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Evaluating the impact of jatropha oil extract against the *Varroa* mite, *Varroa destructor* Anderson & Trueman (Arachnida: Acari: Varroidae), infesting honeybee colonies (*Apis mellifera* L.)

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Abstract

The *Varroa* mite, *Varroa destructor* Anderson & Trueman (Arachnida: Acari: Varroidae), is a severe external parasitic mite of honeybees that causes great losses of colonies globally. Four concentrations (1, 2%, 5, and 10%) of *Jatropha curcas* oil were tested for controlling the *Varroa* mite. Significant effects of reducing percentage of the mite infestation ($P = 0.05$) after second treatment for sealed brood and after third treatment for adult workers in all tested concentrations of *J. curcas* were recorded. The low concentrations 1 and 2% of *J. curcas* were more effective than the higher ones 5 and 10% on reduction of percentage of *Varroa* mite infestation. Moreover, the colonies treated with the lowest concentrations of jatropha oil had the highest amount of brood area (75.75 and 77.50 inch^2) and the highest number of combs covered with bees compared with the colonies treated with the concentrations 5 and 10%. Treated colonies with the concentrations 1, 10, and 5% had a high amount of stored honey and pollen grains, 126.50, 111, and 96 inch^2 and 11.25, 9.75, and 9.75 inch^2, respectively. Obtained results encourage researchers to study deeply the ability of using jatropha oil in the widely field of Apicultural.

Keywords: *Varroa destructor*, *Apis mellifera*, *Jatropha curcas* oil, Control

Background

In the last decades, researchers identified 4 factors significantly affected honeybee populations around the world: exposure to pesticides and pollution, pathogens, parasites, and poor nutrition (Goulson et al. 2015 and Al Naggar et al. 2020). The Varroa mite, *Varroa destructor* Anderson & Trueman (Arachnida: Acari: Varroidae), is an external parasite of the honeybee *Apis mellifera* L. feeds on bee's hemolymph and caused the collapse of heavily infested and untreated colonies in a few years (Ramsey et al. 2019). Synthetic acaricides such as fluvalinate, flumethrin, and amitraz have been used successfully to control the mite (Gracia et al. 2017). Use of these chemicals against *Varroa* mites has led to develop mites resistant to the acaricides and to increase residues in bee products such as wax and honey (Tihelka 2018). Therefore, there is an urgent need to find cheap and safe materials both to humans as well as honeybees for controlling the *Varroa* mites. Several studies have focused on natural products such as plant extracts and essential oils against *V. destructor* in honeybee colonies (Ariana et al. 2002). The efficiency of some essential plant oils such as eucalyptus, thyme, neem, sage, and grape fruit against *Varroa* mites has been reported by many researchers (Dimetry et al. 2005; Rashid et al. 2011; Abd El-Wahab et al. 2012; Islam et al. 2016, and Nowar et al. 2018).

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The *Jatropha curcas* is a drought-resistant multipurpose shrub or a small tree belonging to the Family Euphorbiaceae. It is a native of tropical America but now thrives in many parts of the tropics and sub-tropics in Africa, Asia, and southern America (Gübitz et al. 1999). Jatropha seeds are rich in protein (40%), oil, essential amino acids, and mineral contents (Widiyastuti and Sutardi 2016). The extracts of *Jatropha* have an effect as nematicidal, fungicidal (Sharma and Trivedi 2002), antifeedant (Meshram et al. 1996), molluscicidal (Liu et al. 1997), insecticidal activity (Ohazuruike et al. 2003) and acaricidal activity (Roy *et al.* 2018). Toxicity of *J. curcas* seeds is attributed to several components, including saponins, lectins (curcin), phytates, protease inhibitors, curcalonic acid, and phorbol esters (Kumar et al. 2014).

The present study aimed to evaluate the efficiency of jatropha seed oils against *Varroa* mites and the side-effect of the oil on the biological activities of honeybee colonies.

