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ANTI-INFLAMMATORY EFFECT OF THE MOLECULAR PECTIN COMPLEX WITH ACETYLSALICYLLIC ACID ON A MODEL OF CARRAGEENAN PAW EDEMA IN RATS

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Keywords:

Nonsteroidal anti-inflammatory Properties, Acetylsalicylic acid, Pectin, Carrageenan edema, Leukocytes

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ABSTRACT: The aim of this work is a comparison of anti-inflammatory properties of the molecular pectin complex with 10% acetylsalicylic acid (P-ASA) and acetylsalicylic acid (ASA) in equimolar doses on the model of carrageenan-induced paw edema of rats. The study was carried out on a total of 28 adult male Sprague Dawley rats at 7 months of age. 30 min after injection of 1% aqueous solution of carrageenan to the plantar aponeurosis, studied drugs were orally administered once at the doses of 10, 20, 40 mg/kg ASA and 100, 200, 400 mg/kg P-ASA. The severity of edema, pain sensitivity, leukocyte count, its subpopulation ratio and IL-1β, IL-6, IL-8, TNFα level were determined. Normality, t-test and Mann-Whitney tests were used for statistical analyses. The ulcerogenic effect of P-ASA (dose 400 mg/kg) is significantly less than that of ACA (dose 40 mg/kg). The analgesic effect of P-ASA is comparable to ASA at equimolar doses. The anti-edema effect of ASA at the doses of 10 and 20 mg/kg is more pronounced than that of P-ASA at equimolar doses. P-ASA contributes to normalization of blood leukocyte profile. P-ASA may be recommended to using as anti-inflammatory agent with low ulcerogenic properties.

INTRODUCTION: Studies in the development of anti-inflammatory drugs confirm their high relevance and take place in novel world literature [1, 2].

There is some evidence that about 30 million people in the world use non-steroidal anti-inflammatory drugs (NSAID) daily [3].

The existing problems associated with the occurrence of side effects when using NSAIDs [3], including acetylsalicylic acid (ASA), determine one of the most important areas of research in the development of anti-inflammatory drugs reducing toxicity and side effects. Previously, we have prepared molecular complexes from pectin and

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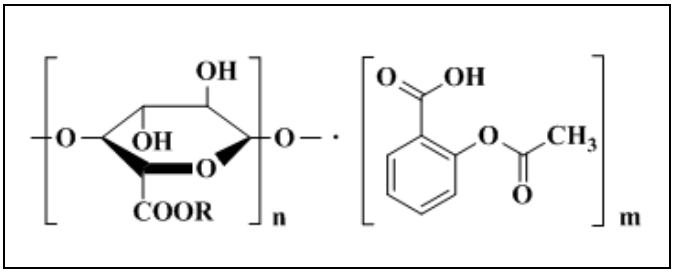
dicarboxylic acids succinic and fumaric [4], by complexation process, as well as complexes from pectin with ASA [5]. The decrease in the toxicity of the studied acids in the composition of the complexes with pectin was shown.

Moreover, for the complex of pectin with acetylsalicylic acid (P-ASA), the structure of which is given on the formula (I), the absence of an ulcerogenic effect on the gastric mucosa was revealed after a ten-day course with a P-ASA dose of 500 mg/kg. This dose corresponds to the equimolar therapeutic dose of ASA 50 mg/kg, which under similar conditions of administration caused the hyperemia of the gastric mucosa and the strengthening of the star-shaped vascular pattern [5, 6].



As has been shown in the literature, the complexation of active substances with biopolymers leads to a change in their physicochemical and biological properties. There are some studies aimed at developing a matrix based on chitosan for the controlled release of active compounds [7]. Pectins are also of interest as a polymer matrix for active substances, since complexation with pectins leads to a decrease in toxicity as well as to an increase in bioavailability and prolongation. These properties of pectins are used in the treatment of a number of pathological states, also, pectins are included in composition of many long-acting drugs. The potentiating and detoxic effects of pectins have been revealed in combination with penicillin, tetracyclines and neomycin [8].

In addition, pectin appears to have immunomodulatory properties [9-11]. Numerous in-progress clinical trials research the possibility of using pectins as a matrix of drug delivery for the treatment of colorectal cancer [12]. High bioavailability of Fe and anti-anemic properties are shown for pectin complexes - Na, Fepolygalacturonate, and Na, Ca, Fe polygalacturonate and for the latter there is no antagonism between Fe and Ca [13] and absence of toxicity [14]. From the foregoing, we can expect that the complexation of ASA with pectin can lead to both an increase and a decrease in biological activity due to changes in bioavailability, as well as to the manifestation of new properties. Since the pharmacological properties of P-ASA, in particular, anti-inflammatory activity, have not been studied, their study and comparison with ASA is of scientific and practical interest. The aim of the work is comparison of anti-inflammatory properties of the molecular pectin complex with 10% acetylsalicylic acid (here in after – P-ASA) and acetylsalicylic acid (here in after - ASA) in equimolar doses on the model of carrageenan-induced paw edema of rats.

MATERIALS AND METHODS:

Test Compounds: The acetylsalicylic acid (ASA) of the analytical purity brand was used. The pectin complex contained 10% of ASA (P-ASA) and presented on the formula (I) was synthesized correspondently to the methods described in works [5, 6].

