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FEATURES OF FRAGMENTATION OF PARACETAMOL IRRADIATED WITH ELECTRONS (18 MeV) ON THE M-30 MICROTRON

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This study investigates the fragmentation characteristics and changes in the biological activity of pure and tableted paracetamol following irradiation with an 18 MeV electron beam in an M-30 microtron. Using aqueous-phase mass spectrometric studies, fragmentation pathways were investigated, characteristic ion peaks were identified, and the influence of excipients in the tablet formulation on the formation of molecular fragmentation products was determined. Changes in the drug's antibacterial and antifungal activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Candida albicans* were evaluated. It was shown that electron irradiation causes significant structural changes in tabletted paracetamol and alters its biological activity. The results obtained indicate the potential of using radiation modification to develop drugs with improved pharmacological properties.

INTRODUCTION

It is well known that the therapeutic effect of drugs, in addition to their chemical composition, is largely determined by the way they are delivered into the body: in powder, tablet, or liquid form, either orally or parenterally via injection.

This offers a wide range of opportunities to improve the therapeutic efficacy of pharmaceutical drugs by modifying their chemical state or physical form using thermal, physicochemical, or nanotechnological methods. One such approach is the fractionation of the active ingredient, which helps accelerate its dissolution and absorption and increase its bioavailability. Recently, there has been active research aimed at improving the biological activity of drugs by mechanically converting them into ultradisperse powders through grinding and micronization. As a rule, these methods are applied to the starting ingredients in the early stages of drug production, before they are tableted or converted into another dosage form. It is assumed that this treatment increases the dissolution rate of the solid by increasing its specific surface area and reducing its average particle size. Studies [1, 2] report that aspirin particles of 1…10 μm were produced, resulting in dosage forms with improved solubility and bioavailability.

The use of ionizing radiation to selectively modify the therapeutic properties of pharmaceutical preparations is a promising area of development in modern pharmaceutical science. This method enables alteration of the physicochemical and biological properties of active pharmaceutical ingredients and excipients through molecular modification, thereby improving their therapeutic properties. Mass spectrometric studies of irradiated bioorganic compounds [3] – which form the basis of many pharmaceuticals – indicate similarities in their fragmentation pathways under the influence of ionizing radiation and during biochemical metabolism. In other words, irradiated compounds may reflect specific stages of their biochemical transformations occurring in the human body. This opens up opportunities for the development of fundamentally new drugs with regulated, selective biological activity based on radiation technologies. In addition, ionizing radiation can be used to sterilize pharmaceuticals, a crucial step in ensuring their microbiological safety.

At the same time, there is practically no information in the literature on the radiation modification of mass-produced pharmaceuticals, in which the irradiation of ready-made dosage forms can modulate their biological activity, complementing traditional manufacturing technologies. This applies primarily to tablet formulations, which account for more than 90 % of the global pharmaceutical market. A distinctive feature of their production is the use of excipients (fillers, binders, disintegrants, and other processing aids), as well as, in many cases, a polymer coating [4]. This composition is optimized for oral applications, but the specifics of the fragmentation of the "active ingredient – additional components" system, the nature of their interaction after irradiation, and changes in biological activity require separate study. The relevance of such studies is also due to the potential of ionizing radiation to enable the creation of modified tablet formulations with controlled release of active ingredients [5].

The present study focuses on paracetamol (Paracetamolum), one of the world's most widely used analgesics and antipyretics, a chemical compound with the formula $C_8H_9NO_2$ [6]. Under numerous trade names, tablet formulations account for more than 60% of all over-the-counter analgesic and antipyretic medications [7].

Investigation of paracetamol fragmentation pathways is of particular importance because one of its metabolites, p-aminophenol, reacts with arachidonic acid to produce AM404 ($C_{26}H_{37}NO_2$) [8], which is considered one of the key mediators responsible for the central analgesic mechanism of paracetamol. Particular

interest lies in studying these processes in tablet formulations to understand the interactions between the active ingredient's fragmentation products and excipients. Such information can be obtained through mass spectrometric analysis of irradiated tablets combined with biological activity studies.

The present work continues our previous investigations [9, 10] performed using the M-30 electron microtron.

