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GLUTEN CONTENT IN PHARMACEUTICALS MARKETED IN THE GAZA STRIP

Mai Ramadan[1*], Mohammed Shbair[2] and Raghda Al-Astal[2]

[1]*Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmacy, Al-Azhar University- Gaza, Gaza, B. O Box: 1277, Palestine*
[2]*Department of Pharmacology and Therapeutics, Faculty of Pharmacy, Al-Azhar University-Gaza, Gaza, B. O Box: 1277, Palestine*

Gluten is a wheat protein which could trigger an immune-mediated inflammatory response causing Celiac disease. Pharmaceuticals are possible source of gluten due to excipients like wheat starch.

This study examined some pharmaceuticals (non-prescription and prescription type) on gluten and partially hydrolyzed gluten fragments content using sandwich and competitive ELISA techniques, respectively.

Partially hydrolyzed gluten fragments were detected in ca. 37% of tested samples (Total: 38). Only 5 samples had gluten concentration above 20 ppm. None of the pharmaceuticals had detectable levels of glutens upon sandwich ELISA application. Oral administration of four dosage forms at recommended doses provided celiacs with a daily gluten intake exceeding the daily tolerance limit. Excipients in patient information leaflet were absent in most cases.

The findings confirmed that pharmaceuticals could increase the burdens of celiac patients. Accordingly, regular examination of pharmaceuticals on gluten content is advised. Further studies are recommended that would include a larger sample size, and analytical methods to determine gluten in pharmaceuticals should be developed. Registration agencies in the Gaza Strip should apply updated regulations of excipients.

Keywords: *Gluten, Celiacs, ELISA, Pharmaceuticals*

INTRODUCTION

Celiac disease (CD) is an immunological disorder that is caused by ingestion of gluten in genetically predisposed people[1]. The prevalence of CD is 1% globally, but only 30% of patients are properly diagnosed[2]. Exposure to gluten in CD patients causes inflammation in the gut, and damage in the small intestine. The damaged mucosa renders it unable to digest and absorb nutrients resulting in mal-digestion and malabsorption[3].

Gluten - major storage protein in the grain – is responsible for viscoelastic properties. It encompasses gliadins (Soluble in ethanol), and glutenins (Soluble in dilute acid or alkali solutions)[4]. The immunogenic T-cell mediated response of gluten is caused by gluten peptides, which are produced by partial digestion of gluten. The most immunogenic gluten peptide is α2-gliadin, which is derived from α- and γ-gliadin[5].

The only way to avoid symptoms in celiacs is to follow a gluten free diet (GFD)[6]. The main sources of gluten are wheat, barley, rye, and triticale[7]. CD patients adhere strictly GFD could intake gluten unintentionally via medicines, vitamins, and supplements due to excipients like starch or pregelatinized starch used in pharmaceutical formulation or contamination during manufacturing process[8&9].

According to FDA (Food and Drug Administration) issue in 2017 pharmaceutical industry can voluntarily labeling oral medications to be gluten free when they are

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*Corresponding author: Mai Ramadan, E-mail: m.ramadan@alazhar.edu.ps

sure that no gluten sources as excipients were used in the products[10]. Few studies are published regarding gluten in medications, and most of them were descriptive studies[11&12].

Diverse analytical methods were developed to detect gluten e.g. polymerase chain reaction (PCR), LC-MS, and immunoassay[13-15]. Immunological methods like ELISAs (Enzyme Linked Immunosorbent Assays) are applied widely due to simplicity, specificity, sensitivity, and cost effectiveness[16&17]. Many factors contribute to differences between methods of gluten analysis e.g. diverse matrices, extraction buffer, extraction time and temperature, calibration standard, and specificity of antibodies[18-21]. ELISA R5 and Mendez Cocktail extraction is applied by Codex Alimentarius as Type I official method since 2006[22].

CD patients on GFD attending Ard El Iinsan Association in Gaza strip showed exacerbated symptoms upon oral administration of some medications. These notifications enforce our team to investigate medications as possible source of gluten using immunoassay.