Materials and methods

Jatropha oil concentrations and treatments

This work was carried out at a private apiary at Dirut region, Assiut Governorate, Egypt, from 28th December 2016 to 20th March 2017. Four concentrations, 1, 2, 5, and 10%, of jatropha oil (Habou et al. 2011) were tested for controlling the *Varroa* mites. Tested concentrations were prepared by mixing 1, 2, 5, and 10 ml of jatropha oil with 0.5 ml of Triton-x (emulsifier) and sufficient water to obtain 100 ml of solution. Nontoxic genotype of *J. curcas* (Michoacán) that cultivated at the experimental farm, City of Scientific Research and Technological Applications (SRTA-City), was used in the experiment. Twenty honeybee colonies of Carniolan hybrid *Apis mellifera* L. heavily infested by *Varroa* mites were used for the experiment. Each colony was housed in Langstroth hive and approximately at the same population (5 combs covered with bees). Experimental colonies were divided into 5 groups (4 colonies/group); each group received one of tested concentrations of jatropha oil. Five milliliters of each oil concentration was sprayed directly/colony (on the combs including adults and immature stages of honeybee). Each tested colony was treated 4 times at 12 days intervals from the beginning of experiment. The last group was considered as control (without any chemical treatment).

The percentage of *Varroa* mite infestation in the tested colonies during experimental period was determined in worker sealed brood (pupae with pigmented eyes) by investigation 50 individual cells for each tested colony (Martin 1994). In adult workers, the percentage of infestation was determined in approximately 100 living adult bee workers picked directly from the combs (De Jong 1988). Infestation percentage was determined 2 times at 3 days intervals before treatments, while during the treatments, infestations were determined every 15 days after each treatment. The reduction percentages were calculated from the collected data according to the equation of Henderson and Tilton (1955).

Biological activities of treated colonies

Biological parameters for each tested colony measured during the experimental period were the area of sealed worker brood, stored bee honey, and pollen per square inch at 12 days intervals (Fresnay 1962 and Delaplane et al., 2013). While, number of combs covered with bees was determined at 12 days intervals (Nour 1992). Dead mites fallen on the hive bottom board, covered with sticky sheet of white paper were collected and counted in all tested colonies at 1, 2 and 15 days after each treatment.

Extraction of *Jatropha curcas* seeds oil

Jatropha seeds of Michoacán variety (a wild nontoxic variety) were collected from the experimental farm, City of Scientific Research and Technological Applications (SRTA-City). Mechanical pressing the oldest and simplest method was used for oil extraction, where there were no chemicals to avoid any contamination of the oil. An amount of 1 kg of the jatropha seeds was pressed at room temperature by using manual mechanical pressing machine. Continuous screw-presses replaced the conventional hydraulic presser equipment (Bargale 1997). Mechanical extraction of the oil is accomplished by exerting sufficient force on confined seed. Under this condition, pressure is high enough to rupture the cells and force oil from the seed to "escape." Extraction is accomplished by compressing the material in a container that has small perforations, either round or slotted, that allow the liquid component to leave (Antony et al. 2011). The extracted oil was allowed to settle for 24 h and then filtered by filter paper.

Statistical analysis

All data were subjected to analysis of variance (ANOVA), using SPSS (Statistical Package for the Social Sciences) computer program (version). Means were compared using Duncan s Multiple Range Test. Significance was expressed at 0.05 level of probability.

Results and discussion

Varroa mite infestations on treated colonies

The percentage of *Varroa* mite infestation on sealed brood and adult workers was clearly reduced at the end of the treatments (4th treatment) in all tested concentrations of jatropha oil. It is clear that there was a significant effect of tested concentrations of the oil for reducing *Varroa* mites infestations on worker brood, when compared with control, especially after 2nd (df = 4, F = 9.04, P = 0.05), 3rd (df = 4, F = 7.65, P = 0.05), and 4th (df = 4, F = 15.90, P = 0.05) treatments. A

significant effect was found on adult workers infestations in the 3rd (df = 4, F = 3.62, P = 0.05) and 4th (df = 4, F = 10.55, P = 0.05) treatment than the control (Table 1). At 4th treatment, honeybee colonies treated with 1 and 2% concentrations of jatropha oil gave the highest reduction percentage of *Varroa* infestation in worker brood (88.75 and 85.60%, respectively), while 10 and 5% concentrations gave least reduction percentages (83.33 and 85%), respectively. The results also showed that the 2 and 1% concentrations reduced the percentages of *Varroa* mite infestation on adult workers by 84.07 and 63.27%. But the 5 and 10% concentrations gave lowest percentage (59.85 and 52.63%) after 4th treatment (df = 4, F = 10.55, P = 0.05) (Table 1).

