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Original article

Evaluating the bioequivalence of levetiracetam brand and generic oral tablets available in the Saudi market in vivo

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ABSTRACT

Background: Epilepsy is a common global neurological disorder. About 30% of epileptic patients are managed with anti-epileptic Drugs (AEDs). Since 2000, Levetiracetam (LEV) has been marketed around the world as an AED under the brand name Keppra, and recently more generics are found in the Saudi market as cheaper alternatives. The objective of this study is to evaluate the bioequivalence of LEV brand and generics available in the Saudi market in mice.

Methods: Pharmacokinetics (PK), liver function test, and behavioral studies were conducted for LEV brand and generic in different groups of Blab/c mice.

Results: PK results show a significance difference in PK parameters mostly evidenced with generic 3, then generic 2. The only significant different between Keppra and generic 1 was in $T_{1/2}$. In addition, Keppra did not significantly increase liver enzymes in comparison to other generics. On the other hand, other generics showed less favorable results in increasing liver enzymes. Keppra reduced the number and intensity of epileptic attacks, had no mortality rate due to epilepsy, and was associated with less sever seizures attacks.

Conclusion: Keppra, the brand form of LEV, has better safety and efficacy profiles in mice compared to 3 generics found in the Saudi market. Therefore, we recommend evaluating the same parameters tested in this study in patients utilizing similar generics and brand to establish the existence of bioequivalence between LEV brand and generics.

1. Introduction

Epilepsy is a globally common neurological disorder with up to 70% of epileptic patients disease being controlled with anti-epileptic drugs (AEDs) (Glauser et al., 2006). Also, to gain more tolerability and better safety profiles than older AEDs such phenobarbital, phenytoin, carbamazepine, and valproate, newer generation of AEDs such as gabapentin, lamotrigine, tiagabine, topiramate, levetiracetam are gaining more popularity (French & Gazzola, 2011). Other add-on indications for AEDs are in the management of bipolar disorder, neuropathic pain, and anxiety disorder (Johannessen Landmark, 2008; Johannessen Landmark, Larsson, Rytter, & Johannessen, 2009).

Although not being chemically relevant to existing AEDs, levetiracetam (LEV), marketed under the brand name Keppra since 2000, has shown efficacy against many phases of seizure and thus is now consider as one of AEDs (Klitgaard, Matagne, Gobert, & Wülfert, 1998) (Mohammadi et al., 2012; Pilli, Savakula, Reddy, Reedy, & Research, 2015).

Levetiracetam is almost completely absorbed (up to 96%) after oral administration (P. N. Patsalos, 2004). When administered with food it delays the normal time of the maximum concentration from 1 to 1.5 hr, and decreases C_{max} by 20%. In addition, LEV does not compete with other drugs for binding site due to its low protein binding (<10%)(P. N. Patsalos, 2004). The volume of distribution ranges from 0.6 to 0.9 l/kg in premature infants and children, and 0.5 to 0.7 l/kg in adults (P. N. Patsalos, 2004). It was reported that only 24% of LEV dose is metabolized by enzymatic hydrolysis of acetamide where two inactive metabolites are formed and are

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renally excreted; the first is via the hydroxylation of 2-oxo-pyrrolidine ring and the second is through opening the 2-oxo-pyrrolidine ring at position 5 (P. N. Patsalos, 2000, 2004).

The exact mechanism of LEV as antiepileptic agent is unknown (Pilli et al., 2015). However, it seems that LEV binds to synaptic vesicle protein 2 A (SV2A) which is noticed to inhibit nerve conduction across synapses (Lynch et al., 2004). Some of the side effects associated with Keppra includes partial onset seizures, asthenia, somnolence, and dizziness which is mainly shown in the first 4 weeks of treatment (Abou-Khalil, 2008; Gambardella, Labate, Colosimo, Ambrosio, & Quattrone, 2008). These side effect could possibly increase or decreased with the use of generic forms of LEV. Although not previously known as a cause increasing hepatic enzymes, several recent case studies have reported elevation in liver enzymes associated with the utilization of LEV either as a monotherapy or in combination with other medications such as temozolomide or Lacosamid (Broli et al., 2010; Chen, Mizrahi, & Nubani, 2015; Gutiérrez-Grobe et al., 2013).

Increasing medical costs in recent years have forced healthcare systems to limit spending and increase cost savings (Andermann, Duh, Gosselin, & Paradis, 2007). As a consequence, cheaper generic medicines were exchanges with brand products, which also included AED (Chow, 2014). However, AEDs generics should meet claim of regulatory Food and Drug Administration (FDA) and should have identical active ingredients, be similar in term of safety and efficacy, and bioavailability i.e., bioequivalent (Chow, 2014). Similar bioavailability is a pivotal part of bioequivalence, since it is greatly unwanted to shift a patient to a generic that might cause a significant changes in plasma drug concentrations, thus safety and efficacy(Krauss, Caffo, Chang, Hendrix, & Chuang, 2011).

With regards to LEV, a previous study by Markoula et la, has evaluated the bioequivalence of LEV trade and generic in Greek market. Results indicated that both trade and generic were bioequivalent (Markoula et al., 2017). However, to our knowledge no previous studies was conducted within the Saudi market. Therefore, the aim of this study is to evaluate the bioequivalence of LEV brand Keppra and generics available in the Saudi market in vivo using mice.

2. Materials and methods

2.1. Chemicals and reagents

LEV with (purity > 99%) was provided by Alfa Aesar (Massachusetts, USA). Internal standard (IS) (Salbutamol, purity > 98%) and high-performance liquid chromatography (HPLC) grade methanol and acetonitrile were purchased from Sigma-Aldrich Corporation (Missouri, USA). Ultrapure water was obtained from Ultrapure water Milli-Q Advantage water purification system, 0.22 μm filter (Millipore, Molsheim, France). Potassium dihydrogen phosphate (KH2PO4) was purchased from Winlab (UK). LEV as brand, Keppra was purchased from GSK (London, UK), 3 LEV generics were purchased from the Saudi Market. Pentylenetetrazol-PTZ was purchased from Shanghai pharmaceuticals (Shanghai, Chania). Liver enzyme UV/Kinetic kits for AST, ALT, GGT and ALP were purchased from United Diagnostic (Dammam, Saudi Arabia).