Ethics Statement: Laboratory animal protocols performed in the present study were in compliance with the regulations of the Local Ethics Committee of Kazan Federal University. All animal experimentations and protocols were approved by the Local Ethics Committee of Kazan Federal University (Protocol No. 4 dated 18 May 2017).

The Design of Experiment: The study was carried out on 42 mature males of Sprague Dawley rats at the age of 7 months weighing 400 g at the start of testing. Animals have been obtained from the research and production enterprise laboratory animal farm based at the branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences (Pushchino).

The care and the use of the animals were in accordance with institutional guidelines [15, 16] in standard conditions in a vivarium with 12 h of daylight and *ad libitum* food and water. The animals were fed with complete feed made according to Specification (protein 22%, fiber 4% max, fat 5% max, ash 9% max, humidity 13.5% max, caloric value 295 kcal/100 g.

Animals were divided into 7 groups of 6 rats each on the analog's principle. To induce an inflammatory reaction accompanied by edema, a 1% carrageenan in aqueous solution was administered to the rats under plantar aponeurosis of the right rear paw. After 30 min, the test compounds were administered once orally in aqueous solutions: Group 1: control (water), Group 2: ASA 10 mg/kg, Group 3: ASA 20 mg/kg, Group 4: ASA 40 mg/kg, Group 5: P-ASA 100 mg/kg, Group 6: P-ASA 200 mg/kg, Group 7: P-ASA 400 mg/kg.

The doses of the various preparations were selected in such a way that the animals of groups 5, 6 and 7 to which the complex P-ASA was administered, received doses (100, 200 and 400 mg/kg) at equimolar to the minimum and optimal therapeutic doses of ASA (10, 20 and 40 mg/kg correspondently), which were used in groups 2, 3 and 4.

Investigation of the Inflammatory Reaction: Evaluation of the inflammatory reaction and anti-inflammatory activity of the studied substances was carried out according to the severity of edema and the level of pain sensitivity, according to work [17], [18]. The inflammatory response was measured by an increase in paw size, expressed as a percentage relative to the initial size, quantified on a plethysmometer (Ugo Basile) by volume of the displaced fluid. The level of pain sensitivity was investigated in the hot plate reaction test on the Hotplate 602000 device (TSE-Systems). The plate temperature was set at 55 °C. We determined the minimum time in seconds before signs of pain, for which they took a threefold withdrawal of the paw from the hot plate. The indicator was also expressed as a percentage relative to the initial pain sensitivity threshold. The measurements were carried out before carrageenan injection (0 h), as well as 2, 5 and 24 h thereafter.

Sample Preparation: Blood samples for research were taken *in-vivo* from the tip of the tail. To study the leukocyte profile, blood samples were collected in 0.2 ml EDTA K3 vacuum tubes (purple cap), then they were immediately explored on the mythic 18 vets automatic hematology analyzer. In order to study biochemical parameters of serum, blood samples were collected in vacuum tubes with gel and coagulation activator, and then they were twice centrifuged. The sampled serum before the biochemical blood test was stored in a freezer at a temperature of -25 °C. The serum samples were defrozen and stirred immediately before the test.

Blood Parameters Investigation: In order to study the physiological processes involved in the inflammatory reaction post carrageenan injection and the anti-inflammatory action of the test substances, the blood parameters leukocyte profile, the level of cytokines and C-reactive protein were investigated. The level of malondialdehyde in the serum was determined as an effective biomarker of lipid oxidation (oxidative processes), and catalase activity of the serum was measured as a tool for the anti-oxidant status evaluation. The leukocyte profile was examined on the automatic hematology analyzer Mythic 18 Vet (Orphee, Switzerland) before the start of the experiment (0 h), 5, and 24 h after carrageenan injection.

The total number of leukocytes (WBC), lymphocytes (LYM), monocytes (MON) and granulocytes (GRA), and ratio of lymphocytes (LYM%), monocytes (MON%), granulocytes (GRA%) in % of the total number of leukocytes were determined. Interleukin IL-1β, IL-6, IL-8 cytokines, tumor necrosis factor TNFα was determined in serum in the control group and in experimental groups with the administration of the maximum dose of preparation (40 mg/kg of ASA and 400 mg/kg of P-ASA). The level of cytokines was studied before the start of the experiment (0 h) and at the peak of inflammation (5 h after carrageenan injection) on a MagPix multiplex analyzer using the Merck kit. C-reactive protein was determined by ELISA analysis using the Vector Best reagent kit. Malondialdehyde and catalase were determined by the methods as described previously 24 before the start of the experiment (0 h), 5, and 24 h after carrageenan injection. Measurements of malondialdehyde, catalase and ELISA tests were performed using an Epoch Bio Tek photometer reader.

Estimate of the Ulcerogenic Effect: The ulcerogenic effect of the drugs was investigated for the maximum doses only (40 mg/kg ASA and 400 mg/kg P-ASA) in comparison with water (control group). The study was performed on 9 rats with 3

animals in each group. The test compounds were administered daily in the morning for 10 days. The control group was administered with water. At the end of the course of administration, the animals were euthanized, similarly to the study [19, 20], after which the stomach was removed. The ulcerogenic effect was studied according to work [21] the stomachs were opened along the lesser curvature, cleaned of the contents, washed with saline, and immediately examined the state of the mucosa under a stereomicroscope.