Data mentioned in Table 2 revealed that the total number of *Varroa* mites fallen on the sheet increased gradually after the first treatment. The total number of the mite fallen on the sheet markedly decreased by the end of treatments (4th treatment) (df = 4, F = 5.25; 11.86 and 5.39, P = 0.05, after 1, 2, and 15 days, respectively), particularly in colonies treated with the concentrations 1 and 2% in comparison with the control colonies. Also, the results showed that treated bee colonies with concentrations 2, 5, and

Table 1 Efficiency of different concentrations of jatropha oil on reduction percentage of *Varroa* mites infestation in honey bee colonies

Treatment	Concentration	Mean percentage of *Varroa* infestation on worker brood		Mean percentage of *Varroa* infestation on adult workers	
		Mean of *Varroa* infestation	% of *Varroa* reduction	Mean of *Varroa* infestation	% of *Varroa* reduction
Before treatment	1%	16.00 a	–	18.75 a	–
	2%	25.00 a	–	17.50 a	–
	5%	20.00 a	–	12.25 a	–
	10%	18.00 a	–	10.75 a	–
	Control	12.00 a	–	10.00 a	–
	F value	1.90^{NS}	–	1.13^{NS}	–
First treatment	1%	9.00 a	64.47	19.00 a	34.02
	2%	14.00 a	64.63	17.75 a	37.5
	5%	15.00 a	52.63	22.75 a	16.07
	10%	12.00 a	57.89	16.75 a	16.90
	Control	19.00 a	–	16.50 a	–
	F value	2.03^{NS}	–	0.41^{NS}	–
Second treatment	1%	9.00 b	62.5	23.50 a	48.84
	2%	7.00 b	81.3	23.75 a	45.77
	5%	8.00 b	73.33	18.50 a	38.35
	10%	8.00 b	70.37	22.00 a	16.44
	Control	18.00 a	–	24.50 a	–
	F value	9.04**	–	0.33^{NS}	–
Third treatment	1%	5.00 b	85.57	8.50 b	74.09
	2%	10.00 b	81.35	8.00 b	73.87
	5%	12.00 b	72.30	11.25 b	47.52
	10%	9.00 b	76.92	9.25 b	50.82
	Control	26.00 a	–	17.50 a	–
	F value	7.65**	–	3.62*	–
Fourth treatment	1%	3.00 b	88.75	10.50 b	63.27
	2%	6.00 b	85.60	4.25 c	84.07
	5%	5.00 b	85.00	7.50 bc	59.85
	10%	5.00 b	83.33	7.75 bc	52.63
	Control	20.00 a	–	15.25 a	–
	F value	15.90**	–	10.55**	–

F value was determined with df (4, 15)
NS non-significant means in a column with dissimilar letters differ significantly at 0.05 level of probability

Table 2 Effect of different concentrations of the jatropha oil on the mean number of dead *Varroa* mites and adult bee fallen on the sheet

