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RESEARCH ARTICLE

Genetic and reproductive toxicity of aqueous extracts of *Telfairia occidentalis* (Hook F.), *Vernonia amygdalina* and their combination on the testicular cells of male mice

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Abstract

Telfairia occidentalis (Hook F.) and *Vernonia amygdalina* are two commonly consumed vegetables, individually and as recipes, for their nutritive and medicinal values in Africa. Data on reproductive toxicity and DNA damage of the combination of these plants on the male reproductive system is scarce. We evaluated the toxic and genotoxic effects of aqueous extracts of *T. occidentalis* and *V. amygdalina* and their combination on the reproductive cells of male Swiss albino mice. Groups of mice exposed to five concentrations (62.5–1000 mg/kg) of each extract or combination as against distilled water and cyclophosphamide (20 mg/kg bwt) as negative and positive control respectively were examined for abnormal sperm morphology, organo-somatic index and pathological changes. Each of the extracts significantly reduced the frequency of aberrant sperm cells, while their combination provoked increasing sperm abnormalities when compared with the negative control. None of the extracts induced adverse effect on body and testes weight of exposed mice. There was no visible histological lesion induced by extracts of *V. amygdalina* but there were residual bodies indicative of increased spermatogenesis. The extracts of *T. occidentalis* induced necrosis only at 1000 mg/kg, while with the combination, there were necrotic cells, mild congestion of blood vessels, and the seminiferous tubules had irregular outlines. These indicates that aqueous extracts of *T. occidentalis* and *V. amygdalina* individually at the tested doses did not evoke significant induction of genetic damage in the sperm head, and pathological changes unlike the combination in mice.

Keywords *Telfairia occidentalis* · *Vernonia amygdalina* · Abnormal spermatozoa · Reproductive toxicity · DNA damage

Abbreviations

DNA	Deoxyribonucleic acid
ACUREC	Animal care and use in research ethics committee
ANOVA	One-way analysis of variance

Introduction

The use of medicinal plants, and/or compounds isolated from medicinal plants, as supplement or for the treatment of diseases is increasing worldwide. This is due to the assumption that they are effective and free from adverse effects (Abbas et al. 2018). However, toxicity of medicinal plant is currently a concern. There are reports of adverse effects arising from the consumption of medicinal plants in animals and man (Oyeyemi et al. 2015; Enfield et al. 2018; Seremet et al. 2018; Ugwah-Oguejiofor et al. 2019). Toxicity can be specific to an organ, multiple organs or to system(s). Toxicity towards the reproductive system in males and females are

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not uncommon and can contribute to infertility in human. Hence, the need to investigate the toxicity of medicinal plants on the reproductive system to ascertain their safety.

Telfairia occidentalis, Hook F., and *Vernonia amygdalina* Del. are often grown around the house as vegetables and commonly consumed for their nutritive and medicinal effects in Nigeria. *T. occidentalis* is known as 'Ugwu' in Igbo language and 'Apiroko' in Yoruba language of Nigeria, while *V. amygdalina* is known as 'Olubu' in Igbo language and 'Ewuro' in Yoruba language. Each of the vegetables can be prepared individually and both can be combined for cooking or as recipes for medicinal uses (Oyeyemi et al. 2019). *T. occidentalis* and *V. amygdalina* are known to possess several pharmacological effects including antioxidant and anticancer effect (Iwalewa et al. 2005; Eseyin et al. 2014; Kadiri and Olawoye 2016; Oyeyemi et al. 2018). Extract of the leaf of *T. occidentalis* was non-toxic to brine shrimp (Olatunbosun et al. 2018), the liver and male reproductive system of rats (Akindele et al. 2018) while extracts of *V. amygdalina* was non-toxic to the liver and kidney of rats (Akowuah et al. 2015; Njan et al. 2008) but was genotoxic in liver and breast cancer cell lines (Yedjou et al. 2008; Kahaliw et al. 2018). The plants have been reported to each exhibit spermatogenic effect at low concentrations and spermatotoxic effect at high concentrations (Saalu et al. 2013; Emmanuel et al. 2018). There is however a dearth of information on the effect of the combination of these plants on the male reproductive system. This study seeks to investigate the genotoxic effect of *T. occidentalis*, *V. amygdalina* and their combination using the mouse sperm morphology assay (Wyrobek et al. 1983; Bakare et al. 2005). In addition we investigated the toxic effect of the extracts on the histology of the testes.