Statistical Analysis: Statistical analysis was performed in such programs as SPSS, Origin 6.0 and STATISTICA. The obtained samples were analyzed for normality of distribution according to the Kolmogorov-Smirnov test. In the case of normal distribution in all subgroups, equality of means was tested with a student T-test, if data were not normally distributed, equality of medians was tested with a Mann-Whitney U-test.

RESULTS:

The Inflammatory Reaction and Anti-Inflammatory Activity of Substances: As a result, it was shown that 2 h after carrageenan injection, the volume of the paw increased (in the control group by 26.7%) and the time of pain decreased in the hot plate test (in the control group by 10%), which is associated with the development of the inflammatory reaction. 2 h after the injection of carrageenan in the experimental groups, to which 40 mg/kg of ASA and 400 mg/kg of P-ASA were administered, the severity of the edema was statistically significantly less ($p<0.05$) than in control **Fig. 1**. However, the studied drugs did not have a significant effect on the pain sensation after 2 h **Fig. 2**.

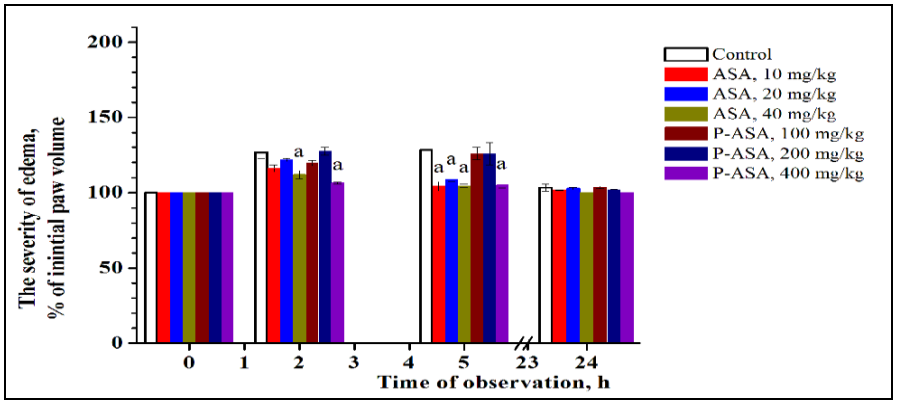


FIG. 1: THE EFFECT OF MOLECULAR COMPLEX P-ASA ON THE SEVERITY OF EDEMA UPON CARRAGEENAN INJECTIONIN COMPARISON WITH EQUIMOLAR DOSES OF ASA. (a) Show statistically significant differences ($p<0.05$) with the control group that did not receive drugs

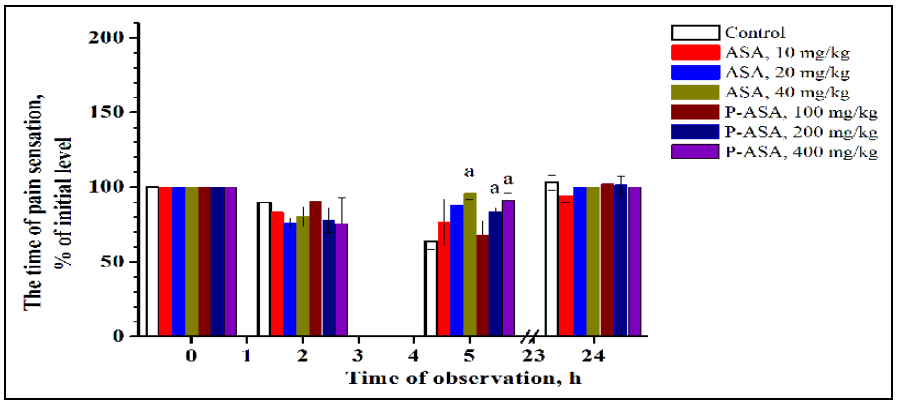


FIG. 2: ANALGESIC EFFECT OF P-ASA WITH CARRAGEENAN INJECTION IN COMPARISON WITH EQUIMOLAR DOSES OF ASA. (a) Show statistically significant differences ($p<0.05$) with the control group that did not receive drugs

After 5 h, in all groups that have been administered with ASA (10, 20, and 40 mg/kg), a perceptible decrease in edema was observed. The paw size was restored and exceeded the initial values by only 4-8%. The differences with the control are significant at $p<0.05$. With the injection of P-ASA, a similar

effect was observed only at a maximum dose of 400 mg/kg **Fig. 1**. ASA and P-ASA in equimolar doses of 20, 40 mg/kg, and 200, 400 mg/kg, respectively, had an equal effect on the manifestation of pain, increasing the time of appearance of pain in the hot plate test **Fig. 2**. The revealed differences with the control group were statistically significant ($p<0.05$) in the groups that were administered with 40 mg/kg of ASA, as well as 200 and 400 mg/kg of P-ASA **Fig. 1**.