Treatment	Concentrations	No. of *Varroa* mites				No. of dead bees			
		24 h	48 h	15 days	Total	24 h	48 h	15 days	Total
Before treatment	1%	7.75 a	–	–	7.75	0.0	–	–	0.0
	2%	10 a	–	–	10	0.0	–	–	0.0
	5%	11.75 a	–	–	11.75	0.0	–	–	0.0
	10%	8.75 a	–	–	8.75	0.0	–	–	0.0
	Control	5 a	–	–	5	0.0	–	–	0.0
F value		1.89^{NS}							
First treatment	1%	6.5 a	6 ab	16.5 a	29	0.0	0.25	0.0	0.25
	2%	11.25 a	10.75 a	25.25 a	47.25	0.0	1.0	0.0	1.0
	5%	9.75 a	11 a	19.25 a	40	0.0	0.25	0.0	0.25
	10%	10.5 a	8.5 ab	24.25 a	43.25	0.0	0.5	0.0	0.5
	Control	3.5 a	3.75 b	12.75 a	20	0.0	0.0	0.0	0.0
F value		2.95^{NS}	3.19*	0.80^{NS}					
Second treatment	1%	7.75 b	8.25 a	21 a	37	0.5	0.0	0.0	0.5
	2%	15 ab	14.25 a	24.75 a	54	0.75	0.0	0.0	0.75
	5%	17.25 a	14.5 a	27.5 a	59.25	0.50	0.0	0.0	0.50
	10%	15 ab	14 a	24.25 a	53.25	0.25	0.25	0.0	0.50
	Control	14.5 ab	14.25 a	21 a	49.75	0.25	0.0	0.0	0.25
F value		1.61^{NS}	1.62^{NS}	0.30^{NS}					
Third treatment	1%	7 b	9.25 b	10.25 a	26.5	0.25	0.0	0.0	0.0
	2%	13.25 ab	10.25 b	21.25 a	44.75	0.25	0.0	0.1	0.35
	5%	11.5 ab	12 b	23.75 a	47.25	0.0	0.0	0.5	0.5
	10%	11.75 ab	11 b	24.5 a	47.25	0.25	0.25	0.5	1.0
	Control	16.5 a	17 a	23.5 a	57	0.0	0.0	0.75	0.75
F value		3.10*	3.77*	1.39^{NS}					
Fourth treatment	1%	7.5 b	9.5 b	7.25 b	24.25	0.0	0.0	0.0	0.0
	2%	8.25 b	9.75 b	11.75 b	29.75	0.25	0.0	0.0	0.25
	5%	15.25 a	9.25 b	11.25 b	35.75	0.0	0.25	0.0	0.25
	10%	10.5 ab	9.75 b	11 b	31.25	0.0	0.25	0.0	0.25
	Control	15.5 a	17.25 a	19.5 a	52.25	0.0	0.0	0.0	0.0
F value		5.25**	11.86**	5.39*					

F value was determined with df (4, 15)
NS non-significant means in a column with dissimilar letters differ significantly at 0.05 level of probability

10% of jatropha oil results higher numbers of fallen *Varroa* mites in most treatments. Treated bee colonies by the highest concentration (5 and 10%) of jatropha oil caused some harmfulness to the honeybee workers than the lowest concentrations (Table 2).

According to the *t* test analysis, obtained data showed a highly significant reduction of the *Varroa* mites infestation on worker brood before and after 4th treatment for 1, 2, 5, and 10% concentrations (t = 3.434, t = 2.752, t = 7.833, and t = 8.510, respectively). Furthermore, *Varroa* mite infestations of adult workers had significant differences at 2 and 10% concentrations of oil (t = 2.545 and 2.640), respectively before and after the 4th treatments (Table 3).

In the present study, honeybee colonies treated with 1 and 2% of jatropha oil had a high reduction percentage of *Varroa* mite infestation in worker brood and adult workers after the 4th treatment. It was noticed that the low concentrations of jatropha oil caused the highest reduction percentage of *Varroa* mite infestations than the highest concentrations. According to Devappa et al. (2010), *J. curcas* seed oil had a toxic chemical of phorbol esters, i.e., tetracyclic diterpenoids. This may attribute

Table 3 *t* test for the comparison between the levels of *Varroa* infestations before and after the four treatments by the different concentrations of jatropha oil

Concentrations	Mean percentage of *Varroa* infestation on worker brood			Mean percentage of *Varroa* infestation on adult workers		
	Before treatments	After fourth treatment	*t* value (df = 6)	Before treatments	After fourth treatment	*t* value (df = 6)
1%	16.00	3.00	3.434*	18.75	10.50	1.291^{NS}
2%	25.00	6.00	2.752*	17.50	4.25	2.535*
5%	20.00	5.00	7.833**	12.25	7.50	2.094^{NS}
10%	18.00	5.00	8.510**	10.75	7.75	2.640*
Control	12.00	20.00	2.828*	10.00	15.25	2.414*

Minimum average risk *t* value ($P = 0.05$) = 2.29

obtained results concerning high reduction of *Varroa* infestation in colonies treated with lower oil concentrations. These results may encourage using jatropha oil extract in management programs of *Varroa* mites in honeybee colonies.