2.2. Experimental animals

Healthy male Balb/c mice weighing (20–28 g) were obtained from animal house, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. Animals were kept on a 12/12-hr light/dark cycle and were housed at a temperature maintained at 20–25 °C and at humidity of about 50% with access to food and water. Daily observation of all mice was required to ensure that all animals maintained good health. All animal experiments strictly followed the guidelines of the Ethical Committee for Performing Studies on Animals, King Saud University, Riyadh, Saudi Arabia, protocol number KSU-SE-19–115.

2.3. LEV chromatographic conditions

Plasma samples were diluted as described previously (Engelbrecht, Grobler, & Rheeders, 2017), then LEV concentrations were determined from plasma samples using HPLC system, The chromatography was applied on a Venusil XBP C18, 250 × 4.6 m m, 5 μm particle size column (Agela technologies company, Tianjin, China) protected by a security guard precolumn (Agela technologies company, Tianjin, China) with a graphite filter. The mobile phase was a mixture of 50 mM KH2PO4 buffer (6.8045 g/L) with acetonitrile (90:10, v/v). The pH of the mobile phase was set at ± pH 5.5, with sodium hydroxide (NaOH), with a flow rate of 1 ml/min, an injection volume of 10 μL (Engelbrecht et al., 2017). Quantitative determination of LEV was conducted using a linear calibration curve between the range of 7.5––200 ng/ml with a correlation coefficient of 0.996 previously evaluated as an important validation parameter during method development and validation, did not exceed 15% for concentrations above lower limit of quantification (LLOQ) and 20% for concentrations at LLOQ level. Method validation parameters are described in the supplementary materials.

2.4. Pharmacokinetic study

Twenty four animals were randomly divided into four groups (n = 6) and treated once with a LEV dose of 54 mg/kg, brand or generic, through oral gavage (LEV tablet 500 mg/dissolved in 50 ml of water)(Benedetti et al., 2004). Immediately before dosing (0 time) and at specific time intervals (0.5, 1, 2, 4, 8, 24, and 48 h) blood samples (0.2 ml) were drawn from each mouse through tail vein and processed as previously reported(Almomen, Maher, Alzoman, Shehata, & Alsubaie, 2020). Briefly, acquired samples were immediately centrifuged after being withdrawn at 4,500 rpm and 4 °C for 30 min to yield plasma, which was then kept at 20 °C until analysis. LEV then was extracted, and concentrations were evaluated (Benedetti et al., 2004; Engelbrecht et al., 2017).

2.5. Liver functions study

Thirty-five animals were randomly divided into five groups (n = 7), and received 54 mg/kg of LEV, either brand or generics, through oral gavage once daily for two weeks.(Benedetti et al., 2004) Animals were then sacrificed, blood samples were collected in heparinized coated tubes and centrifugate at 3500 r.p.m. Plasma were then withdrawn and stored at −20 °C until the time of analysis. liver enzymes AST, ALT, GGT, and ALP were analyzed as per manufacturer protocol. Mice tissue were also trimmed and sent for further evaluation.

2.6. Histological evaluation of mice tissues

Mice livers from the liver function study were trimmed at the time of sacrifice and tissues were send for histological evaluation using hematoxylin and eosin stain (H&E) at the Dept. of Pathology, King Saud University-Riyadh, Saudi Arabia. In addition to liver, heart and kidneys were also sent for histological evaluation.

2.7. Behavioral study

Thirty-five mice were randomly divided into 5 groups (n = 7). Group I served as control, group II received Keppra, group III received generic 1, group IV received generic 2, and group V received generic 3. Groups II-V received a single dose of 580 mg / kg through oral gavage (Gower, Hirsch, Boehrer, Noyer, & Marescaux, 1995).To induce convulsions, animals in all groups received 75 mg/kg of Pentylenetetrazol PTZ dissolved in distilled water and administered intraperitoneally (IP) 30 min post LEV administration (ERKEÇ & Arihan, 2015). The convulsions protective effect of LEV, brand and generic, were conducted using three camera (Canon EOS 90D DSLR Camera, Tokyo, Japan,). Each camera was focused on animals' cages from a 60 cm distance.. Seizure latency, seizure duration, number of attacks, and the time of last attack were recorded (ERKEÇ & Arihan, 2015; Kitano et al., 2005; Klitgaard et al., 1998; Vohora, Pal, & Pillai, 2000)Further, epileptic seizure scoring was evaluated as follows: case 0 normal behavior, no abnormality; case 1 immobilization, lying on belly; case 2 head nodding, facial, forelimb, or hindlimb myoclonus; case 3 continuous whole-body myoclonus, myoclonic jerks, tail held up stiffly; case 4 rearing, tonic seizure, falling down on its side; case 5 tonic-clonic seizure, falling down on its back, while rushing and jumping; case 6 deaths (ERKEÇ & Arihan, 2015; Shimada & Yamagata, 2018).

2.8. Statistics analysis

Statistical analysis was performed using GraphPad prism 8 edition (GraphPad Software company, San Diego, California, USA) and SPSS ver. 22.0 (IBM, Chicago, IL, USA). Data were represented as mean and ± SEM. One-way ANOVA and Tuky's or Bonferroni's post hoc analysis were used. Statistical significance was obtained with p-values ≤ 0.05.

3. Results

Our study is intended to study the bioequivalence of the LEV brand, Keppra, with generics available in the Saudi market. In this study PKs, liver function tests, liver histology, and behavioral evaluation were conducted in vivo using mice.