Leukocyte Profile: When studying the leukocyte profile, it was shown that after the carrageenan injection WBC increased in all groups **Table 1** and a change in the ratio of leukocyte subpopulations was observed: absolute and differential monocyte and granulocyte counts were increased (MON, MON%, GRA, and GRA%), *i.e.* cells involved in phagocytosis, and the lymphocyte count (LYM and LYM%) was decreased. The most pronounced changes in the leukocyte profile were observed at the peak of inflammation, *i.e.* 5 h after the carrageenan injection. After 24 h, when the manifestations of the inflammatory reaction (edema, pain sensitivity) returned to normal **Fig. 1** and **2**, the leukocyte profile indices partially or completely recovered **Table 1**.

As a result of the study, the peculiarities of changes in the leukocyte profile depending on the administered drugs were identified, which is presented in **Table 1**. In the control group, the maximum increase in WBC was observed 5 h after carrageenan injection and remained at the same level after 24 h. With the injection of ASA in the doses of 10, 20, and 40 mg/kg and P-ASA in the doses of 100 and 200 mg/kg, on the contrary, the peak of WBC count was at 24 h.

Differences with baseline values in all these groups, except for 10 mg/kg, were statistically significant ($p<0.05$). With the P-ASA administration at the dose of 400 mg/kg, the peak of WBC counts was at the time of the peak of inflammation, *i.e.* at 5 h (differences with baseline values are statistically significant at $p<0.05$), and after 24 h, the WBC count was fully restored to its initial values **Table 1**.

When analyzing the ratio of leukocyte sub-populations, it was shown that in the control group, the absolute count of GRA at the 5^{th} h demonstrated a statistically significant increase ranging from 1.3 to 5.0 × 109 / L. The change in absolute counts of LYM and MON was not significant **Table 1**. As the percent change decreased at the fifth hour in total LYM count to baseline level 1.6 times ($p<0.05$), GRA% level was increased 3 times ($p<0.05$), and changes of MON% were insignificant ($p>0.05$). At the doses of ASA 20 mg/kg and P-ASA 100 and 200 mg/kg, the decrease in LYM count at 5 h was more pronounced, and the increase in GRA, on the contrary, was less pronounced compared to the control.

In all groups that were administered with preparations, a significant increase in MON count compared with the initial level ($p<0.05$) was observed **Table 1**. At the same time, the percentage of LYM% in the groups that were administered 10 and 20 mg/kg of ASA and 100, 200 and 400 mg/kg of P ASA did not differ from the control, and when administered ASA 40 mg/kg, LYM% was higher.

MON% in all experimental groups, except for the group 4 (40 mg/kg of ASA), was higher ($p<0.05$), and GRA%, except for the group 2 (10 mg/kg of ASA), was lower ($p<0.05$) compared to the control **Table 1**. After 24 h, despite the higher compared to the initial level, the percentage ratio of lymphocytes (LYM%) in the control and in experimental groups 2-6 was reduced ($p<0.05$) **Table 1**.

Both absolute and differential monocyte counts MON and MON% after 24 h were higher than baseline values in the control group and groups 2-6. The absolute count of granulocytes GRA was higher than the initial values in the control and in experimental groups 2-6 (in groups 2, 3, and 4 which were administered with ASA these differences were statistically significant at $p<0.05$).

The percentage ratio of GRA% was restored in all groups, except for the second (10 mg/kg of ASA). In the group 7, which was administered with P-ASA at the dose of 400 mg/kg, unlike other groups, a complete recovery of all the studied parameters of the leukocyte profile was observed after 24 h **Table 1**, which may indicate an immunomodulating effect of the complex due to the presence of pectin.