Materials and methods

Plant collection and extraction

The leaves of *T. occidentalis* were obtained commercially at Bodija market Ibadan, Nigeria while the leaves of *V. amygdalina* were obtained within the premises of University of Ibadan, Ibadan, Nigeria. Both were identified and authenticated at the University of Ibadan Herbarium, and voucher specimens UIH-22711 and UIH-22710 were deposited for *T. occidentalis* and *V. amygdalina* respectively. Aqueous extract was prepared by juicing the fresh leaves as previously described by Akinsulire et al. (2007). Briefly 1000 g of the leaves of each plant or the combination of the two (1:1) were infused in 1000 mL of distilled water and filtered using 110 mm filter (Whatman® no. 42). The filtrate was taken as the stock and stored at 4 °C until use. The extracts were designated aqueous extract of *T. occidentalis*, aqueous extract of *V. amygdalina* and aqueous extract of the mixture.

Acquisition and care of experimental mice

Male Swiss albino mice (*Mus musculus*, 11–15 weeks old; 28 ± 2 g) were obtained from the animal breeding unit of the Department of Physiology, University of Ibadan, Ibadan, Nigeria. The animals were kept in a pathogen-free experimental animal facility of the Department of Zoology, University of Ibadan, Ibadan, Nigeria, in plastic cages and subjected to the natural light-dark cycle before and during the experimental period. Standard pelleted feed (Vital Feed®, Nigeria) and drinking water were supplied *ad libitum*. Animals were cared for according to standard guidelines (ILAR 2011). The experiment was approved by the Animal Care and Use in Research Ethics Committee (ACUREC) of the University of Ibadan, Ibadan, Nigeria (UI-ACUREC/18/0061).

Experimental design

For each of the extracts or combination, mice were randomly divided into 7 groups (n = 5). Group I served as negative control and received 0.2 mL of distilled water by oral gavage daily for 35 days. Group II served as positive control and received cyclophosphamide (20 mg/kg, i.p) for 5 consecutive days. Groups III–VII received 62.5, 125, 250, 500 and 1000 mg/mL of each of the extracts or combination by oral gavage daily for 35 days. Body weight of each mouse in each group was taken on the first and last day of the experiment. All animals were sacrificed on the 35th day by cervical dislocation. The caudal epididymes and testes were surgically excised. Sperm smears were prepared from the epididymes as previously described (Wyrobek et al. 1983; Bakare et al. 2005; Oyeyemi et al. 2015). For each mouse, 1000 sperm cells were assessed for morphological abnormalities according to standard (Wyrobek and Bruce 1975; Wyrobek et al. 1983; Oyeyemi et al. 2015).

Relative organ weight of the testes and histopathology

Testes were rinsed in physiological saline, blotted with filter paper and weighed, after which they were fixed in Bouin's fluid. Sections were prepared and processed for histopathological examination as earlier described (Oyeyemi et al. 2017). The relative organ weight was calculated as:

$$\text{Relative Organ Weight} = \frac{\text{Absolute organ weight (g)} \times 100}{\text{Final body weight of animal prior to sacrifice (g)}}$$

Statistical analysis

Data were presented as mean ± S.E. Significance difference between groups was tested using one-way analysis of variance (ANOVA) at 5% significant level followed by the Dunnett's multiple comparison post-hoc test. Values were considered statistically significant at $p < 0.05$.

Results

Body weight, organ index and histopathology

An increase in body weight was observed in mice exposed to different concentrations of the extracts and controls for 35 days (Table 1). The testes weight in mice treated with the aqueous extracts of each of *T. occidentalis* and *V. amygdalina* and their combination was similar to that observed in the negative control. The positive control however, caused a reduction in the body weight, testes weight and relative testes weight. The architecture of the testes in the negative control mice showed uniformly sized seminiferous tubules with regular outlines and predominant stages of spermatocytes, but in the positive control the spermatocytes were necrotic and haphazardly arranged (Fig. 1a–f). In the testes of mice treated with *T. occidentalis,* no visible lesions were observed except at 1000 mg/mL where foci of necrosis of spermatogenic cells were observed. There was no visible lesion at the tested concentrations of *V. amygdalina* but there were residual bodies indicative of increased spermatogenesis. In the testes of mice treated with the combination, there were necrotic cells and mild congestion of blood vessels, and the seminiferous tubules had irregular outlines.

