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(RESEARCH ARTICLE)

# The ameliorative effect of *Aloe Vera* on cholelithiasis

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## Abstract

**Aims**: The aim of this study was to evaluate the effect of *Aloe Vera* (AV) on gallstone formation by evaluating its effect on substances which are involved in the pathogenesis of gallstones, and this includes biliary calcium, bilirubin and cholesterol concentration.

**Methodology**: A total of thirty-five (35) albino wistar rats weighing between 200 and 250g were obtained from the Animal house, Department of Physiology. The animals were randomly divided into 5 groups of 7 animals each. Group I served as control, group II was fed ca2+ in cow' s milk and High fat diets (HFD) while group III and IV received low dose and high dose of *Aloe Vera* gel extract respectively in addition to ca2+ and high fat diet. Afterward a period of 12 weeks, the animals were sacrifices and bile collected for analysis of biliary flow rates and constituents (biliary calcium and bilirubin), while blood for calcium analysis was collected by cardiac puncture and placed in lithium heparinised tubes respectively.

**Results**: Analysis of the various parameters revealed a reduction in biliary and plasma calcium ion, Increased in biliary flow rate, reduction of total cholesterol concentration. But an increase in total bilirubin and conjugated bilirubin, but a reduction in unconjugated bilirubin.

**Conclusion**: these results are indicative of the efficacy of *Aloe Vera* gel to obviate derangement of biliary parameters that lead to cholelithiasis, hence *Aloe Vera* may be used to prevent formation, reoccurrence of gallstones.

**Keywords:** *Aloe Vera*; Cholelithiasis; Bilirubin; Cholesterol; Calcium

## 1. Introduction

Gallstone diseases (GSD) also known as cholelithiasis is a fairly widespread disorder that occurs in most parts of the world [1] and one of the most common gastrointestinal diseases seen in clinical practices [2] which is also a major indicator for most abdominal surgery [1].

The Prevalence of GSD has shown to be higher in female, aged, higher body mass index (BMI), alcohol consumption, and diabetes mellitus all have been accounted for as predisposing factors for GS formation [3,4,5]. Gallstone is a worldwide problem [6]. Though, the condition is rare among indigenous Africans. However, a recent epidemiologic study of gallstone incidence in Nigeria suggests an increasing trend of the condition owing to the westernization of dietary habits among Nigerians [7,8].

The chief constituents of gallstones are cholesterol, bilirubin and calcium [9]. Other constituents may include fatty acids, triglycerides, polysaccharides and protein [10].

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Generally, Gall stones can be classified based on analysis of its constituents into pure gallstone of cholesterol or calcium bilirubinate (Pigment stones), mixed gallstones (Cholesterol, calcium carbonate, calcium bilirubinate) and a combination of stones with a nucleus of one type and a shell of another substance [11]. Analysis of these composites reveals excess bilirubin, cholesterol and calcium as major constituents of these stones. The quantification and evaluation of these constituents in gallstones enables the understanding of its etiology, origin, metabolic basis, and pathogenesis of the disease [12,13].

However, the pathogenic mechanism(s) by which gallstones forms is generally agreed to be due to alteration in the composition of bile, stasis and infection [14].

Anticholelithiatic plants are plants used to manage or prevent gallstones formation. Various anticholelithiatic plants have been employed in the past before imagining current medicines for treating gallstones and to avoid their recurrence [15]. Although medicinal plants produce slow recovery, they are affordable and less expensive, evidence based traditionally proven to dissolute or eliminate gallstones, less relapse of cholelithiasis, their successful prophylactic use, less side effects does not only reveal their therapeutic potential but encourages patient's belief and increasing their interest in traditional practices to find a herbal treatment for gallstones. The use of anticholelithiatic plants comes in form of decoction,juice, infusion, powder with water, raw eaten are less expensive than current medication and procedures [16].Expanding interest for restorative plants has become as one of the main territories of exploration and *Aloe Vera* is one of such plant of interest.

The botanical name of *Aloe Vera* is Aloe barbadensis miller. It belongs to Asphodelaceae (Liliaceae) family, and is a shrubby or arborescent, perennial, xerophytic, succulent, pea- green color plant. It grows mainly in the dry regions of Africa, Asia, Europe and America [17]. *Aloe Vera* has been used to manage a wide range diseases including Seborrheic dermatitis, psoriasis vulgaris, genital herpes, skin burns, type II diabetes, HIV infection [18,19,20]. Moreso, traditional use and ayurvedic therapies of *Aloe Vera* has shown to be used to dissolve small gallstones [18,21]. But there is paucity of data on the effect this plant has on the pathogenesis of gallstone. Hence, this present study was objectified to investigate the effect of *Aloe Vera* on bilirubin concentration, biliary electrolytes and biliary cholesterol concentration

## 2. Material and methods

### 2.1. Animals

A total of 35 albino wistar rats weighing between 200- 250g were obtained from the animal house, Department of Physiology, University of Calabar. They were housed in plastic cages and maintained under standard laboratory conditions. They were fed standard rat chow(Bendel Feeds and Flour Mills, Ewu, Nigeria) and were allowed free access to water. All the animal experiments were conducted according to the ethical norms approved by the Institutional Ethical Committee.