TABLE 1: INFLUENCE OF THE MOLECULAR COMPLEX P ASA AND ASA ON THE LEUKOCYTES PROFILE IN RATS WITH CARRAGEENAN PAW EDEMA

Indicators	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
WBC, $\times10^9$/L							
0 h	11.1 ± 1.1	12.3 ± 2.2	10.4 ± 0.5	8.5 ± 0.8	9.7 ± 0.2	11.0 ± 0.8	10.5 ± 0.8
5 h	14.2 ± 1.4	16.5 ± 4.7	9.5 ± 0.8	11.5 ± 1.2	11.9 ± 0.4b	11.2 ± 0.2	13.8 ± 0.5b
24 h	14.5 ± 1.5	23.4 ± 4.7	14.0 ± 1.3b	15.9 ± 1.8b	13.3 ± 1.1b	17.3 ± 1.5b	10.3 ± 1.8
LYM, $\times10^9$/L							
0 h	7.8 ± 0.6	6.3 ± 0.8	7.1 ± 0.4	5.9 ± 0.6	7.0 ± 0.3	8.1 ± 0.6	7.2 ± 0.6
5 h	6.4 ± 0.5	6.6 ± 1.8	4.1 ± 0.5a, b	6.1 ± 0.7	5.2 ± 0.4b	5.0 ± 0.2b	6.7 ± 0.7
24 h	8.5 ± 0.6	9.1 ± 2.6	7.4 ± 0.9	9.3 ± 1.2	8.7 ± 0.6b	11.2 ± 0.8a,b	7.2 ± 1.4
MON, $\times10^9$/L							
0 h	2.0 ± 0.4	1.6 ± 0.3a	1.0 ± 0.0b	0.9 ± 0.1	1.8 ± 0.2	2.0 ± 0.2	2.4 ± 0.1
5 h	2.9 ± 0.2	4.4 ± 1.0b	2.9 ± 0.2b	2.7 ± 0.3b	3.7 ± 0.1a, b	3.3 ± 0.2b	3.8 ± 2.4b
24 h	3.5 ± 0.5	4.3 ± 0.4b	3.5 ± 0.3b	3.8 ± 0.4b	3.2 ± 0.4	4.1 ± 0.5b	2.0 ± 0.3a
GRA, $\times10^9$/L							
0 h	1.3 ± 0.2	4.4 ± 1.2a	2.3 ± 0.2a	1.7 ± 0.1	0.9 ± 0.1	1.0 ± 0.2	0.9 ± 0.1
5 h	5.0 ± 0.7b	5.6 ± 2.0	2.4 ± 0.2	2.8 ± 0.2a, b	3.0 ± 0.2a, b	2.8 ± 0.3a, b	3.3 ± 0.1a, b
24 h	2.5 ± 0.6	10.0 ± 1.2a, b	3.2 ± 0.2b	2.8 ± 0.2b	1.4 ± 0.2	2.1 ± 0.4a	1.1 ± 0.1a
LYM,%							
0 h	71.2 ± 2.1	53.4 ± 3.3a	68.0 ± 0.5	69.2 ± 0.9	72.3 ± 1.8	73.2 ± 2.1	68.8 ± 1.0
5 h	44.9 ± 1.6b	41.7 ± 4.0	43.2 ± 1.5b	52.5 ± 1.4a, b	43.6 ± 1.7b	45.2 ± 1.9b	48.4 ± 3.3b
24 h	59.4 ± 2.9b	37.8 ± 3.6a, b	52.0 ± 1.7b	58.5 ± 1.1b	65.9 ± 2.2	64.8 ± 2.4b	69.3 ± 2.4a
MON,%							
0 h	17.5 ± 1.7	12.8 ± 0.2a	10.2 ± 0.3a	10.6 ± 0.3a	18.4 ± 1.3	17.7 ± 0.9	22.5 ± 0.8a
5 h	20.5 ± 0.7	28.2 ± 2.1a, b	31.7 ± 0.4a,b	23.0 ± 1.1b	31.4 ± 0.9a, b	29.9 ± 1.4a,b	27.6 ± 2.7a
24 h	24.0 ± 2.0b	19.7 ± 2.1b	24.9 ± 0.5b	23.9 ± 0.5b	23.8 ± 1.6b	23.3 ± 1.1b	19.9 ± 0.9b
GRA,%							
0 h	11.3 ± 0.1	33.9 ± 3.4a	21.9 ± 0.6a	20.2 ± 1.0a	9.3 ± 1.1	9.1 ± 1.2	8.7 ± 0.7
5 h	34.7 ± 2.2b	30.2 ± 5.1	25.1 ± 1.6a	24.5 ± 1.6a	25.0 ± 1.4a, b	24.9 ± 2.8a,b	24.1 ± 0.7a,b
24 h	16.7 ± 2.8	42.5 ± 2.9a	23.1 ± 2.1	17.6 ± 0.8	10.4 ± 0.7	12.0 ± 1.3	10.8 ± 2.1

a - differences with the control group 1 are significant ($p<0.05$), b - differences with initial values are significant ($p<0.05$)

Level of Cytokines: The study of cytokines showed that at 5 h later on the carrageenan injection, the changes were minor. Thus, no significant difference in the level of IL-1β and TNFα was shown before the experiment and 5 h after carrageenan injection, as well as between groups. IL-6 in the control and in group 4 (40 mg/kg of ASA) increased by only 0.2% after 5 h, but the differences with the baseline level were statistically significant. In group 7 (400 mg/kg of P-ASA), the increase of IL-6 was 0.7% only and was not statistic significantly **Table 2**. In the control group, IL-8 was increased by 1% (differences with baseline are not statistically significant, $p>0.05$). In both experimental groups 4 and 7, the level of IL-8 did not differ from initial values, wherein, in the group 4, differences of IL-8 values with the control were statistically significant ($p<0.05$).

TABLE 2: INFLUENCE OF THE MOLECULAR COMPLEX P ASA AND ASA ON THE CYTOKINES LEVEL IN THE BLOOD SERUM OF RATS WITH CARRAGEENAN PAW EDEMA

Indicators after 5 h	Before injection of carrageenan (0 h)	Group 1, 5 h (Control)	Group 4, 5 h (ASA 40 mg/kg)	Group 7, 5 h (P ASA 400 mg/kg)
IL-1β, pg/ml	0.776 ± 0.007	0.765 ± 0.003	0.774 ± 0.011	0.771 ± 0.005
IL-6, pg/ml	2.905 ± 0.005	2.910 ± 0.002b	2.910 ± 0.004b	2.907 ± 0.001
IL-8, pg/ml	0.623 ± 0.003	0.629 ± 0.003	0.618 ± 0.001a	0.623 ± 0.003
TNFα, pg/ml	2.954 ± 0.009	2.979 ± 0.010	2.975 ± 0.010	2.985 ± 0.012

a - differences with the control group 1 are significant ($p<0.05$), b - differences with initial values are significant ($p<0.05$)