3.1. Generics increase the exposures to LEV in comparison to Keppra

The plasma concentrations-time curve is shown in Fig. 1 and PK parameters were thereafter calculated using PK Solver Add-In Excel 2010 (Fig. 2). The PK parameters calculated were maximum plasma concentration (C_{max}), time taken to reach the maximum plasma concentration (T_{max}), half-life ($T_{1/2}$), the area under the curve from 0 to the last sampling time t ($AUC_{0\text{-}48}$), and from 0 to ∞ ($AUC_{0\text{-}\infty}$), and the apparent oral clearance rate (CL/F). Results showed that a significant difference in PK parameters was mostly found between Keppra and all generics and was mostly found with generic 2 and 3. Generic 3 showed an significant increase in T_{max}, $T_{1/2}$, $AUC_{0\text{-}48}$, $AUC_{0\text{-}\infty}$ which possibly means an increase in drug exposure. Generic 2, however, showed a significant decrease in C_{max}, AUCs and an increase in T_{max} which might indicate a decrease in exposure and delay in the antiepileptic effect. The only significant difference in PKs between Keppra and generics 1 was in C_{max} and T_{max} which might indicate a decrease in the extent and rate of drug absorption (Han, Lee, & Pang, 2018).

3.2. LEV brand exhibits a better safety profile on the liver in comparison to LEV generics

Here, we attempted to evaluate if LEV generics retain the same safety profile on the liver as brand. In terms of hepatic function tests, there was no significant difference in hepatic enzyme levels between the control and Keppra. However, there was a significant increase in at least three out of the four enzymes tested in all 3 generics relative to control; in generic 1 and 3, a significant increase was found in ALP, AST, and ALT; generic 2 showed a significant increase in AST, ALT, and GGT (Fig. 3 and Table 1). In comparison to Keppra, there was a significant increase in ALP, AST, and ALT found with generic 1; a significant increase in AST, ALT, and GGT with generic 2; and a significant increase in ALP and ALT with generic 3 (Fig. 3 and Table 1). Regarding differences among generics, there are also significant differences in hepatic enzyme levels (Table 1).

Histopathological analysis of mice showed a mononuclear centrilobular inflammation (mainly lymphocytic) in mice treated with LEV. The degree of inflammation varied between minimal or mild in mice treated with generic 2 and generic 3 to moderate in mice treated with Keppra and generic 1. Acidophilic bodies were seen in most mice (Fig. 4. A-B). Spotty necrosis was seen in mice treated

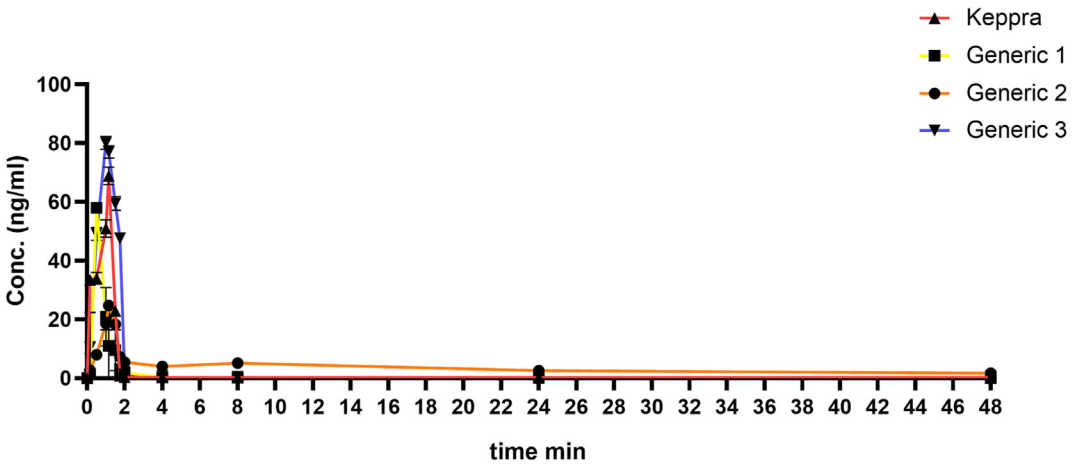

Fig. 1. Concentration time curve for levetiracetam trade (Keppra) versus 3 Generics available in the Saudi market (n = 7) over 48 hr.