OBJECT OF STUDY AND EXPERIMENTAL CONDITIONS

The study focused on samples of pure paracetamol in powder and tablet form. The structure of the paracetamol molecule (Fig. 1) consists of a benzene ring to which two functional groups are attached in the para position (1,4): a phenolic hydroxyl group (–OH) and an acetamide group (–NH–CO–CH_3). The structural formula of paracetamol ($C_8H_9NO_2$), shown in Fig. 1, indicates that it is a derivative of para-aminophenol in which the amino group is acetylated to form an amide bond. The presence of phenolic and amide functional groups attached to the benzene ring determines the molecule's physicochemical properties, its reactivity, and the characteristics of its transformations under the influence of ionizing radiation [11].

Paracetamol tablets were used in the form of the medication "Paracetamol-Darnitsa." Each tablet weighed 600 mg, of which 500 mg was the active ingredient – paracetamol ($C_8H_9NO_2$) – and 100 mg was the excipients: pregelatinized starch ($(C_6H_{10}O_5)_n$), povidone ($(C_6H_9NO)_n$), microcrystalline cellulose ($(C_6H_{10}O_5)_n$), croscarmellose sodium ($(C_6H_7O_2[OCH_2COONa]_2)_n$), and calcium stearate ($C_{36}H_{70}CaO_4$). Excipients serve technological functions as fillers, binders, disintegrants, and lubricants, ensuring the mechanical strength of the tablets, their stability during storage, and the necessary disintegration and release characteristics of the active ingredient.

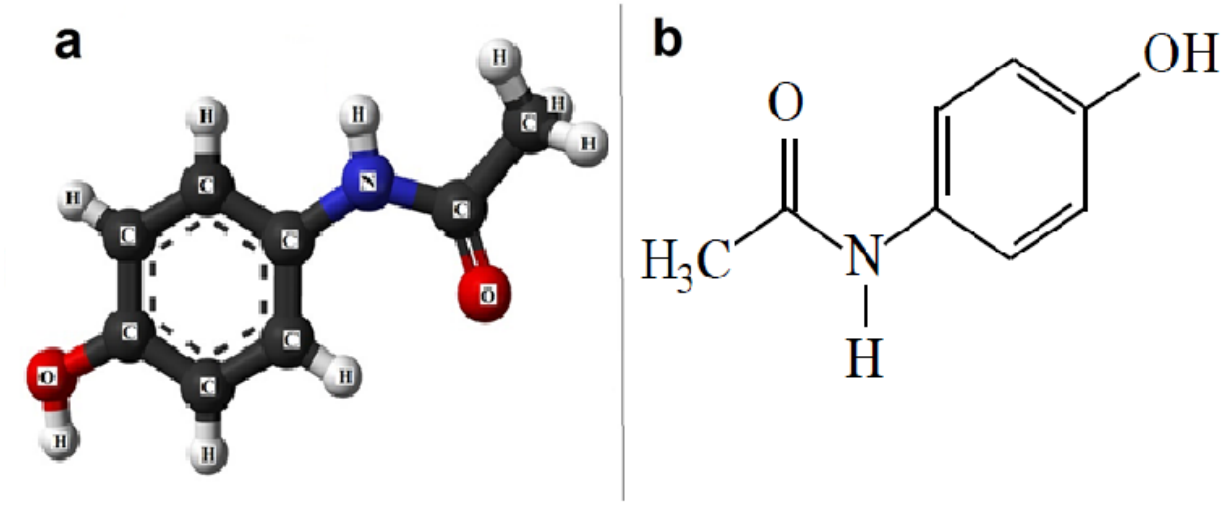


Fig. 1. Structural formulas of paracetamol ($C_8H_9NO_2$): a – 3D view and b – structural formula [12]

The samples under study were irradiated in an M-30 microtron at 18 MeV. The samples were placed in airtight plastic containers 50 cm from the electron source. The uniformity of the irradiation field over an area of 100 cm² was no worse than 5%; the irradiation conditions did not cause the samples to heat up. The flux density over the irradiation area was $2.25 \cdot 10^{10}$ electrons$\cdot$cm$^{-2}\cdot$s^{-1} and was controlled by a Faraday cylinder. Two series of irradiation experiments were conducted on the samples with fluences D1 = $2.02 \cdot 10^{13}$ electrons/cm² and D1 = $4.04 \cdot 10^{13}$ electrons/cm²; with reference absorbed dose estimates of 2.0 and 4.0 kGy.