### 2.2. Preparation of *Aloe Vera* leaf pulp extract

The *Aloe Vera* plant over 3 years old was harvested from a local garden in Calabar, Cross River State, Nigeria. It was authenticated by the chief botanist of university of Calabar. Afterwards, the gel was extracted using a spoon and homogenized with a homogenizer (Ultra-Turrax T25, IKA Labortechnik, Germany), it was mixed with equal volume of phosphate buffer saline (0.1M, Ph 7.0), then homogenized again and filtered. The fresh yield pup was kept at refrigerator temperature of about -10 °C until used. It was administered as low dose *Aloe Vera*(LD AV) and high(HD AV)dose*Aloe Vera* at 0.4ml/100g and 0.8ml/100g respectively.

### 2.3. Formulation of high fat diet (HFD) and calcium supplement.

High fat diet (HFD) to induce Diet-induced hypercholesterolemia was by similar method used by Eddy et al., [22], we fed experimental rats with standard rat chow supplemented with 4% cholesterol. While calcium ions were administered by a similar method by Varnaiet al., [23]. Calcium was administered to the rats in cow's milk by addition of 3% calcium as $CaHPO_4 \times 2H_2O$ suspension to increase the daily calcium intake 3 times above controls values.

### 2.4. Animal grouping

The animals were randomly divided into 5 groups of 7 rats each.

- Group I served as control, it was administered: rat chow and water freely.

- Group II was administered: Ca2+ (3% calcium in cow's milk) and High fat diet (HFD) (rat chow supplemented with 4% cholesterol).
- Group III was administered: ca2+ and HFD + LD AV (0.4 ml/100g)
- Group IV was administered: ca2+ and HFD + HD AV (0.8 ml/100g)

### 2.5. Sample collection

After 12 weeks of the treatment, some animals were sacrifices by cervical dislocation and blood samples for calcium analysis was collected into lithium heparinised tubes for analysis of blood calcium ion concentration.

### 2.6. Measurement of Biliary cholesterol concentration

Cholesterol was determined using the method of Allain et al., [24]. This is an enzymatic method is described for determination of total cholesterol by use of a single aqueous reagent. The method requires no prior treatment of sample and the calibration curve is linear to 600 mg/dl. Cholesterol esters are hydrolyzed to free cholesterol by cholesterol ester hydrolase (EC 3.1.1.13). The free cholesterol produced is oxidized by cholesterol oxidase to cholest-4-en-3-one with the simultaneous production of hydrogen peroxide, which oxidatively couples with 4-aminoantipyrine and phenol in the presence of peroxidase to yield a chromogen with maximum absorption at 500 nm. The method is reproducible.

### 2.7. Measurement of calcium ions

Calcium ions in bile was measured in duplicate at similar body temperature of 37 °C with an ICA 1 analyzer (Radiometer, Copenhagen, Denmark), which provides simultaneous measurements for the activity of Ca2+ and PH as well.

Measurement of plasma calcium ions involved fasting the animals for a period of 18hrs, then blood was collected by cardiac puncture into lithium heparin containers and sent to the laboratory for analysis.

### 2.8. Collection of bileand biliary flow rate

At the end of the extract administration, bile was collected by the method of Vickers et al [25]. The animals were starved for 18hrsprior to the procedure. The rats were then weight and anaesthetized with sodium thiopentone at a dose of 6 mg/100 g body weight. They were fastened onto a board for tracheostomy to clear the airways. An incision was made along the linea alba to expose the stomach. A laparotomy was performed and the liver lobes deflected to expose the common bile duct. The common bile duct was cannulated with a portex cannula of 0.5 mm in diameter; thereafter a small incision was made and a cannula was fastened with a white cotton thread. The bile was collected for 3 h for each rat to obtain bile and bile flow rate per unit time [25].

### 2.9. Measurement of biliary bilirubin concentration

The bilirubin concentration in bile was determined by colorimetric method as describe by Jendrassik and Grof [26] and modified by Sherlock [27].

### 2.10. Statistical analysis

Data are presented as mean ± standard error of the mean. Data was analyzed using GraphPad Prism 8.02 version (GraphPad Statistical Software Inc., San Diego, CA, USA). Comparisons between the groups were made using one-way analysis of variance and Turkey's post hoc test was employed to test the significance of the difference between groups, and $P < 0.05$ was considered as statistically significant.