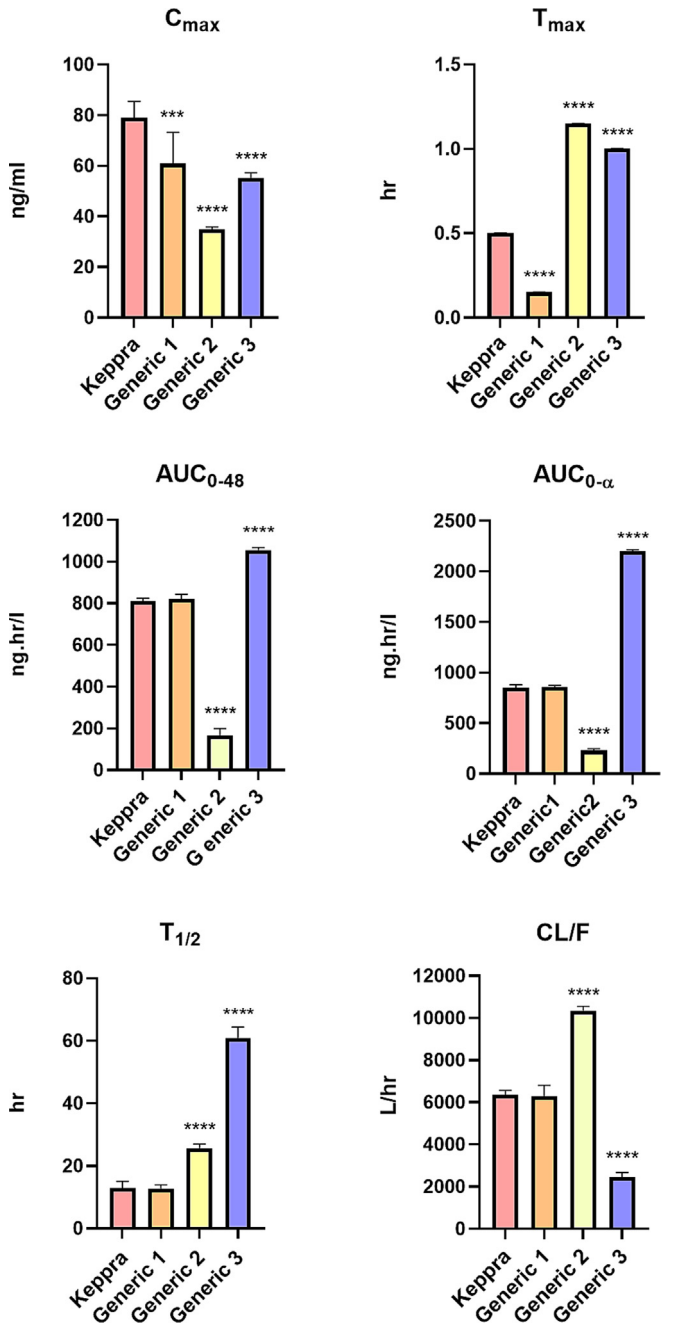

Fig. 2. Comparison of pharmacokinetic parameters Cmax, Tmax, AUC0-48, AUC0-∞, T1/2, and CL/F between Keppra and Levetiracetam generics (n = 7). The significance shown in the graph is relative to Keppra.. p-values of 0.05 were considered statistically significant, where ** p < 0.005, *** p < 0.001, and **** p < 0.0001.

with generic 1, and a single mouse treated with generic 3 (Fig. 4. C). All cases showed reactive/regenerative hepatocytes with large nuclei, binucleation, or mitotic figures in addition to scattered atypical, bizarre hepatocytes (Fig. 4. D and E). Furthermore, ill-defined granulomas were seen in some mice treated with Keppra, generic 1, and generic 3 (Fig. 4 F). Portal inflammation ranged from minimal or mild to moderate in some cases. No massive/submassive necrosis was seen. No fibrosis or steatosis was noted. No bile duct injuries or vascular injuries were present. Supplementary Table 2 includes a detailed histopathological analysis of mice in different treatment groups.

3.3. Keppra has a more protective effect against seizure attacks than generic

Results show that although there was a difference in the onset of epilepsy between Keppra and generic 1, 2, and 3 (≈ 3.057, 1.429, 2.471, 2.843, respectively) these differences were statistically insignificant. The only significant difference was between the onset of attack between the control and Keppra-treated groups, where attacks started at about 3 min in Keppra treated group compared to about 0.3 min with the control (Fig. 5. A). However, seizure durations were shorter and statistically significant in Keppra and generic 1 compared to control (0.485 and 0.342 vs 3.47 min, respectively) (Fig. 5. B). Furthermore, there was a significant difference in the length of attacks between Keppra versus generic 2 and 3 (0.485 vs 3.18 and 2.1 min, respectively), where Keppra exhibited short attack duration (Fig. 5. B and Table 2). Concerning differences between generic groups, generic 1 exhibited a shorter attack duration in comparison to generic 2 and 3, and differences were significant (Fig. 5. B and Table 2).

Regarding the number of attacks, this was monitored from the time of receiving the PTZ dose up to 30 min or at animal death. Fig. 5. C shows that there was no significant difference between the number of attacks between the control group and generic 1 versus Keppra. This can be mainly explained by the high mortality rate seen with the control group, and generic 1 during the 30 min of monitoring (Fig. 5. C) (control deaths occurred between 1.7 and –8 min, generic 1 death occurred between 15.1 and 24.5 min). However, there was a significant difference in the number of attacks between Keppra and generic 2 and 3 (6.42 vs 14.71 and 11.71 attacks). A similar pattern was found at the time of the last attack. The last recorded seizure attack in the control group occurred at around 4.3 min and no more attacks could be recorded due to animals' deaths. A similar scenario was found between Keppra and genetic 1 where statistical significance could not be drawn due to the high mortality seen with generic 1. However, there was a significant difference between the time of the last attack between Keppra (≈13.6 min) and the generic 2 groups (24.57 min) (Fig. 5.D and Table 2). No significant difference in the time of the last attack between generic 3 and the other treatment groups. Lastly, Fig. 5.E also shows the death rate among control, Keppra, generic 1, 2, and 3 were 100, 0, 71.4, 42.85 and 57.14%, respectively, which indicates that Keppra has a superior capability in reducing mortality due to seizures, followed by generic 2, then generic 3. Generic 1, however, was associated with a high mortality rate.

3.4. Keppra is associated with less severe cases of seizure compared to LEV generics

Scoring seizure attacks was conducted to evaluate the protective potency of LEV trade and generic against different types of seizure based on intensity. Results show that all 5 groups experience case 1 with no significant difference among groups. Case 2, however, was mostly seen with generic 2 which was significantly different from Keppra, generic 1, and generic 3 (Fig. 6, and Table 3) indicating Keppra, generic 1, and generic 3 are equally effective in protecting against case 2 of epileptic attacks. Case 3, on the other hand, was mostly found with generic 3 which was significantly different from Keppra, generic 1, and generic 2 (Fig. 6 and Table 3). No