Mass spectrometric studies were performed using an MX-7304A quadrupole mass spectrometer, a dynamic mass analyzer. The recorded mass range was 0…160 Da with a mass resolution of $\Delta M \leq 1$ Da. The experiment was carried out using the crossed electron–molecular beam technique, in which the electron and molecular beams intersect at 90°. The electron source operated at a fixed electron energy of 10…70 eV while maintaining a stabilized beam current of 0.05…0.5 mA. In the present study, the ionizing electron energy was set to 70 eV.

The experimental setup and measurement procedure have been described in detail elsewhere [13, 14]. In the present experiments, the molecular beam was generated by transferring aqueous solutions of pure and tableted paracetamol from the liquid phase into the gas phase. The molecular concentration in the interaction region was within the range of $5 \cdot 10^{10} \ldots 10^{11}$ cm^{-3}. The ions produced by electron-impact ionization were separated by mass-to-charge ratio (m/z) and recorded using an automated data acquisition system with digital registration of ion intensity and mass-to-charge ratio.

Data acquisition and processing were performed automatically using specialized software. Particular attention was paid to mass scale calibration, which was carried out using reference mass spectra of argon, krypton, and xenon. Their principal and isotopic peaks were used to calibrate the mass scale.

Microbiological studies were conducted at the Research and Training Center for Molecular Microbiology and Mucosal Immunology at Uzhhorod National University. The biological effects of irradiated and non-irradiated tablet samples were evaluated based on their antibacterial, antifungal, or growth-stimulating activity against selected test microorganisms [15, 16]. Irradiated and non-irradiated paracetamol tablets were dissolved in 20 ml of MRS broth. The investigated microorganisms included clinical isolates of opportunistic pathogens: Gram-negative rods

Escherichia coli (lac+), Gram-positive cocci *Staphylococcus aureus*, Gram-positive spore-forming rods *Bacillus subtilis 090*, and yeast-like fungi *Candida albicans*. In total, four test microorganisms were included in the study.

OBTAINED RESULTS AND THEIR DISCUSSION

The high-energy ionizing radiation causes extensive destruction of bioorganic compounds, leading to molecular fragmentation and the formation of numerous structural fragments whose composition depends on the irradiation conditions. The subsequent evolution of these fragmentation products may proceed through various decomposition and recombination pathways, leading either to partial reconstruction of the parent molecule or to the formation of new chemical compounds. Under identical irradiation conditions, the probability of forming a particular reaction product is governed by temperature, the composition and concentration of molecular fragments, and their relaxation time. Figuratively, this process resembles the assembly of a Lego construction set [17], where the same structural building blocks can be rearranged to reconstruct the original molecule or generate entirely new molecular structures depending on the subsequent interaction conditions.

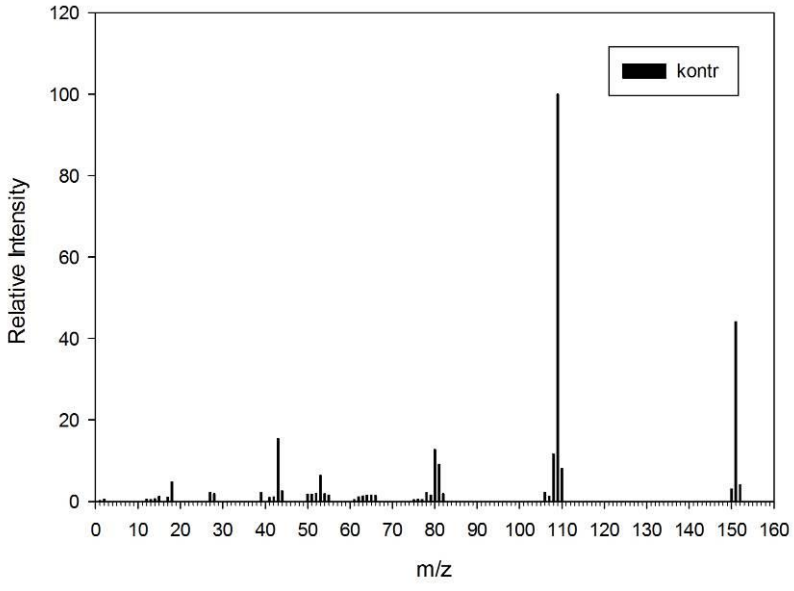
The mass spectrometric technique employed in this work is based on the ionization of molecular beams in vacuum mostly by 70 eV low-energy electrons. Prior irradiation of the samples modifies the composition of the generated fragmentation products, which is reflected in changes in the intensities and relative abundances of individual peaks in the recorded mass spectra [18].