Sperm morphology assay

Each of the three extracts induced different types of aberrant sperm cells in mice. These include sperm cells with banana

Table 1 Changes in body weight and relative organ weight of mice exposed to aqueous extracts of *Telfairia occidentalis*, *Vernonia amygdalina* and their combination (1:1) for 35 days

Conc. (mg/mL)	*Telfairia occidentalis*			*Vernonia amygdalina*			Combination of TO and VA		
	Percentage increase in body weight	Absolute testes weight (g)	Relative testes weight	Percentage increase in body weight	Absolute testes weight (g)	Relative testes weight	Percentage increase in body weight	Absolute testes weight (g)	Relative testes weight
NC	14.24 ± 3.53	0.22 ± 0.01	0.86 ± 0.03	10.94 ± 3.53	0.24 ± 0.01	0.86 ± 0.03	14.24 ± 3.44	0.22 ± 0.02	0.86 ± 0.07
62.5	23.33 ± 3.79	0.19 ± 0.01*	0.82 ± 0.03	11.20 ± 7.96	0.20 ± 0.01	0.89 ± 0.06	18.53 ± 1.39	0.20 ± 0.01	0.89 ± 0.04
125	16.52 ± 4.67	0.22 ± 0.02	0.85 ± 0.04	9.62 ± 4.54	0.21 ± 0.01	0.72 ± 0.05	9.71 ± 1.59	0.21 ± 0.01	0.88 ± 0.05
250	9.76 ± 3.13	0.24 ± 0.02	0.82 ± 0.06	18.39 ± 2.69	0.63 ± 0.42	2.79 ± 1.89	7.14 ± 2.15	0.20 ± 0.02	0.86 ± 0.09
500	14.33 ± 2.82	0.20 ± 0.01	0.85 ± 0.02	22.32 ± 1.35	0.21 ± 0.02	0.84 ± 0.06	16.47 ± 1.35	0.21 ± 0.01	0.95 ± 0.03
1000	22.89 ± 3.76	0.20 ± 0.01	0.88 ± 0.03	6.05 ± 2.80	0.23 ± 0.02	0.88 ± 0.05	13.41 ± 2.53	0.23 ± 0.01	0.88 ± 0.06
PC	8.73 ± 5.13	0.17 ± 0.02	0.64 ± 0.09	8.73 ± 5.13	0.17 ± 0.02	0.64 ± 0.09	8.73 ± 5.13	0.17 ± 0.02	0.76 ± 0.10

NC Negative control (distilled water), *PC* positive control (cyclophospahmide, 20 mg/kg), *TO Telfairia occidentalis*, VA *Vernonia amygdalina*