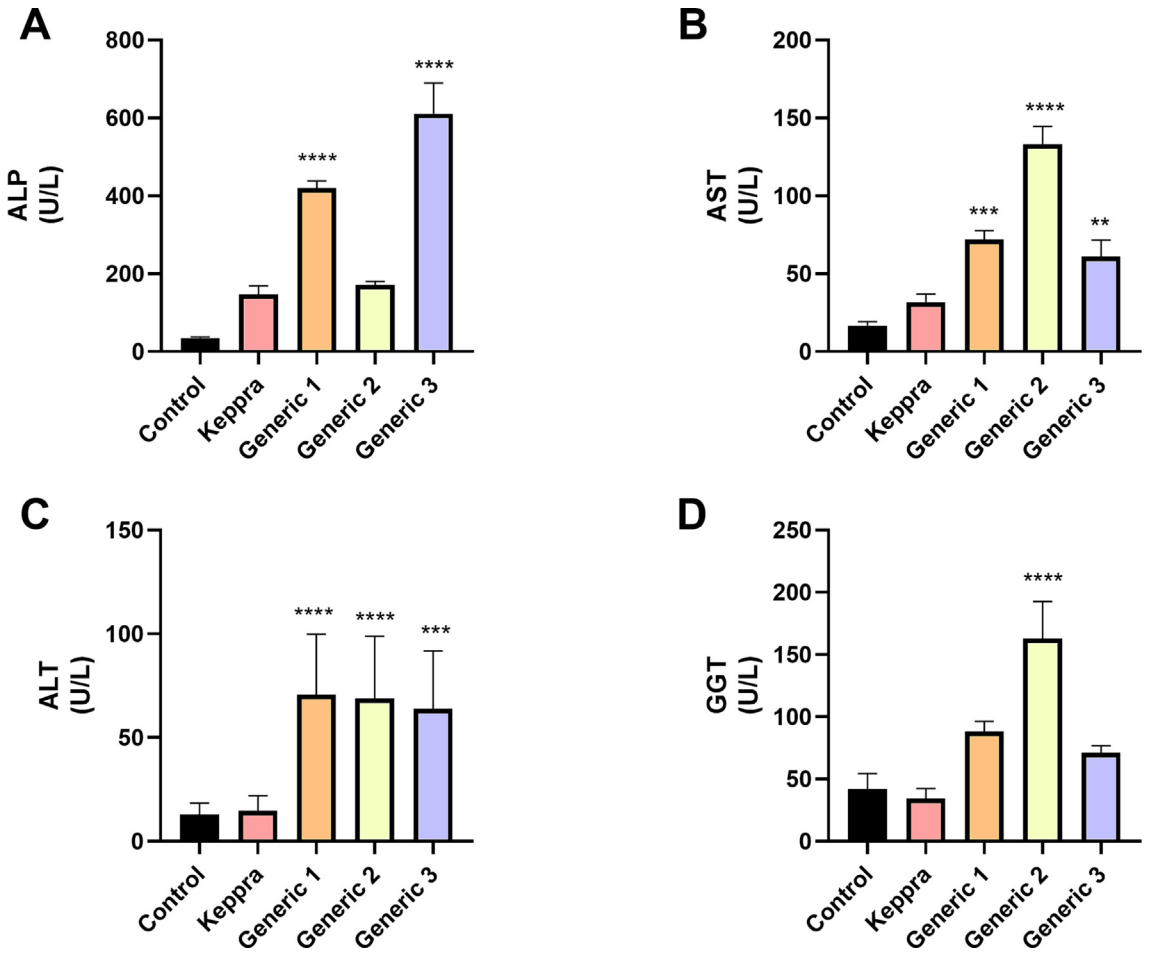

Fig. 3. Levetiracetam generics elevate levels of hepatic enzymes, ALP(A), AST (B), ALT (C), and GGT (D) with mostly statistical significance relative to control and Keppra (n = 7). The significance shown in the graph is relative to the control. p-values of 0.05 were considered statistically significance, where ** $p < 0.005$, *** $p < 0.001$, and **** $p < 0.0001$.

Table 1
Statistical significance among treatment groups, brand (Keppra) vs generic in the hepatic function test evaluation.

	ALP	AST	ALT	GGT
Control vs. Keppra	ns	ns	ns	ns
Control vs. Generic 1	****	***	****	ns
Control vs. Generic 2	ns	****	****	****
Control vs. Generic 3	****	**	***	ns
Keppra vs. Generic 1	***	**	****	ns
Keppra vs. Generic 2	ns	****	****	****
Keppra vs. Generic 3	****	ns	***	ns
Generic 1 vs. Generic 2	***	****	ns	*
Generic 1 vs. Generic 3	**	ns	ns	ns
Generic 2 vs. Generic 3	****	****	ns	**

p-values of 0.05 were considered statistically significance, where * $p < 0.05$, ** $p < 0.005$, *** $p < 0.001$, and **** $p < 0.0001$.

significant difference was found between generic 1 and 2 with Keppra. Although generic 2 exhibited more of case 4, attacks, the difference was not significantly different than other treatment groups. Generic 3 however, exhibited more attacks of case 5 which was significantly different than Keppra and generic 2.

4. Discussion

To lower financial burdens on both patients and healthcare institutions, the use of generics alternatives became highly encouraged(Andermann et al., 2007). However, in many cases it became