Previous studies have shown that the toxicity of *J. curcas* oil is due to presence of several steroids and diterpenes (phorbol esters), which are the most toxic molecules in the plant (Kumar et al. 2014) and considered jatropha oil as an insecticidal and antifeedant against a wide range of insects (Phowichit et al. 2008). Studies conducted by Habou et al. (2011) revealed that jatropha oil concentration of 5% makes it possible to reduce aphids by 10% and thrips and bugs by 50%. However, the 7.5% of jatropha oil concentration makes it possible to reduce aphids by 50% and thrips and bugs by 75%. Ratnadass et al. (1997) reported that the raw oil extracted from *J. curcas* seeds had a larvicidal effect, at concentrations of 0.01% and 1% on *Busseola fusca* and *Sesamia calamistis*, respectively.

Biological activities of treated colonies

Area of reared brood

Treated honeybee colonies infested with *Varroa* mites by different concentrations of jatropha oil recorded remarkably increasing in the area of sealed brood (inch^2) and number of combs covered with bees than the control ones, particularly during 3rd and 4th treatments. After the last treatment (4th), the highest areas of sealed brood (75.75 and 77.50 inch^2) were in colonies treated with 1 and 2% concentrations of jatropha oil, respectively, while concentrations of 5 and 10% produced the lowest area of sealed brood (53.75 and 57.50 inch^2), respectively, (df = 4, $F = 0.87$, $P = 0.05$). After the 4th treatment, mean number of combs covered with bees was clearly increased, especially in the colonies treated with 1 and 2% of jatropha oil (Table 4).

The results clearly showed that after treatment, the brood area increased in all treated colonies. This may be due to the protein content of jatropha seeds. The state of honeybee colonies depends on the queen's reproductive and health. Thus, the activity of queen, health, and physiological conditions of worker bees can affect the colony through protein nutrition "pollen" (Fine et al. 2018). Jatropha seeds content about 33% protein, rich in various macro-elements and micro-elements as Mn, Fe, K, Mg, P, and Na (Abou-Arab and Abu-Salem, 2010). Therefore, the obtained results showed that the increase in brood area (inch^2) may relate to the content of protein and nutrient components of jatropha oil.

In general, the majority of honeybee activities increased gradually after the treatments as a result of curing the colonies from infestation with *Varroa* mites using the tested concentrations of jatropha oil. Dimetry et al. (2005) reported that, in all cases control of *Varroa* mites, using natural plant products are more recommended than other chemical acaricides to keep the biological activities.

Stored bee honey and pollen grain

Data obtained in Table 5 indicated that the mean amount of stored bee honey and pollen increased

Table 4 Mean area of sealed worker brood (inch^2) and number of combs covered with bees in honeybee colonies treated by different concentrations of the jatropha oil

No. of treatments	Area of worker brood (inch^2)/colony						Number of combs covered with bees/colony					
	Jatropha oil concentrations					*F* value	Jatropha oil concentrations					*F* value
	1%	2%	5%	10%	Control		1%	2%	5%	10%	Control	
Before treatment	28.25 a	27.25 a	23.75 a	23.75 a	28.00 a	0.34^{NS}	3.25 a	2.75 a	3.00 a	3.00 a	3.25 a	1.16^{NS}
First treatment	30.25 a	26.50 a	26.75 a	31.25 a	32.25 a	0.25^{NS}	3.25 a	3.00 a	2.75 a	3.00 a	3.00 a	1.25^{NS}
Second treatment	22.00 a	12.75 a	15.50 a	19.25 a	18.50 a	0.82^{NS}	2.50 a	2.50 a	2.25 a	2.25 a	2.25 a	0.26^{NS}
Third treatment	45.25 a	36.00 a	28.25 a	38 a	28.25 a	0.72^{NS}	3.25 a	2.75 a	2.75 a	2.75 a	2.50 a	1.12^{NS}
Fourth treatment	75.75 a	77.50 a	53.75 a	57.50 a	42.00 a	0.87^{NS}	4.00 a	3.50 a	3.25 a	3.25 a	3.25 a	0.48^{NS}

NS non-significant means in a row with dissimilar letters differ significantly at 0.05 level of probability

Table 5 Effect of different concentrations of jatropha oil extract on the honey and pollen grains stored in the tested honeybee colonies