MATERIALS AND METHODS

Chemicals and reagent

ELISA kits (RIDASCREEN GLIADIN) for Competitive assay (R7021) and for Sandwich assay (R7001), and Cocktail patented (R7006 / R7016) were purchased from R-Biopharm, Germany. Ethanol p.a. grade was obtained from (Merck, Germany) and distilled water was prepared by Milli-Q purification system (Merck, Germany).

Samples

Pharmaceutical dosage forms (Tablet, film coated tablet, powder ready for reconstitution, hard gelatin capsule) were collected from community pharmacies in Gaza Strip. Sample size was 38 dosage forms, which were reported by CD patients in Gaza Strip to cause gluten related reaction, along with some over the counter medications (OTC) and prescription medications.

Preparation of the samples

For competitive ELISA assay, tablets were crushed using mortar and pestle, then one gram of each sample was weighed and put in a 15ml test tube.

Hard gelatin capsules were emptied and one gram was weighed including the powder and the hard shell. Capsules containing granules were emptied and the granules were crushed before being placed in the test tube.

Powder ready for reconstitution was used directly in weighing (1g) since the product was already homogenized.

From liquid dosage forms including syrups and suspensions 1 mL was transferred in test tube.

The same procedures were followed in sandwich ELISA assay except 0.25 g for solid dosage forms and 250 µl for liquid dosage forms were used.

Analysis of the samples

In sandwich ELISA to the samples (0.25 g for solid dosage forms and 250 µl of liquid dosage forms in test tubes) 2.5 mL of cocktail patented were added, and mixed well. Samples were incubated at 40 minutes at 50°C. After cooling 7.5 mL 80% ethanol were added, then centrifuged for 10 min at 2500 g and room temperature. The samples were diluted 1:12.5 with sample diluent and 100 µL were used in assay. The procedure followed the instructions of manufacturer[23].

For Competitive ELISA assay 10 mL ethanol (60%) were added to solid dosage forms and 9 mL were added to liquid dosage forms. Samples were mixed for 30 seconds using vortex mixer and were shaken well up-side down for 10 minutes. Hard gelatin capsules were incubated in a water path at 50°C until complete dissolution of the shell. After that samples were centrifuged for 10 min at 2500 g, room temperature. Dilute the supernatant (1:50) using the buffer, then transfer 50 µL into ELISA well and follow the instructions of manufacturer[24].

ELISA reader (MR-96A, Mindary, China) a microtiter plate spectrophotometer was used to measure absorbance at 450 nm. A calibration curve was constructed using standard series. The calibration curve was then used to determine gliadin concentration in samples, following which the values were multiplied by a dilution factor to express gliadin in mg/kg (ppm unit). The obtained results were multiplied by a factor of 2 in order to obtain the

gluten concentration in each sample as indicated in the kit manual[23&24].

RESULTS AND DISCUSSION

Sample characterization

The drugs selected in this study were 38 samples. 17 samples were OTC and the rest (21 samples) were prescription drugs (Rx). The samples included fourteen dosage forms indicated by CD patients to cause gluten related reaction, which were OTC of non-steroidal anti-inflammatory drugs (NSAIDs) and anti-cough dosage forms, and Rx of antibiotic and antiviral dosage forms. Selection of other samples depended on its' frequent dispensing[25]. Most of the samples were locally manufactured, and dosage forms have a patient information leaflet without specifying the excipients. The patient information leaflet guidelines are not followed by local authorities[26&27]. The questionable safety of excipients and reports on its' potential adverse events led to development of many regulations[28]. Lack of excipient information section in leaflet make recognition of medications as possible source of gluten to guide CD patients in Gaza Strip unpractical. A description of the samples is given in **Table 1**.

Table 1: Characters of tested samples on gluten (n = 38).