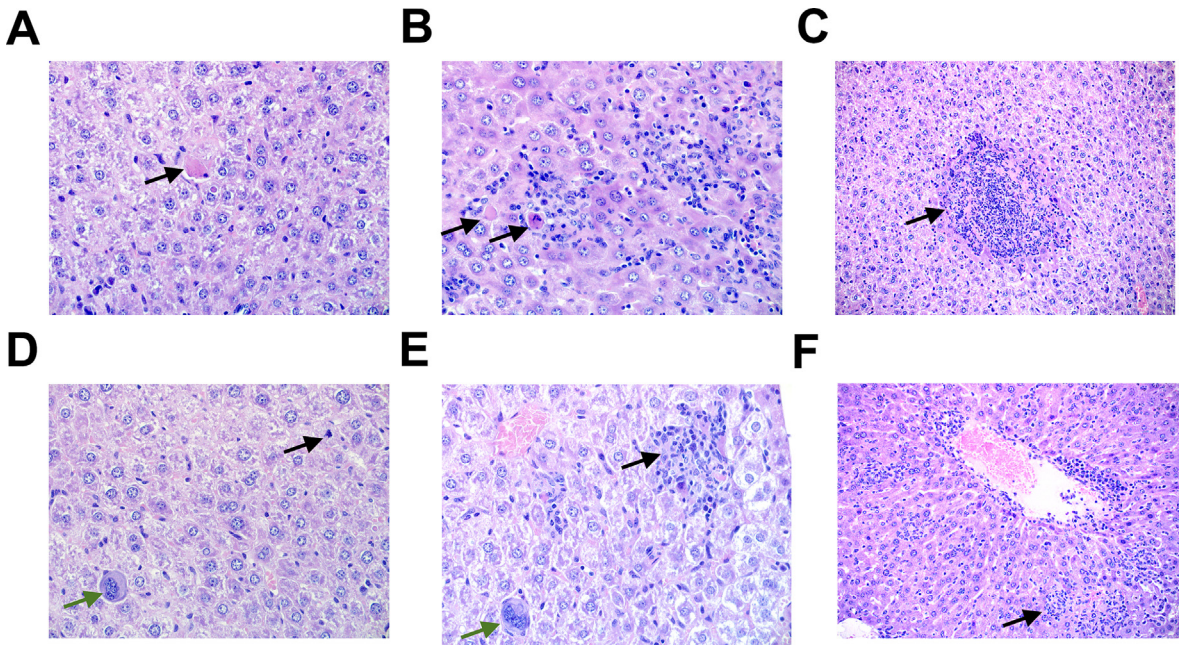

Fig. 4. Histopathology (A) A photomicrograph showing liver tissue with an acidophilic body (arrow) in a mouse treated with generic 2 (H&E stain, magnification x400). (B) Acidophilic bodies (arrow) are also noted in a few mice treated with generic 1 (H&E stain, magnification x400). (C) Another larger necrotic focus (arrow) was seen in a mouse treated with generic 3 (H&E stain, magnification x200). (D) A photomicrograph showing a mitotic figure (black arrow) and a bizarre cell (green arrow) in a mouse treated with generic 2 (H&E stain, magnification x400). (E) Another image shows a focus on lobular inflammation (black arrow) and a bizarre cell (green arrow) in a mouse treated with generic 1 (H&E stain, magnification x400). (F) A low-power magnification shows a small granuloma (arrow) in a mouse treated with Keppra (H&E stain, magnification x200).

obvious that branded AEDs are more effective and superior to generic ones (Andermann et al., 2007)**.** Moreover, available data on AEDs generic are not reliable because they mainly depend on physician's and patient's surveys, and the reported interchangeability effect/side effect experienced by patients (Makus & McCormick, 2007). Therefore, it became important to assess the safety and efficacy profiles of available generics and to evaluate whether they can be considered bioequivalent to brands.

The bioequivalence of LEV brand and generic was evaluated previously in Greece by Markoula et.al, using Keppra and a local Greek generic on human subjects. Results showed that brand and generic were bioequivalent in terms of PKs, seizure protection, and safety profile (Markoula et al., 2017). However, our results indicate that there was lack of bioequivalence between LEV trade and generics in the Saudi market.

Despite some contending that extrapolating the results of experiments on animal's PK and bioavailability to that of humans is not always appropriate due to differences in some CYP enzyme isoforms, Matsubara et al. found that there can be similarities between humans and rats, such as the similarity between human CYP3A4 and rat CYP3A62 isoform (Matsubara et al., 2004). Also, and extensive literature analysis by Musther et al., indicated that although human quantitative bioavailability cannot be predicted by correlating to animal bioavailability, qualitative bioavailability in term of high and low can be used to reflect human scenario (Musther, Olivares-Morales, Hatley, Liu, & Rostami Hodjegan, 2014). In our study there were differences in the PK parameters between Keppra and other generics mostly pronounced with generic 2 and3. The differences seen specially in both AUCs' indicates that the animal exposure to both drugs can be different, thus efficacy as well as safety profiles might be impacted.

LEV is not likely to be significantly accompanied by pharmacokinetic interactions because it is not metabolized in the liver and is not bound to plasma proteins (P. J. P. Patsalos & therapeutics, 2000). The hepatic cytochrome P450 system is not involved in the primary metabolic pathway of LEV. Hepatic enzymes are neither inhibited nor stimulated by LEV. About more than half of LEV's administered dose is eliminated unaltered in the urine, and only 24% is converted into an inactive metabolite that is both excreted in the urine and detectable in the blood (Selvaraj, Madabushi, Gunasekar, & Singh, 2016). Following LEV therapy, elevated liver enzymes are recorded in<1% of individuals (Lin et al., 2015). LEV is also thought to be helpful in individuals with seizures after liver transplantation because of its reduced likelihood of drug-drug interactions or with those epileptic patients that have chronic hepatic diseases (Bilo et al., 2008; Lin et al., 2015).

The most common markers used in the detection of hepatocellular injury are elevations in liver enzymes i.e., aspartate aminotransferase (AST) and alanine aminotransferase (ALT)(Lala, Goyal, & Minter, 2021). However, AST can be found in red blood cells, skeletal muscles, heart, kidney, and brain thus, it is a less specific marker for hepatic injury. On the other hand, ALT is more suggestive of liver injury because of its low levels in kidney and skeletal muscle (Lala et al. (2021). Elevation of both ALT and AST can indicate either hepatic or extrahepatic diseases, nevertheless, in hepatic injuries, AST and ALT tend to be elevated for longer periods, unlike extrahepatic injuries where the markers rapidly decrease after 12–72 h (Lala et al., 2021). Although not much histological differences between Keppra and other generics were detected, significant increase in both AST and ALT in generic compared to Keppra might indicate possible occurrence of possible hepatocellular disease or cholestatic disorder since the increase in these markers extended beyond 72 h (Lala et al., 2021). Thus, Keppra may retain a better hepatic safety profile in comparison to other generics. It has been reported that excipients used in medicine formulations might be linked to liver damage (Belayneh, Tadese, & Molla, 2020). For example, propylene glycol, a solvent used in some pharmaceuticals, and parabens, a preservative used in some medications, are two medicinal additives that have been related to liver damage(Lim, Poole, Pageler, & therapeutics, 2014). Thus, it might be important to carefully assess the safety profiles of drug additives.