Mass spectrometric analyses: pure acetaminophen. The data from mass spectrometric analyses of aqueous solutions of pure acetaminophen are presented in Fig. 2. A common feature of all these spectra is the presence of five groups of characteristic peaks, among which the peaks with m/z = 43, 53, 80, 109, and 151 Da are the most intense. Their positions fully correspond to the NIST reference values [19].

The presence of the main characteristic peaks in the mass spectrum of paracetamol upon interaction with electrons allows us to propose a fragmentation scheme, which is shown in Fig. 3. The mechanism of its formation is as follows: intramolecular proton transfer occurs via the transfer of a hydrogen atom from the methyl group to a nitrogen atom, leading to α-cleavage of the amide bond, the formation of the ketene molecule C_2H_2O (m/z = 42), and subsequently the $C_7H_5ON^+$ ion. Subsequent rearrangement, followed by loss of a formaldehyde radical, leads to the formation of $C_3H_4O_2^+$, and ionization of the amine oxygen occurs via α-cleavage, forming the CH_3CO^+ cation.

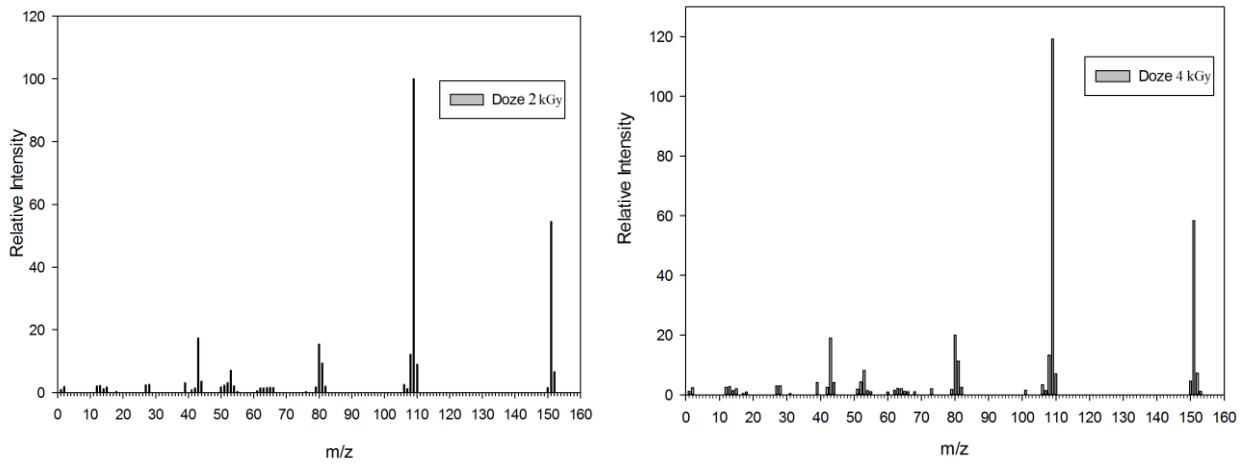
Let us analyze the fragmentation scheme of pure acetaminophen (see Fig. 3):

- The neutral fragment (ketene) C_2H_2O m/z = 42 in the mass spectrum of acetaminophen obtained by electron impact is the ejection of a neutral molecule. In contrast, the molecular (parent) ion $C_8H_9NO_2^+$ (m/z = 151) decomposes via cleavage from the acetamide group, forming the base peak, aminophenol $C_7H_5ON^+$ (m/z = 109).
- The $C_2H_3O^+$ fragment ion with m/z 43 (acetyl cation) is one of the most common and characteristic fragments in organic mass spectrometry; it indicates the presence of a carbonyl group, particularly methyl ketone. This peak originates from two main components: the acyl cation and the propyl or isopropyl cation.



a

Fig. 2. Mass spectra of aqueous solutions of pure acetaminophen: a – unirradiated sample



b *c*

Fig. 2. Mass spectra of aqueous solutions of pure acetaminophen: b – sample irradiated with dose D1; c – sample irradiated with dose D2