Influence of Preparations on Catalase Activity and Malondialdehyde Level in Serum Blood: It was shown that inflammatory reactions and the action of anti-inflammatory substances might correlate with changes in the state of the pro / anti-oxidant system [22, 23, 24]. In this regard, the dynamics of changes in the malondialdehyde level in the blood serum, a parameter characterizing oxidative

processes, namely, the intensity of lipid peroxidation and the activity of catalase, an enzyme that protects cells from free radicals and characterizes the state of the endogenous anti-oxidant system, was studied. It was shown that the initial (background) values of studied parameters differed between some groups. In groups 2, 3, 4, and 7, catalase activity was higher than in groups 1, 5, and 6 ($p<0.05$). The level of malondialdehyde in groups 3 and 4 was higher than in other groups ($p<0.05$). In the control group, after carrageenan injection, the catalase activity was increased after 24 h **Fig. 3** ($p<0.05$), but the level of malondialdehyde was not changed during the day of observation **Fig. 4**.

At the peak of inflammation (5 h after injection of carrageenan), in all experimental groups, except for group 5 (100 mg/kg of P-ASA), the catalase activity was decreased compared to the initial level ($p<0.05$), but the differences from the control were not statistically valid. After 24 h, catalase activity was lower than the initial level in groups 2 and 4, which were administered with 10 and 40 mg/kg ASA, and in the group 3 (20 mg/kg ASA), catalase activity significantly exceeded both the initial level and control indices **Fig. 3**. In groups 5 and 6 (100 and 200 mg/kg of P-ASA), an increase in catalase activity was observed (dynamics is similar to the control group) and in the group 7 (400 mg/kg) the catalase activity did not exceed the initial level of activity **Fig. 3**.

At the peak of inflammation (5 h after carrageenan injection), the malondialdehyde level was decreased compared to the initial level in groups 3 and 4 (20 and 40 mg/kg ASA), which indicates a decrease in the intensity of lipid oxidation processes. In group 6 (200 mg/kg P-ASA), on the contrary, a sharp increase in malondialdehyde was observed **Fig. 4**. In other groups that were administered with P-ASA, the level of malondialdehyde remained at the control and baseline levels for 5 h. In group 2 (10 mg/kg ASA) the level of malondialdehyde increased compared to the control ($p<0.05$) **Fig. 4**.

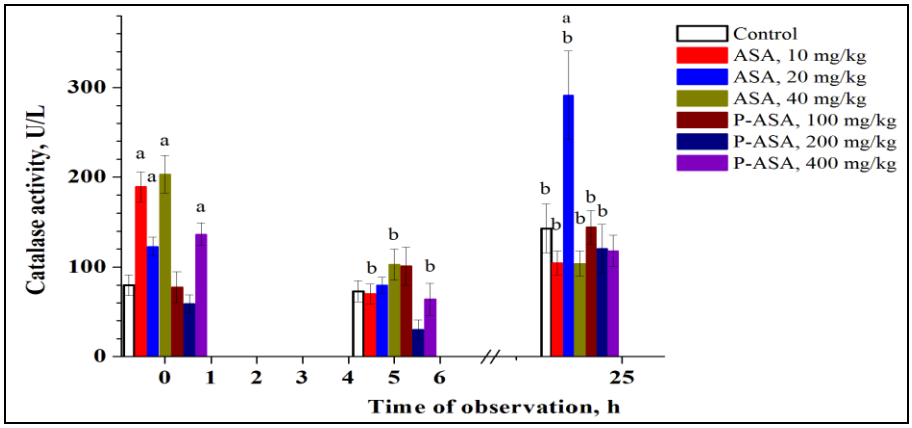


FIG. 3: CATALASE ACTIVITY OF SERUM BLOOD IN TESTED GROUPS OF RATS. a - differences with the control group 1 are significant ($p<0.05$), b - differences with initial values are significant ($p<0.05$)

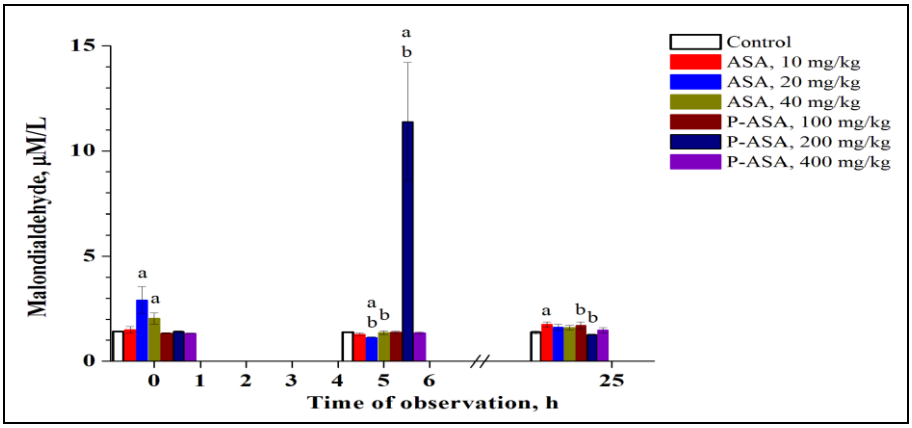


FIG. 4: MALONDIALDEHYDEOF SERUM BLOOD IN TESTED GROUPS OF RATS. a - differences with the control group 1 are significant ($p<0.05$), b - differences with initial values are significant ($p<0.05$)