No. of treatment	Area of honey (inch^2)/colony					*F* value	Area of pollen grains (inch^2)/colony					*F* value
	Jatropha oil concentrations						Jatropha oil concentrations					
	1%	2%	5%	10%	Control		1%	2%	5%	10%	Control	
Before treatment	95.00 a	82.50 a	72.50 a	85.00 a	95.00 a	0.66^{NS}	0.50 a	0.00 a	0.25 a	0.50 a	0.50 a	0.80^{NS}
First treatment	12.50 a	24.50 a	38.25 a	18.25 a	23.00 a	1.16^{NS}	1.00 a	1.25 a	1.00 a	1.00 a	1.00 a	0.27^{NS}
Second treatment	11.00 a	7.75 b	8.25 b	8.75 ab	6.75 b	3.84*	1.50 a	1.25 a	1.00 a	1.25 a	1.25 a	0.35^{NS}
Third treatment	28 a	27 a	28 a	27 a	27 a	0.10^{NS}	1.75 a	1.25 a	1.50 a	1.50 a	1.75 a	0.42^{NS}
Fourth treatment	126.50 a	93 a	96 a	111 a	96 a	1.55^{NS}	11.25 a	9.75 a	9.00 a	9.75 a	6.75 a	1.20^{NS}

NS non-significant means in a row with dissimilar letters differ significantly at 0.05 level of probability

gradually in colonies treated with different concentrations, particularly after 3rd and 4th treatments. At the end of experiment, the mean amount of stored honey area reached to 126.50, 111, and 96 inch^2 for concentrations 1, 10, and 5% (df = 4, F = 1.55, P = 0.05) of tested colonies, respectively. The results also indicated that stored pollen grains reached to 11.25, 9.75, and 9.75 inch^2 when the concentrations 1, 2, and 10% were used (df = 4, F = 1.20, P = 0.05), respectively.

Obtained results indicated that treated colonies with jatropha oil slightly increased in the area of stored honey and pollen. Since *Varroa* mite has devastating effects on honeybee colonies because it causes a physical damage and transmit pathogens, especially viruses and resulted weaken colonies (Strauss et al. 2014). Like infected colonies failed collecting nectar and pollen (Bagheri and Mirzaie 2019). Yousif-Khalil et al. (2009) reported that *Varroa* mite infestation affected honey production of infested honeybee colonies, whereas the diseased colonies have workers characterized with lower ability of flying to visit the flowers, lower capacity of loading nectar and pollen. Moreover, Begna et al. (2016) investigated negative correlation ($p < -0.513$) between mite population and bee brood, stored honey, stored pollen, and population of workers as well.

Recently, controlling of *Varroa* mites in honeybee colonies is challenging for beekeepers because *Varroa* mites have developed high resistant to synthetic acaricides, contamination, and increase residues of these acaricides in the honeybee products and the cost of treatments as well (Rosenkranz et al. 2010). Therefore, beekeepers look for alternative, safety, and natural treatments for controlling *Varroa* mites to produce bio-bee products that became more interest for consumers and beekeepers. These results strengthen one of the recent recommendations for the control of *V. destructor* using natural and eco-friendly products. However, using jatropha oil for controlling *Varroa* mites needs further studies to investigate its toxicity (even use nontoxic genotype) to both immature stage (larvae and pupae) and adult bees.

Conclusion

Obtained findings encourage researchers to study deeply the ability of using suitably concentrations of jatropha oil in the field of Apicultural and management programs of *Varroa* mites in honeybee colonies. Further studies to investigate its toxicity (even use nontoxic genotype) to both immature stage (larvae and pupae) and adult bees are necessary.

Abbreviations

Varroa: *Varroa destructor*; Jatropha: *Jatropha curcas*; JatroMed project: Evaluation of the energy crop *Jatropha curcas* as a mean to promote renewable and sustainable energy for the Mediterranean region

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Authors' contributions

The first author, SHDM, take care of Jatropha plants, collected seeds, extract oil, and prepare it for treatment, and contributed in writing the MS. The second author, TEA, designed the experiment in the apiary, followed the treatment and analyzed data, and contributed in writing the MS. The third author, MR, is the PI of JatroMed project, and he followed all process for oil extract and purification. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this article.

Ethics approval and consent to participate

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Consent for publication

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Competing interests

We, the authors, do not have competing interests.

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