*$p < 0.05$ significant when compared with the negative control

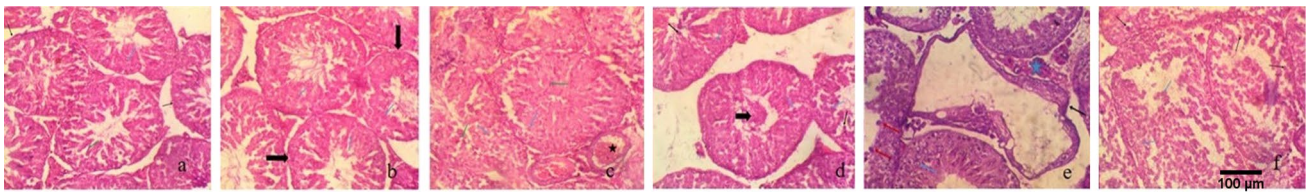

Fig. 1 Histopathologic lesions in testes of mice exposed to combination of aqueous extracts of *Telfairia occidentalis* and *Vernonia amygdalina*, **a** no visible lesion; seminiferous tubules with regular outlines (black arrow) having predominant stages of spermatocytes (blue arrow), **b** no visible lesion; seminiferous tubules with predominant stages of spermatocytes (blue arrow) and residual bodies indicative of increased spermatogenesis (black arrow), **c** seminiferous tubules with regular outlines (blue arrow) having predominant stages of spermatocytes, elongated spermatids (green arrow) and congested blood vessels (black star), **d** small-sized seminiferous tubules with regular outlines (blue arrow) having predominant stages of spermatocytes and foci of necrosis of spermatogenic cells (black arrow), **e** seminiferous tubules with irregular outlines(black arrow) having predominant stages of spermatocytes (blue arrow), mild congestion of blood vessels (blue star) and necrotic spermatogenic cells (red arrow), **f** haphazardly arranged spermatocytes (blue arrow); obscured lumen; necrotic spermatogenic cells (black arrow). Magnification: ×400

head, knobbed hook, pin head, wrong-tail attachment, no hook and folded spermatozoa (Fig. 2a–l). The frequency of aberrant sperm cells induced by different concentrations of aqueous extracts of *T. occidentalis* and *V. amygdalina* were significantly ($p < 0.05$) lower compared to the negative control except at 62.5 and 1000 mg/kg of *T. occidentalis*, where it was similar to the negative control (Table 2). The frequency of aberrant sperm cells in mice exposed to the combination of the two plants increased with increasing concentration and was significantly higher ($p < 0.05$) than those observed in the negative control at 1000 mg/kg. The frequency of aberrant sperm cells in mice receiving the combination is higher than in mice receiving the corresponding dose of either *T. occidentalis* or *V. amygdalina* except at 6.5 mg/mL.