Ulcerogenic Effect of Preparations: The study of ulcerogenic action was carried out, and it was shown that rats treated with the pectin complex P-ASA at the dose of 400 mg/kg, the gastric mucosa in all areas was pale pink and did not differ from the control **Fig. 5A**, *i.e.* it conforms with a normal state. Hyperemia is either not detected, or local hyperemic areas are detected, occupying no more than 1-3% of the surface of the gastric mucosa. The observation under the binocular showed the moderately pronounced vascular pattern **Fig. 5B**. In rats treated with ASA at the dose of 40 mg/kg, the mucosa in both body and pyloric areas of the gastric was hyperemic **Fig. 5 C**. The most pronounced hyperemia was detected in the area of the body along the lesser curvature of the stomach. Microscopic examination revealed a pronounced star-shaped vascular pattern. The area of hyperemia of the gastric mucosa ranged from 70 to 100% of the entire surface **Fig. 5D**.

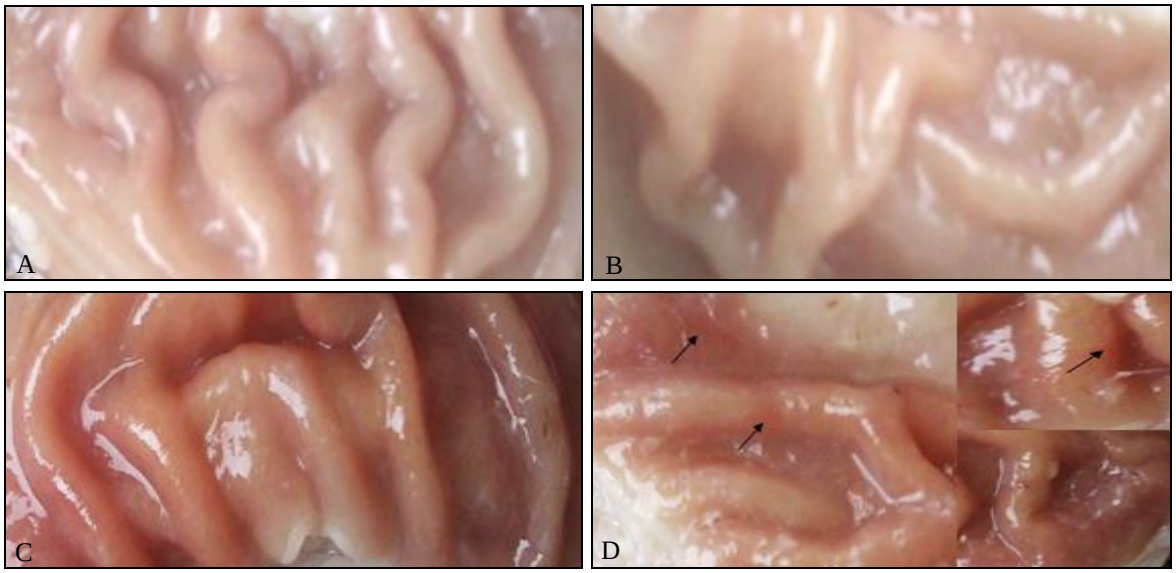


FIG. 5: STATE OF THE GASTRIC MUCOSA: A - CONTROL GROUP, B - GROUP TREATED WITH 400 mg/kg OF P-ASA, C, D - GROUP TREATED WITH 40 mg/kg OF ASA. (a - hyperemia of mucosa, b - star-shaped vascular pattern indicated by the arrow)

DISCUSSION: Thus, the results of a comparative evaluation of the anti-inflammatory activity and the ulcerogenic effect of the molecular complex of pectin with acetylsalicylic acid (P-ASA) and the individual substance acetylsalicylic acid (ASA) showed that P-ASA at the maximum therapeutic dose of 400 mg/kg has almost no ulcerogenic effect, in contrast to ASA in an equimolar dose of 40 mg/kg, causing hyperemia of the gastric mucosa and increased stellate pattern. These results are comparable with our previous data on the comparative assessment of the toxicity of P-ASA and ASA [5, 6] as well as with literature data confirming the ability of pectin to reduce the toxic and side effects of biologically active and medicinal substances during complexation [8]. This property of pectin complexes may be due to the enveloping effect of pectin, as well as the gradual release and absorption of the active drug component into the gastrointestinal tract. A comparative analysis of anti-inflammatory activity revealed some features of the action of P-ASA and ASA. Thus, the effect on the severity of carrageenan-induced edema, the effectiveness of P-ASA, is similar to the effect of ASA only with a maximum administered dose of 400 mg/kg (equivalent to 40 mg/kg of ASA). At the minimal doses of P-ASA 100 and 200 mg/kg, no effect on the edema reduction was observed, while ASA in equimolar doses of 10 and 20 mg/kg caused a significant decrease in the severity of edema.