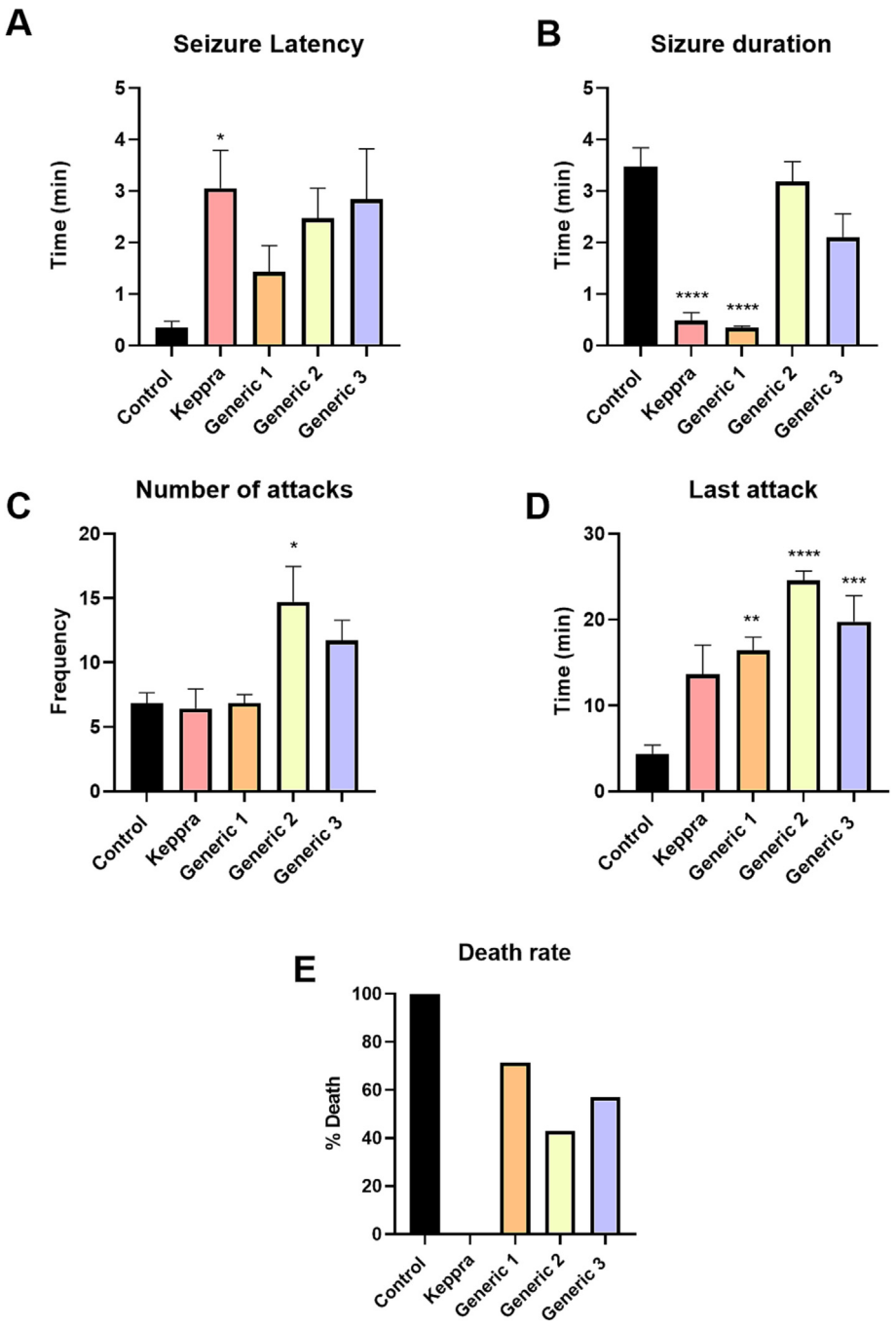

Fig. 5. Behavioral study of mice treated with Keppra and generics in terms of seizure latency (A), length of attacks (B), number of attacks (C), last attack (D), and death rate (F) (n = 7). p-values of 0.05 were considered statistically significance, where * p < 0.05, ** p < 0.005, *** p < 0.001, and **** p < 0.0001. The significance shown in the graph is relative to control.

Table 2
Statistical significance among treatment groups, brand (Keppra) vs generic in the behavioral study.

	Seizure Latency	Attack duration	Number of attacks	Last attack
Control vs. Keppra	*	****	ns	****
Control vs. Generic 1	ns	****	ns	ns
Control vs. Generic 2	ns	ns	*	****
Control vs. Generic 3	ns	*	ns	****
Keppra vs. Generic 1	ns	ns	ns	****
Keppra vs. Generic 2	ns	****	*	***
Keppra vs. Generic 3	ns	*	ns	*
Generic 1 vs. Generic 2	ns	****	*	****
Generic 1 vs. Generic 3	ns	**	ns	****
Generic 2 vs. Generic 3	ns	ns	ns	****

p-values of 0.05 were considered statistically significance, where * p < 0.05, ** p < 0.005, *** p < 0.001, and **** p < 0.0001.