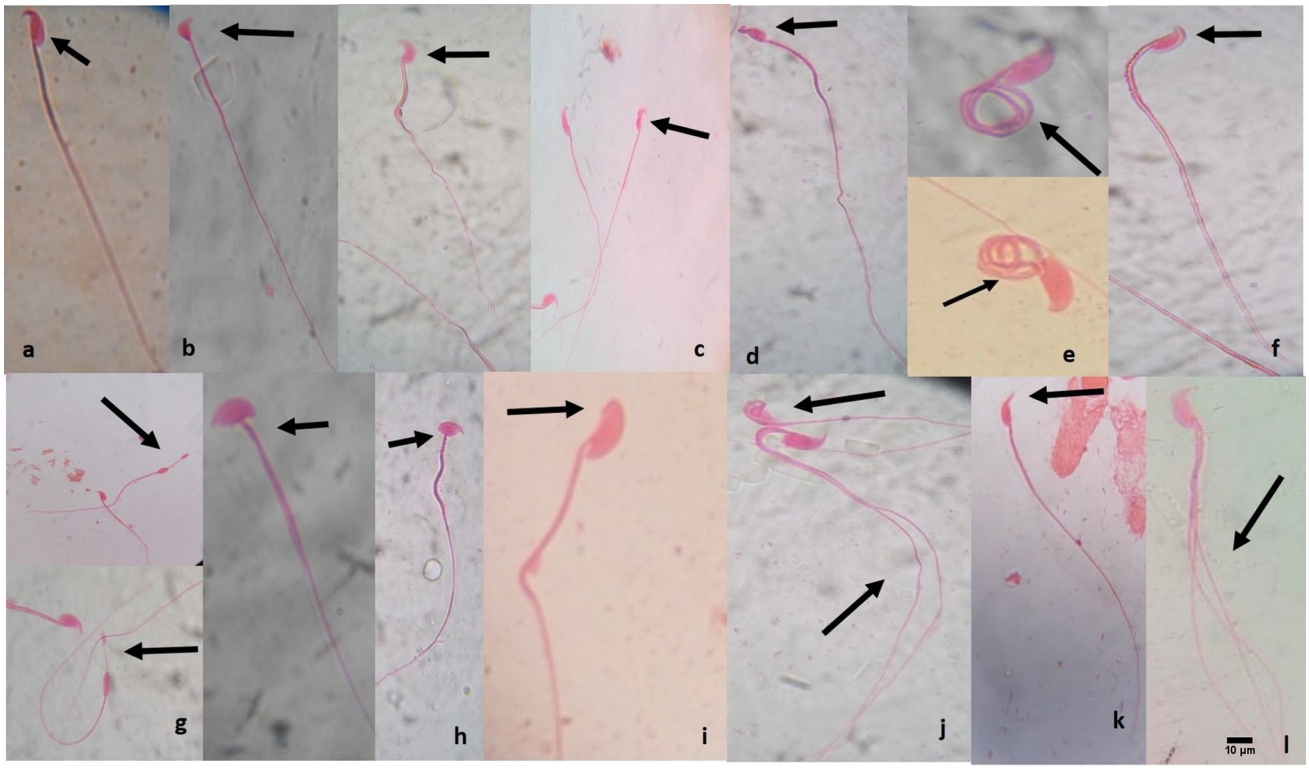

Fig. 2 Abnormal sperm cells induced in mice exposed to aqueous extracts of *Telfairia occidentalis, Vernonia amygdalina* and their combination. **a** Normal sperm cell, **b** amorphous head, **c** banana head, **d** knobbed hook, **e** folded sperm cells, **f** short hook, **g** sperm cells with pin head, **h** wrong-tail attachment, **i** no hook, **j** double tails, **k** hook at wrong angle, **l** triple tails. Magnification: ×1000

Table 2 Frequency of abnormal sperm cells induced in mice exposed to aqueous extracts of *Telfairia occidentalis*, *Vernonia amygdalina* and their combination (1:1) for 35 days

Conc. (mg/mL)	*Telfairia occidentalis*	*Vernonia amygdalina*	Combination of TO and VO
	Mean ± SE	Mean ± SE	Mean ± SE
NC	16.34 ± 1.05	16.34 ± 1.05	16.34 ± 2.18
62.5	18.20 ± 4.60	10.86 ± 1.36*	11.16 ± 0.47*
125	8.70 ± 0.90*	12.43 ± 1.10*	13.98 ± 0.97
250	9.64 ± 1.78*	9.00 ± 1.18*	14.25 ± 5.54
500	11.38 ± 2.79*	8.48 ± 1.42*	17.20 ± 1.19
1000	16.64 ± 1.15	10.03 ± 1.23*	22.23 ± 1.28*
PC	34.33 ± 8.21*	34.33 ± 8.21*	34.33 ± 8.21*

NC Negative control (distilled water), *PC* positive control (cyclophosphamide 20 mg/kg bwt)

*$p < 0.05$ significant when compared with the negative control

Discussion

The use of herbs for the prevention and/or management of diseases is increasingly gaining acceptance all over the world. However, there is a heightened concern on the safety of these herbs. The health of the reproductive system is vital to fertility; agents that impairs the health of the reproductive system significantly impact fertility. Plant extracts have been reported to be able to interfere with or promote the structural integrity and functional competence of the sperm cells (Parveen et al. 2003; Mbongue et al. 2012; Bakare et al. 2015; Oyeyemi et al. 2015). In this study, we investigated the potential toxic effect of two commonly consumed vegetables in Nigeria, *T. occidentalis* and *V. amygdalina*, and their combination on the testicular cells of male mice. Body weight and organ index are two simple but valuable indicator of toxicity. Increase in body weights and insignificant alteration of testes weight index of mice exposed to different concentrations of the tested extracts implied that the extracts did not interfere with growth nor caused a gross alteration in the testes.

Sperm morphology is an important criterion in achieving pregnancy (Berkovitz et al. 2007; Tasaka et al. 2009), hence any agent that induces abnormality in sperm morphology can negatively impact fertility. Herein, the extracts of the individual plants did not induce an increase in the frequency of sperm morphology but rather reduced the background frequency. Frequency of aberrant cells have been associated with the antioxidant status of the cells and apoptotic markers (Ammar et al. 2020). This implies that each of the plant probably increased the antioxidant status of testicular cells and reduced the level of the background reactive oxygen species (ROS) since their antioxidant activities have been earlier demonstrated (Iwalewa et al. 2005; Oboh et al. 2006). This shows that the individual plant extract did not impact spermatogenesis negatively; an observation which is in concert with previous report that the leaves of *T. occidentalis* can improve sperm parameters (Salman et al. 2008; Emmanuel et al. 2018) in rats.