That is, the formation of a complex with pectin led to a decrease in the anti-exudative component of the anti-inflammatory effect of ASA. On the other hand, in all studied doses of 100, 200, and 400 mg/kg, P-ASA exerted an analgesic effect comparable to the effect of ASA in equimolar doses. Based on the obtained results, it can be assumed that the formation of the ASA complex with pectin leads to partial blocking of the main mechanism of ASA action associated with the

suppression of the activity of the cyclooxygenase (COX) enzyme, which is confirmed by a decrease in the anti-edema effect. On the other hand, the complexation of ASA with pectin does not reduce the impact of ASA on the production of prostaglandins and other biologically active substances that play a key role in the occurrence of pain during inflammation, because the effect of P-ASA on reducing the pain sensation is similar to ASA.

A significant reduction in the ulcerogenic effect of P-ASA compared with ASA associated with inhibition of COX-1 activity, combined with a pronounced analgesic effect, suggests that the complexation of pectin with ASA leads to a predominant decrease in the effect of the drug on COX-1. The study revealed some features of the action of ASA and P-ASA on the response of immune cells of rats with carrageenan-induced edema. The main feature of the effects of drugs was less pronounced compared to the control increase in both GRA and GRA% counts at the peak of inflammation, which is associated with the anti-inflammatory pharmacological effect of ASA, including the suppressive effect on immune cells neutrophils [24], which are most rapidly activated in response on the injection of an antigen. In the groups that were administered with ASA, 24 h after administration of carrageenan, when the effect of the drug disappears, the number of neutrophils either continues to increase or remains elevated.

At the same time, in the groups that were injected with P-ASA, the number of GRA is increased only at the peak of inflammation, and after 24 hours it is fully normalized, which may be due, on the one hand, to prolongation, and, on the other hand, to immunomodulating properties, that is, with the peculiarities of the action of pectin [9-11]. The decrease in the intensity of oxidative processes at the peak of inflammation (5 h after carrageenan injection) under the action of ASA, assessed by the level of malondialdehyde in serum, can be explained by a decrease in the activity of neutrophils as a result of the immunosuppressive effect of ASA [26, 27] or by the manifestation of its antioxidant properties which is in agreement with a report [28]. In the groups that were treated with the molecular complex P-ASA, the level of malondialdehyde was not decreased, and when P-ASA was administered at the dose of 200 mg/kg, at the peak of inflammation, malondialdehyde was increased sharply, which may be due to the activation of oxidative processes occurring primarily in neutrophils in response to the injection of an antigen. This effect may be due to the immunomodulatory properties of pectin. On the other hand, it is possible that the complexation of pectin with ASA has led to a decrease in the antioxidant properties of ASA. A sharp increase in the activity of catalase 24 h after the administration of ASA at a dose of 20 mg/kg was revealed as the effect of ASA on the antioxidant system.

The results obtained are comparable with the literature data [27], confirming the anti-oxidant properties of ASA in a low dose of 30 mg/kg, namely, its ability to increase the activity of the antioxidant enzyme superoxide dismutase (SOD).

Analyzing the results obtained for catalase activity in the groups that were injected with P-ASA, it can be concluded that the complexation of pectin with ASA reduced the effect of ASA on the antioxidant catalase enzyme: on a decrease in its activity at the peak of inflammation and on an increase in its activity after 24 h later on administration, which is especially noticeable at a low dose of P-ASA of 100 mg/kg. In reports [24, 28, 29], changes in the level of cytokines TNFα, IL-6, IL-1β, IL-8 are mentioned in conjunction with the study of the intensity of inflammatory reactions.

It is noted that the level of these cytokines decreases when anti-inflammatory agents affected arthritis induced by Freund's adjuvant 24 or collagen-induced arthritis [28]. In work [29] it is noted an increase in IL-6 and IL-8 in osteoarthritis. In our study, in response to the carrageenan injection, a significant increase in IL-6 and a tendency to increase in IL-8 and TNFα were observed in the control group, which at the level of changes in these biomarkers confirms the development of inflammation.

The absence of differences with the initial level of IL-6 and IL-8 at the peak of inflammation in the group administered with P-ASA at the dose of 400 mg/kg confirms the effectiveness of the molecular complex P-ASA as an anti-inflammatory agent.

CONCLUSION: The results of the study prove that the formation of a complex of acetylsalicylic acid with pectin increases the safety of its use, as it leads to a decrease in ulcerogenic properties. The anti-inflammatory properties of acetylsalicylic acid with the use of the complex at the dose of 400 mg/kg are completely equivalent to the effectiveness of an equimolar dose of acetylsalicylic acid 40 mg/kg, but under the action of the complex more rapid normalization of the leukocyte profile is observed. When using low doses of the complex of 100 and 200 mg/kg, there is a decrease in the effectiveness on the severity of edema compared with equimolar doses of acetylsalicylic acid 10 and 20 mg/kg, but the analgesic effect of the complex and acetylsalicylic acid in equimolar low doses are equivalent.

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