- The $C_4H_5^+$ fragment ion (m/z = 53) is an important and widely encountered cation in the mass spectrometry of organic compounds; it arises from the electron ionization of aliphatic hydrocarbons and cyclic structures, and in mass spectra, it indicates the presence of branched or cyclic structures containing this fragment.
- The $C_4H_5^+$ fragment ion (m/z = 80) is a cyclopentadienylidene ammonium cation. It is formed as a result of the cleavage of a ketene molecule from the parent paracetamol ion, resulting in a stable p-aminophenol ion with m/z = 109, which undergoes intramolecular rearrangement followed by narrowing of the benzene ring (see Fig. 1) to a five-membered ring. This is followed by the loss of a CHO radical (m/z = 29), which leads to the formation of the cyclopentadienylidene ammonium conjugate cation:
- The $C_7H_5ON^+$ fragment ion (m/z = 109) is the para-aminophenol radical cation, corresponding to the aromatic fragment with an amino group – the protonated radical cation of hydroquinone. This fragment is very stable due to the combination of an unshared pair of nitrogen electrons with the aromatic ring (see Fig. 1). Upon further decomposition, the ion loses a molecule of carbon monoxide (CO) or a formaldehyde radical, transforming into ions with m/z = 81 or 80.

Thus, the proposed fragmentation scheme for the paracetamol molecule reflects the basic principles of mass spectrometry and allows a clear determination of the fragmentation mechanism under electron influence. It should be noted that fragments of the paracetamol molecule may influence the biological activity of n-acetylaminophenol-based drugs in certain ways.

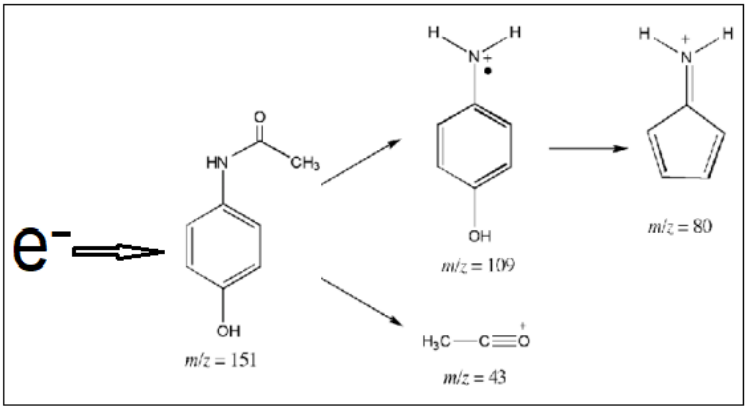
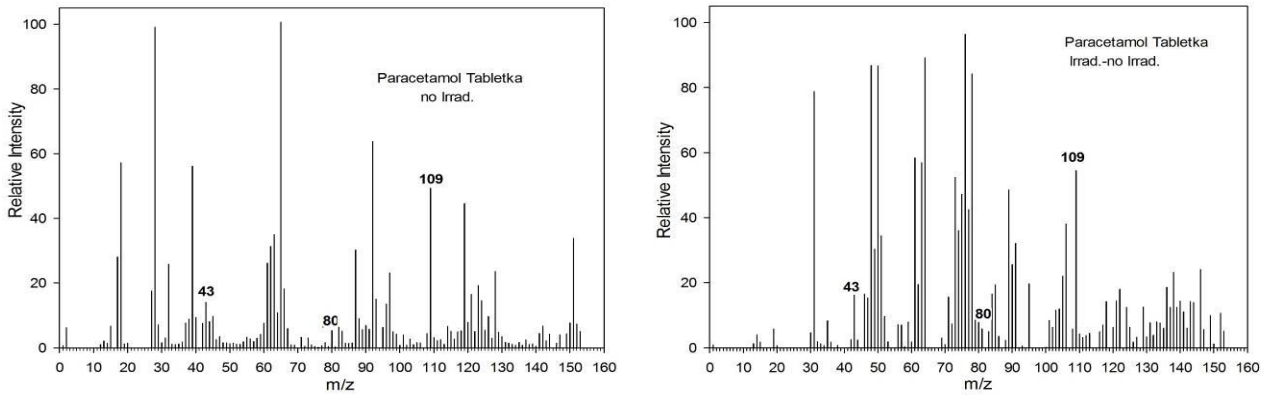


Fig. 3. Schematic representation of paracetamol molecule fragmentation

Analysis of the mass spectra shown in Fig. 2 indicates that the main difference in irradiated paracetamol is an increase in intensity and the appearance of several additional peaks. Radiation exposure does not alter the number or overall structure of the fragment groups resulting from the cleavage of paracetamol molecules. It shows a slight, within the range of 20…30%, tendency toward an increase in the peaks of light fragments from 28 to 65 in m/z units.