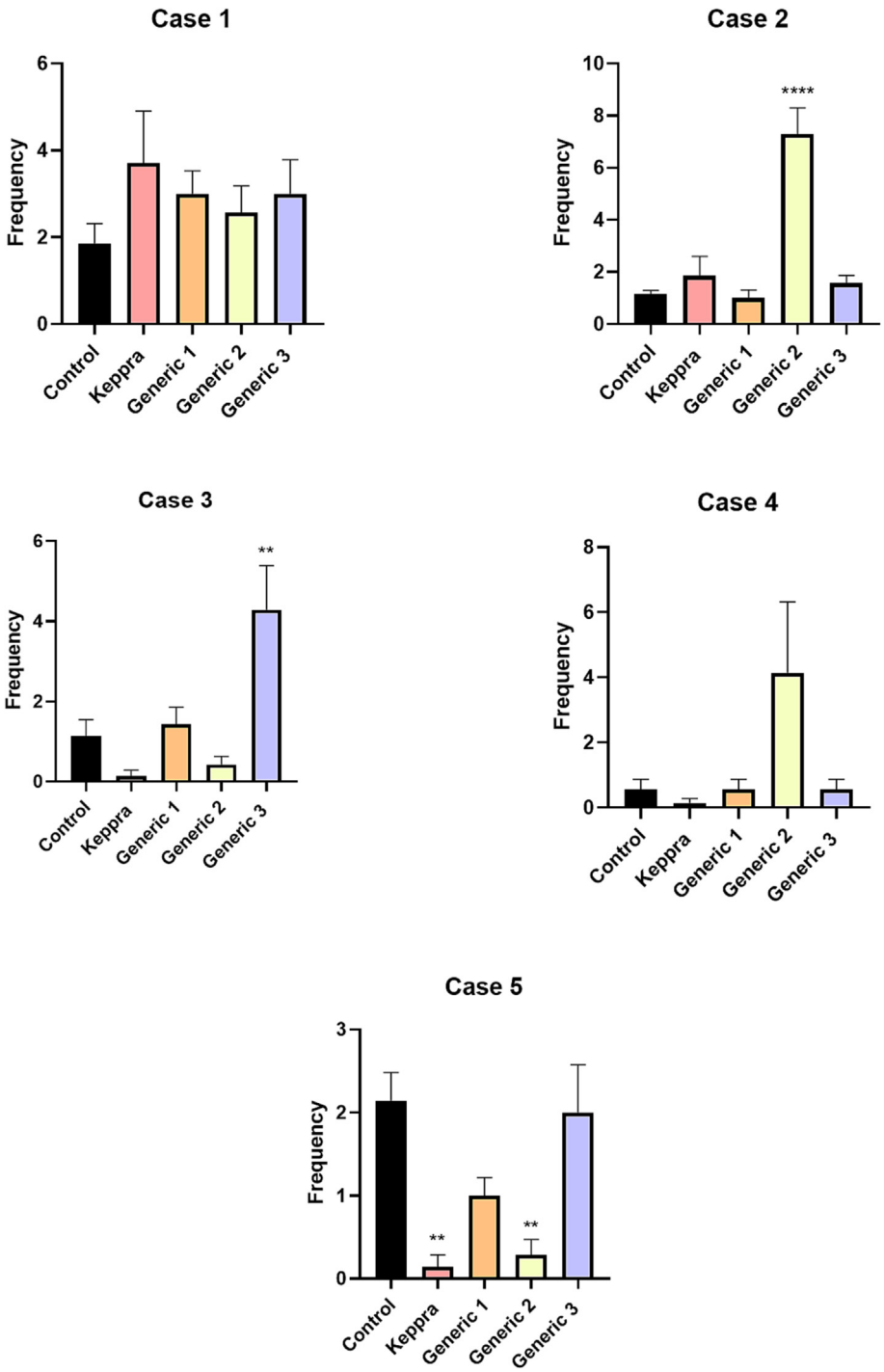

Fig. 6. The mean score of behaviors for the respective seizure of all LEV treatment groups (n = 7) p-values of 0.05 was considered statistically significant, where w ** $p < 0.005$, *** $p < 0.001$, and **** $p < 0.0001$.

To evaluate the protective effect of Keppra versus generics, a behavioral study examining time of first epileptic attack, duration of attack, frequency of attack, last attack, and death rate in an epileptic mouse model was conducted. Although no significant differences between the onset of attacks between Keppra and other generics were apparent, animals receiving Keppra had fewer attacks with shorter durations as well as earlier epileptic attack cessation and no deaths due to seizure compared to other generics. The results of epiliptic scoring study which inidicate the protective potency of Keppra and generics went in parallel with behavioral study results showing the animal receiving Keppra experienced less severe cases of seizure compared to LEV generics. Altogether, results from the behavioral study indicate that Keppra might have superior antiepileptic effect and is more potent in protecting against more severe seizure attacks. It is noteworthy to mention that the high mortality rate seen with generic 1 might hinder statistical significance differences with Keppra.

Table 3
Statistical significance among treatment groups, brand (Keppra) vs generic in in protective potency study.

	Case 1	Case 2	Case 3	Case 4	Case 5
Control vs. Keppra	ns	ns	ns	ns	**
Control vs. Generic 1	ns	ns	ns	ns	ns
Control vs. Generic 2	ns	****	ns	ns	**
Control vs. Generic 3	ns	ns	**	ns	ns
Keppra vs. Generic 1	ns	ns	ns	ns	ns
Keppra vs. Generic 2	ns	****	ns	ns	ns
Keppra vs. Generic 3	ns	ns	***	ns	**
Generic 1 vs. Generic 2	ns	****	ns	ns	ns
Generic 1 vs. Generic 3	ns	ns	*	ns	ns
Generic 2 vs. Generic 3	ns	****	***	ns	**

p-values of 0.05 were considered statistically significance, where * $p < 0.05$, ** $p < 0.005$, *** $p < 0.001$, and **** $p < 0.0001$.

5. Conclusion

In conclusion, our study indicates that two of three LEV generic, generic 2 and 3, available in Saudi market have different safety and efficacy profile when compared to LEV brand, Keppra. It is noteworthy to mention that even though there were no significant differences between Keppra and generic in many cases in this study, generic 1 was associated with high mortality rate. Results in this study should emphasize the importance of applying more stringent criteria on bioequivalence studies before assuming that generic and brand can safely be interchanged.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/xxx/s1, methods validation parameters, Figure S1. and Table S1., S2, and S3. **Table S4** Histopathological evaluation of mouse liver; **Figure S2.**: A photomicrograph showing renal cortical tissue with an ill-defined granuloma in a mouse treated with Keppra (H&E stain, magnification x400); **Table S5**. Heart and kidney histopathology analysis.

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Institutional review board statement

The study was conducted in accordance to the the Ethical Committee for Performing Studies on Animals guidelines, King Saud University, Riyadh, Saudi Arabia, following protocol KSU-SE-19–115.

CRediT authorship contribution statement

Danah ALRabeeah: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Aliyah Almomen:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. **Nourah Alzoman:** Formal analysis, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing. **Maria Arafah:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2023.101758.

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