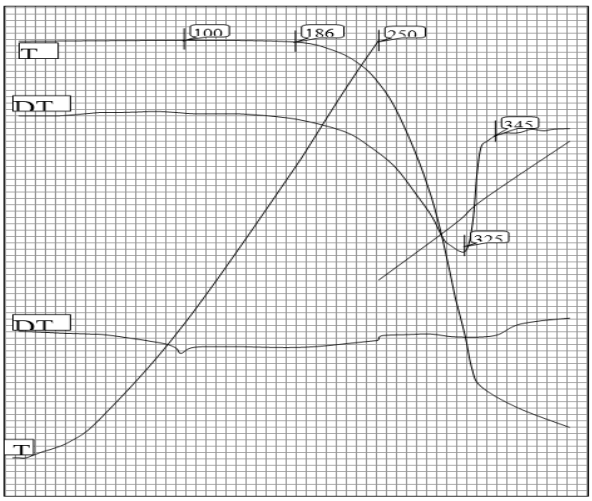

Fig 1: *Plantaglyutsyd thermogram*

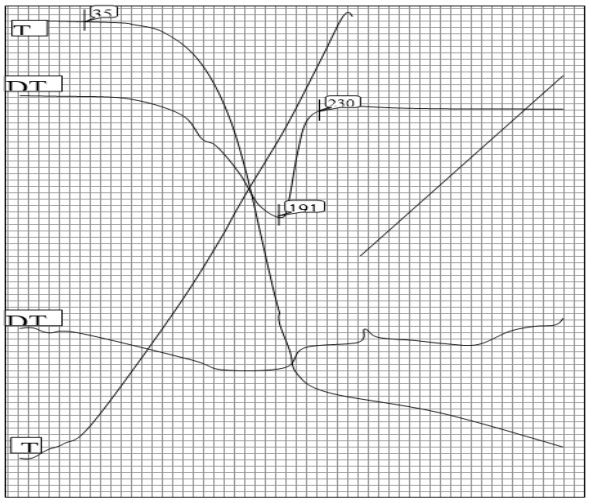

Fig 2: *PPHP thermogram*

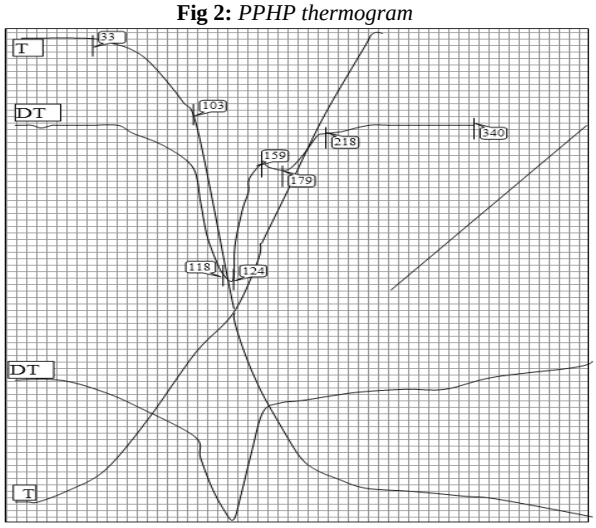

Fig 3: *Thermogram of a standardized honey power*

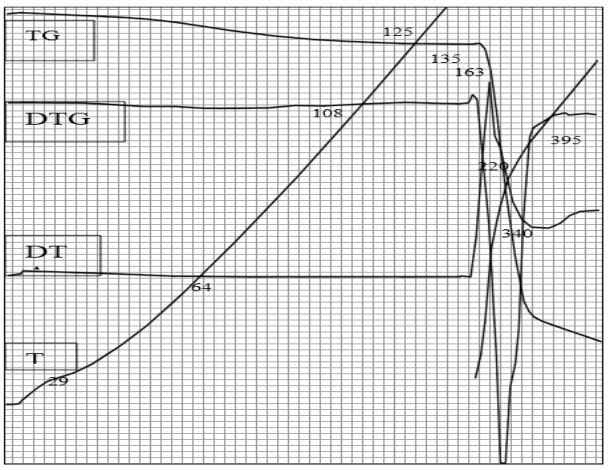

Fig 4: *Thermogram of the investigational drug made in the form of granules*

Table 5: Results of the determination of antimicrobial activity of the investigational drug

Sample name	Inhibition zone, mm				
	S. aureus	E. coli	P. aeruginosa	B. subtilis	C. albicans
Investigational drug	–	–	–	–	–

The studies have shown that test samples do not have antimicrobial activity in relation to the gram positive (S. aureus, B. subtilis), gram negative (E. coli, P. aeruginosa) bacteria, and fungi of Candida type.