Mass spectrometric studies: tableted paracetamol. Fig. 4 shows the mass spectra of tableted paracetamol, before and after irradiation with a dose of 4 kGy, obtained from the liquid phase.



a *b*

Fig. 4. Mass spectra of aqueous solutions of tableted paracetamol: a – unirradiated sample; b – sample irradiated with dose D2; the residual lines shown were obtained by subtracting the mass spectrum of the irradiated sample from that of the unirradiated sample (see a)

In contrast to the previous result, this figure demonstrates a significant effect of radiation on the fragmentation spectrum of tableted acetaminophen. Specifically, the mass spectra shown contain the main paracetamol peaks at m/z = 43, 53, 80, 109, 151, and 152, as well as fragment ion peaks from the tablet excipients: calcium stearate ($C_{36}H_{70}CaO_4$), sodium croscarmellose ($C_6H_7O_2[OCH_2COONa]_2)n$, microcrystalline cellulose ($C_6H_{10}O_5)n$, corn starch ($C_6H_{10}O_5)n$, and povidone ($C_6H_9NO)n$. The complex structural formulas of these components undoubtedly account for the fragment-rich mass spectrum of the Tablets.

It is worth noting the trend in the transformation of the mass spectrum of the tableted sample after irradiation (see Fig. 4,b) – an increase in the intensity of the paracetamol molecular fragment peaks, at m/z = 43, 53, 80, and 109 against a background of a series of peaks from the organic components of the excipients, m/z = 15, 30, 60, 90, and 120. This can be explained by the high self-regeneration capacity of irradiated paracetamol, whereas the excipient components do not possess such a capacity. Biological activity. Unirradiated and irradiated paracetamol tablet samples were tested for biological activity using the results of a physical experiment. A bacterial suspension was prepared from a 24-hour culture of the corresponding microorganisms to a turbidity standard of 0.5 McFarland units ($1.5 \cdot 10^8$ CFU/ml), as determined using a Den-1 densitometer. The purity of the culture, specifically the morphological and tinctorial properties of the test microorganisms, was determined by bacterioscopy (Gram stain) using immersion microscopy with a light microscope (Primo Star iLED, Carl Zeiss). The number of microorganisms, expressed as colony-forming units per 1 ml (CFU/ml), was determined using the plate method to prepare a tenfold serial dilution series [16, 20]. Exposure time (co-cultivation) was 1 h in a thermostat at 36.8…37 °C. The suspensions were inoculated onto appropriate culture media (Nutrien Agar, Mannitol Salt Agar, Sabouraud Dextrose Agar, Endo Agar manufactured by Biolife, Italy). The study was conducted in two stages: stage 1, immediately after irradiation, and stage 2, 24 h later.

Characteristics of the biological effects of pure and tableted paracetamol – both untreated and irradiated with 18 MeV energy using the M-30 microtron – on a population of selected bacteria; the dose parameters D1 and D2 are given in the text