Their combination however, led to an increase in sperm with aberrant morphology. This can be due to interactive effects of phytochemicals present in the two vegetables. The two plants are rich in alkaloids and tannins (Lyumugabe et al. 2017) which are known for their harmful effects on living cells. Hence, the combination might have led to interactive effects of the phytochemical constituents leading to an increase in abnormal sperm morphology. Henkel et al. (2018) reported that excess intake of dietary antioxidants was associated with reductive stress with significant negative effects on cells and male fertility. The combination of the extracts having higher concentration of phytochemicals could have hindered metabolic processes and hence vital funtions in the male reproductive system (Al Joudi 2013). Increase in sperm abnormality has a positive correlation with increase in the DNA damage (Ammar et al. 2020). The combined phytochemicals possibly induced DNA damage in the spermatozoa by increasing the oxidative stress in testes which resulted in increase in the frequency of abnormal sperm cells (Kumar et al. 2002, Ammar et al. 2020).

Histopathological analysis revealed that *T. occidentalis* increased spermatogenesis at the lowest tested concentration, but necrosis at the highest tested concentration. This implies an imbalance in the cell death in seminiferous tubules which will subsequently lead to decrease in spermatogenesis (Niknafs et al. 2015). This corroborate the data on sperm morphology test that showed a significant increase in aberrant sperm morphology at the highest tested concentration. Induction of necrosis and increased abnormal sperm cells confirmed that *T. occidentalis* was toxic and depleted the antioxidant enzymes at high concentration. This can be due to the presence of high level of cyanide and tannin in the leaves of *T. occidentalis* (Akwaowo et al. 2000). The dynamics of phytochemical interaction changes with changing concentration. Thus, the concentration of the phytochemicals probably increased with increasing concentration resulting in the deleterious effect observed at the high concentration. Hence, it is not uncommon for extract which are safe at low concentrations to be toxic at high concentration (Nurul et al. 2018; Ugwah-Oguejiofor et al. 2019; Yang et al. 2019). Consumption of high concentration of *T. occidentalis* could thus be harmful to the sperm cells and can impair the male reproductive system even though it may be safe and beneficial at low concentrations (Akindele et al. 2018; Emmanuel et al. 2018).

V. amygdalina enhanced spermatogenesis at the tested concentrations and no visible histological lesion was observed across the groups. This corroborate the decline in the frequency of background aberrant sperm cells, and implies that it protected the sperm cells from DNA damage possibly by scavenging the free radicals. Considering this, consumption of *V. amygdalina* has a protective effect on the male reproductive system and could be beneficial for male fertility. This is in consonance with the report of Ibrahim et al. (2000) that the leaves of *V. amygdalina* did not alter the testicular architecture of rats exposed for 65 days. But it is in contrast to the findings of Saalu et al. (2013) who reported poor testes histo-morphometric profiles in male rats exposed to it for 60 days.

Conclusion

This study showed that extracts of *T. occidentalis* and *V. amygdalina* at the tested doses did not induce significant abnormal sperm cells and histological alterations, but the combination caused adverse reproductive effect in male mice. The individual aqueous extract did not provoke significant induction of DNA damage in the sperm head but rather preserved its integrity. This suggest potential benefit of consumption of the individual vegetable above the combination on the male reproductive system.

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Authors' contributions AAB, OIT and OOM designed the study. OOM, OOE and ASA carried out all laboratory experiments and analysed the data. AAB and OIT wrote the manuscript with contributions from all authors. All authors have read and approved the manuscript.

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Data availability The datasets generated during the current study are available in the postgraduate research projects supervised by the corresponding author. In the Department of Zoology, University of Ibadan, Ibadan, Nigeria. These datasets were however summarised/analysed and used in the manuscript.

Compliance with ethical standards

Ethical statement Animals were cared for according to standard guidelines (ILAR, 2011). The experiment was approved by the Animal Care and Use in Research Ethics Committee (ACUREC) of the University of Ibadan, Ibadan, Nigeria (UI-ACUREC/18/0061).

Conflict of interests O. E. Oyinleye has no conflict of interest. S. A. Adeniran has no conflict of interest. O. M. Ogunsuy has no conflict of interest. I. T. Oyeyemi has no conflict of interest. A. A. Bakare has no conflict of interest.

Informed consent All authors gave their consent to participate in the study and publication of this article.

Code availability Not applicable.

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