OTC drugs			Rx drugs		
Generic name	**No.**	**Dosage form**	**Generic name**	**No.**	**Dosage form**
Paracetamol**	3	Tablet	Esomeprazole	1	Enteric coated tablet
Ibuprofen**	5	Tablet, film coated tablet, caplet	Amoxicillin**	3	capsule
Loratidine	2	Tablet	Amoxicillin/ clavulanic acid**	3*	Powder for oral suspension, tablet
Chlorphineramine/ Pseudoephedrine	1	Capsule	Azithromycin	3	capsule
Chlorphineramine/ Phenylephrine/ Paracetamol/ Vit. C / Caffeine	1	Tablet	Acyclovir**	2	Suspension tablet
Triprolidine/ Pseudoephedrine	2	syrup	Metformin/ glimepiride	1	Tablet
Diphenhydramine/ Ammonium chloride/ Sodium citrate/ Menthol**	1	Syrup	Metronidazole	2	Tablet
Diphenhydramine/ Ammonium chloride	1*	Syrup	Diclofenac sodium / potassium	3*	Tablet, slow release tablet, film coated tablet
Hyoscine-N-Butylbromide/ Paracetamol	**1**	Tablet	Diclofenac/ Paracetamol	2	Tablet
			Orphenadrine citrate/ Paracetamol	1	Tablet

(*): One dosage form was imported.
(**): Mentioned by celiacs to produce gluten related adverse effects.

Results of ELISA assay

Sandwich enzyme-linked immunosorbent assay ELISA for quantitation of gliadin is based on R5 monoclonal antibodies for detection of the prolamines from wheat (gliadin), rye and barley in raw and processed foods. This method is recognized by the Association of Official Analytical Chemists (AOAC) as the official method for gluten analysis (licensed No. 120601). Upon application of Sandwich ELISA, no sample showed gliadin at a concentration above limit of quantitation (LOQ) of the kit 5 ppb[23].

Competitive ELISA assay was used for the detection of partially hydrolyzed gluten. The kit has been approved by AOAC[24]. The kit contains specific R5 monoclonal antibodies that detect potentially toxic peptide fragments of gluten. The results of competitive assay are listed in **Table 2**.

Table 2: Data of gluten content using competitive ELISA assay, and comparing with the gluten daily tolerance limit.

Sample* & specification	Gluten content (ppm)	Gluten content per dosage unit (mg)	Daily intake (mg)**	% Daily tolerance limit ***
Pseudoephedrine/ Chlorpheniramine Capsule containing granules	16.6	$8.3x10^{-3}$	0.017	4.15%
Ibuprofen Caplet	13.8	$11x10^{-3}$	0.044	11%
Ibuprofen Tablet	74.5	$48.5x10^{-3}$	0.194	48.5%
Metronidazole Tablet	19.6	$8.8x10^{-3}$	0.070	17.6%
Metronidazole Tablet	14.5	$11.3x10^{-3}$	0.045	11.3%
Diclofenac sodium Film coated tablet	21.5	$13.1x10^{-3}$	0.0393	9.8%
Azithromycin Capsule	29.2	$21.0x10^{-3}$	0.084	21%
Azithromycin Capsule	13.3	$10.6x10^{-3}$	0.042	10.6%
Triprolidine / Pseudoephedrine Syrup	17.1	$85.5x10^{-3}$ / 5ml	0.689	171%
Triprolidine /Pseudoephedrine/ Dextromethorphan Syrup	18.6	$93.0x10^{-3}$ / 5ml	0.744	186%
Amoxicillin / clavulanic acid Tablet	299	0.344	1.03	258%
Amoxicillin/ Clavulanic acid powder for reconstitution	28.5	$17.0x10^{-3}$ / 5ml suspension	0.318	318%
Hyoscine HBr / Paracetamol Tablet	15.3	$56.6x10^{-3}$	0.566	141%
Orphenadrine citrate / Paracetamol Caplet	17.7	$9.9x10^{-3}$	0.029	7.4%

(*): Only samples having gliadin at level > 10 ppb (LOQ of competitive ELISA).

(**): Daily intake depends on recommended daily dose as given by manufacturer.

(***): Daily percentage intake of gluten by a medication on the basis of the tolerance limit 0.4 mg gluten daily[29].

Peptide fragments of gluten were estimated in 14 of the tested samples at levels above 10 ppb, which is LOQ of the kit. Gluten concentration was in five samples of them above 20 ppm. The threshold for gluten free food is 20 ppm[28]. Samples were NSAIDs- and antibiotic dosage forms of local production. Two of them were mentioned by celiacs to cause adverse events upon administration, which can explain their complaint. Some dosage forms had high gluten levels and were not mentioned by CD patients and vice versa. This could be explained by testing different batches, variation in excipient sources, or possible contamination during manufacturing process. In addition, reporting of uncontrolled CD status might have other causes rather than medications[2].