No	The name of the solution	Initial concentration, CFU/ml	Concentration, CFU/ml			
			Bacillus subtilis 090		*Escherichia coli (lac+)*	
			stage 1	stage 2	stage 1	stage 2
1	Paracetamol, control	$1.5 \cdot 10^8$	$(1.0 \pm 0.2) \cdot 10^4$	$(>1.0 \pm 0.3) \cdot 10^{10}$	$(4.0 \pm 0.6) \cdot 10^6$	$(6.0 \pm 0.4) \cdot 10^5$
2	Paracetamol, dose D1	$1.5 \cdot 10^8$	$(1.0 \pm 0.2) \cdot 10^4$	$(>1.0 \pm 0.4) \cdot 10^{10}$	$(<1.0 \pm 0.2) \cdot 10^4$	$(<1.0 \pm 0.2) \cdot 10^4$
3	Paracetamol, dose D2	$1.5 \cdot 10^8$	$(1.0 \pm 0.2) \cdot 10^4$	$(1.0 \pm 0.3) \cdot 10^8$	$(<1.0 \pm 0.2) \cdot 10^4$	$(<1.0 \pm 0.2) \cdot 10^4$
			Staphylococcus aureus		*Candida albicans*	
			stage 1	stage 2	stage 1	stage 2
1	Paracetamol, control	$1.5 \cdot 10^8$	$(1.0 \pm 0.3) \cdot 10^6$	$(1.0 \pm 0.3) \cdot 10^6$	$(<1.0 \pm 0.2) \cdot 10^4$	$(7.0 \pm 0.5) \cdot 10^5$
2	Paracetamol, dose D1	$1.5 \cdot 10^8$	$(<1.0 \pm 0.2) \cdot 10^4$	$(3.0 \pm 0.4) \cdot 10^8$	$(<1.0 \pm 0.2) \cdot 10^4$	$(1.0 \pm 0.2) \cdot 10^6$
3	Paracetamol, dose D2	$1.5 \cdot 10^8$	$(<1.0 \pm 0.2) \cdot 10^4$	$(<1.0 \pm 0.2) \cdot 10^4$	$(<1.0 \pm 0.2) \cdot 10^4$	$(2.0 \pm 0.3) \cdot 10^6$

According to the study results, during the first stage (stage 1), the samples under investigation exhibited pronounced inhibitory activity against most of the microorganisms tested, demonstrating antibacterial and antifungal properties. Escherichia coli (lac+) and Staphylococcus aureus proved to be the most sensitive to paracetamol, with a significant reduction in viable cell concentration observed in these strains.

In the second stage of the study (stage 2), antifungal activity against Candida albicans persisted, although growth was partially restored. Among the bacterial cultures, a pronounced inhibitory effect persisted

against Escherichia coli (lac+), indicating a prolonged antibacterial effect of the samples under study.

Of particular note is the Paracetamol sample (dose D2), which demonstrated sustained antibacterial activity of tableted paracetamol against Staphylococcus aureus, with this effect persisting throughout the entire observation period. The results indicate that the antimicrobial activity of irradiated tabletted paracetamol is dose-dependent.

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CONCLUSIONS

The results obtained indicate complex fragmentation patterns for both pure and tableted paracetamol in aqueous solutions, both with and without irradiation by high-energy (18 MeV) electrons. It should be noted that the obtained mass spectra indicate probable pathways for cleavage of the paracetamol molecule during its transformations in biological processes. While it is possible to compare mass spectra of pure paracetamol obtained from the solid and liquid phases, we obtained the mass spectra of the tableted substance for the first time, both for unirradiated and irradiated samples.

The research results demonstrate the high ability of paracetamol molecules to self-recover after exposure to ionizing radiation. For tablet samples, the fragmentation channels of both paracetamol and the excipient components overlap, resulting in a complex overall mass spectrum. It has been shown that radiation treatment promotes the ordering of organic-molecule fragmentation processes and leads to the dominance of characteristic paracetamol fragmentation channels over the general background.

The study also demonstrated a change in the biological activity of tableted paracetamol following radiation treatment. It was established that irradiation enhances both the antibacterial and antifungal activity of the drug and exerts a selective effect on microorganisms from various taxonomic groups, with this effect sustained over time. The results indicate the potential to use ionizing radiation to modify the biological activity of pharmaceutical drugs specifically.

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ОСОБЛИВОСТІ ФРАГМЕНТАЦІЇ ПАРАЦЕТАМОЛУ, ОПРОМІНЕНОГО ЕЛЕКТРОНАМИ (18 МеВ) НА МІКРОТРОНІ М-30

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Досліджено особливості фрагментації та зміну біологічної активності чистого та таблетованого парацетамолу після опромінення електронним пучком з енергією 18 МеВ на мікротроні М-30. За допомогою мас-спектрометричного аналізу досліджено канали фрагментації, ідентифіковано характерні іонні піки та встановлено вплив допоміжних компонентів таблетованої форми на формування мас-спектрів. Оцінено зміни антибактеріальної та протигрибкової активності препарату щодо *Escherichia coli, Staphylococcus aureus, Bacillus subtilis* та *Candida albicans*. Показано, що електронне опромінення викликає суттєві структурні зміни молекул таблетованого парацетамолу та сприяє модифікації його біологічної активності. Отримані результати свідчать про перспективність використання радіаційної модифікації для створення лікарських препаратів із покращеними фармакологічними властивостями.