3. Conclusion

1. We have discovered a strong anti-ulcer effect of the drug made in the form of granules using alcohol and prednisolone model by performing therapeutic injections. Thus, this drug can be used to treat and prevent recurrence of the disease and during exacerbation period.
2. We have made a thermal gravimetric analysis of the Plantaglyutsyd substances, PPHP, standardized honey power, and investigational drug. The results show that there are no chemical reactions between them, allowing us to set a temperature of the manufacturing process, which does not exceed 108ºC.
3. We have made microbiological studies of the drug and proven that its anti-ulcer effect does not have any antimicrobial properties, which makes it safe for the development of normal microflora of an intestinal tract.

4. References

1. Ivashkin VT. Rational pharmacotherapy of gastrointestinal disorders / V.T. Ivashkin; under general editorship of V.T. Ivashkin. – M. : Lit- terra, 2003, 38.
2. Kalinin AV. Gastroesophageal reflux disease. Diagnostics, therapy, preventive care / A.V. Kalinin // Pharmateka 2003; 7(70):45-50.
3. Kalinin AV. Symptomatic gastroduodenal ulcers and gastroduodenal ulcers in peptic ulcer disease / A.V. Kalinin // Russian magazine of gastroenterology, hepatology and coloproctology 2004; 14(3):22-32.
4. Kurkin VA. Phytotherapy of gastritis and peptic ulcer disease / V.A. Kurkin // Russian pharmacies 2006; 6:12-14
5. Mayev IV. Modern methods of treatment for peptic ulcer disease / I.V. Mayev. // Consulting physician 2003; 5:4-8
6. Pre-clinical studies of pharmaceutical drugs: method, rec. / under the editorship of member-corr. of Academy of Medical Sciences of Ukraine O.V. Stefanov. – K.: "Avitsenna", 2001, 528
7. Zupanets IA. The rationale for using a combination of ethanol and prednisolone for screening gastroprotectors / I.A. Zupanets, L.V. Yakovleva, V,V, Propysnova // Clinical pharmaceutics 1998; 2(3):29-33.
8. Baraboy VA. Bio-antioxidant / V.A. Baraboy. – K.: Knyha plus, 2006, 324.
9. Hryha IV. Medicinal plants in pharmacology: the tutorial on Phytotherapy/ I.V. Hryha, V.I. Hryha, Ph. D. Hamor. – Uzhhorod : Zakarpattya, 2001, 107.
10. Zorya AV. Characteristics of pathophysiologic changes during exacerbation of peptic ulcer disease / A.V. Zorya // Gastroenterology: interdepart. digest. – Ed 2000; 31:32-36.
11. Experience in the diagnostics and treatment of peptic ulcer disease / M.O. Vatsyk, I.V. Kovalchuk, M.R. Ivaniv, etc. // Transport medicine of Ukraine 2008; 2:68-70.
12. Babak OYa. Modern pharmacotherapy of peptic ulcer disease/O.Ya. Babak, H.D. Fadeenko. – Kharkiv, 1997, 24.
13. Kauffman G. Aspirin-induced gastric mucosal injuri: Lessons learned from animal* models / G. Kauffman // Gastroenterol 1989; 96(2):606-614.
14. Kohly J. Effect of hyperxygenation and of mycolytic agent, pronase on aspirin-induced erosions / J. Kohly, C. Pfeiffer // IV Intern. Cont. for exp. Ulcer. Tokyo, 1980, 90.

15. Kontureck SJ. Gastric cytoprotection / S.J. Kontureck // Mount Sinay J. Med 1982; 5:355-369.
16. Kuipers EJ. The prevalence of Helicobacter pylori in peptic ulcer disease / E.J Kuipers, J.C Thijs, H.P.M. Festen // Aliment Pharmacol. Ther 1995; 9(2):56-69.
17. Labenz J. Highly significant change of the clinical course of relapsing and complicated peptic ulcer disease after cure of Helicobacter pylori infection / J. Labenz, G. Borsch // The American Journal of Gastroenterology 1994; 89:1785-1788.
18. Lee A. The aging stomach or the stomach of the ages. Changing gastric acid secretion. The key to Helicobacter pylori and gastroduodenal disease / A. Lee, S.V. Van Zanten//Gut 1997; 41:575-576.
19. Magistretti MJ. Antiulcer activity of an anthocyanidin from vaccinum myrtillus / M.J. Magistretti, M. Conti, A. Cristoni // Arzneim. Forsch. - Bd 1988; 38(5):S452-454.
20. Naranjo P. Art und Sitz des experimentellen Magengeschwurs und antiulcerose Akti-vitat verschiedener Arzneimittel. Arzneimitt / P. Naranjo, G. Ridalgo, E. Banda- Naranjo // Forsch 1961; 7:S662-664.