The daily intake of gluten through medication administration at recommended doses was calculated (**Table 2**). Office of food safety center in food and drug administration (FDA) estimated a maximum limit of 0.4 mg daily intake of gluten to control CD status and prevent morphological effects[29]. In some samples the tolerance limit was exceeded. Excipients like starch, pregelatinized starch, dextrins or dextrates, brown rice syrup, and caramel may be sourced from wheat, barely, or rye[30]. It was obvious that frequent intake of some tested samples (Anticough, antibiotic, antispasmodic) would result in clinical effects or even morphological effects that is expected to exacerbate CD status. These drugs are applied for a short period, so the effect might be insignificant[31].

It was noticed that several dosage forms - having gluten – were made by a local pharmaceutical company. This indicates an inappropriate quality of excipients or high risk of contaminations in the company.

Conclusion

The findings of this study indicate that despite most of tested drugs are devoid of gluten, some may contain gluten in amounts that would provoke adverse clinical effects or even worsen the morphological status of celiac patients in Gaza Strip. The results showed the necessity to regulate and update registration requirements taking a consideration about excipients' quality and package leaflet. It is recommended to examine regularly pharmaceuticals on gluten content for celiac centers in Gaza Strip.

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نشرة العلوم الصيدلية
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تحديد محتوى الغلوتين في المستحضرات الصيدلانية المتداولة في قطاع غزة

مي رمضان[1*] – محمد شباير[2] – رغدة الأسطل[2]

[1] قسم الكيمياء الصيدلية والعقاقير ، كلية الصيدلة ، جامعة الأزهر ، غزة ، ص.ب ، ١٢٧٧، فلسطين

[2] قسم علم الأدوية والعلاجات ، كلية الصيدلة ، جامعة الأزهر ، غزة ، ص.ب ، ١٢٧٧، فلسطين

الغلوتين (Gluten) هو بروتين القمح الذي يمكنه تحفيز جهاز المناعة محدثا التهابات وهو المرض المعروف بالداء الزلاقي. يمكن للمستحضرات الصيدلانية ان تكون مصدرا للغلوتين نتيجة لاستخدام نشا القمح كمادة مساعدة في التصنيع الدوائي.

تناولت الدراسة فحص بعض المستحضرات الصيدلانية (الواجبة الصرف بروشتة طبية أو الممكنة الصرف بدونها) على كمية الغلوتين (Gluten) وببيتيدات الغلوتين المتحلل جزئيا (Partially hydrolyzed gluten fragments) باستخدام تقنية الإليزا (ELISA) من نوع الشطيرة (Sandwich) , والطريقة التنافسية (Competitive) لكل منهما على التوالي.

تم الكشف عن ببيتيدات الغلوتين المتحلل جزئيا في حوالي ٣٧% من العينات (عدد العينات الكلي: ٣٨). وسجل لخمس عينات منها فقط (١٣%) حدا من الغلوتين الذي تجاوز ٢٠ جزءاً في المليون. عند استخدام تقنية الإليزا من نوع الشطيرة لم يسجل الغلوتين في أي عينة.

أظهرت الدراسة ان تعاطي اربعة اصناف دوائية حسب الجرعات الموصي بها يوميا يمنح مرضي الداء الزلاقي كمية من الغلوتين تفوق الحد اليومي الآمن من الغلوتين. اضافة الي ذلك فان معلومات المواد المساعدة في النشرات الطبية المرفقة بالأدوية غير مذكورة في معظم العينات.

هذه النتائج تؤكد أن المستحضرات الصيدلانية يمكن ان تساهم في تفاقم الأعراض لدي مرضى الداء الزلاقي. وبناءا على ذلك فإنه يوصى بتحديد مستوي الغلوتين في الادوية وباجراء مزيد من الدراسات على الغلوتين باستخدام عددا أكبر من العينات في المستحضرات الصيدلانية، وتطوير طرق تحليل الغلوتين فيها. كما يوصي باتباع الإجراءات الحديثة لدي دوائر تسجيل الأدوية في قطاع غزة الخاصة بالمواد المساعدة في التصنيع الدوائي.