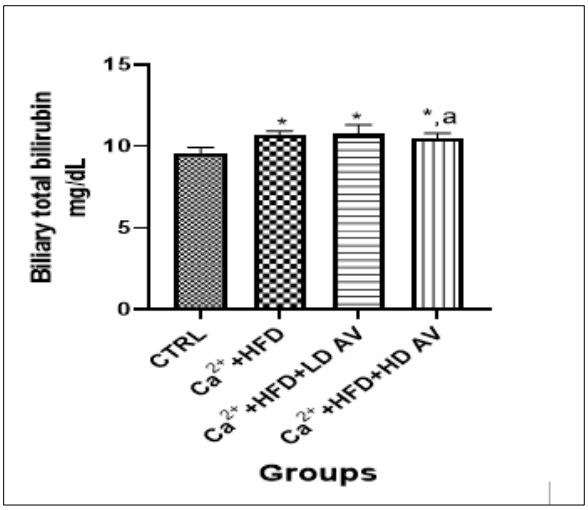
## 3. Results

### 3.1. Bile concentrations of total, conjugated and unconjugated bilirubin

Figure 1. Biliary total bilirubin concentration in control, Ca2++HFD, Ca2++HFD+LD AV and Ca2++HFD+HD AV groups.

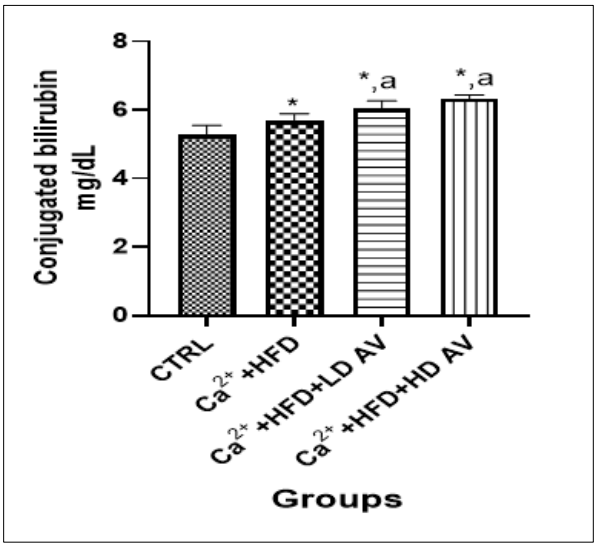
The mean and SEM values were 9.586±0.1370, 10.71±0.08571, 10.76±0.2114, 10.50±0.1155 for control, Ca2++HFD, Ca2++HFD+LD AV and Ca2++HFD+HD AV groups respectively.

The result showed a significant increase across groups compared to control group and a significant decrease in Ca2++HFD+HD AV compared to Ca2++HFD.




Values are expressed as mean ± SEM, N=7; * = p<0.05 vs control; a = p<0.05 vs Ca2++HFD

**Figure 1** Biliary total bilirubin concentration in the different experimental groups



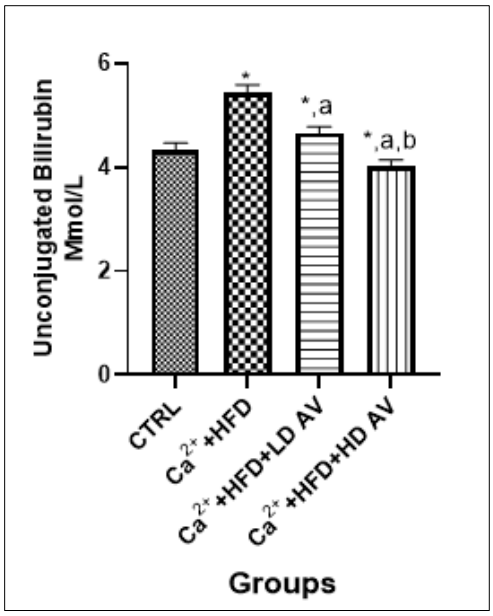

Values are expressed as mean ± SEM, N=7; * = p<0.05 vs control; a = p<0.05 vs Ca2++HFD

**Figure 2** Biliary conjugated bilirubin concentration in the different experimental groups

Figure 2. Biliary conjugated bilirubin concentration in control, Ca2++HFD, Ca2++HFD+LD AV and Ca2++HFD+HD AV groups.

The mean and SEM values were 5.286±0.1010, 5.671±0.08371, 6.043±0.08411, 6.329±0.04206 for control, Ca2++HFD, Ca2++HFD+LD AV and Ca2++HFD+HD AV groups respectively.

The result showed a significant increase across groups compared to control group as well as a significant increase in treated groups compared to Ca2++HFD.




Values are expressed as mean ± SEM, N=7; * = p<0.05 vs control; a = p<0.05 vs Ca2++HFD; b = p<0.05 vs Ca2++HFD+LD AV

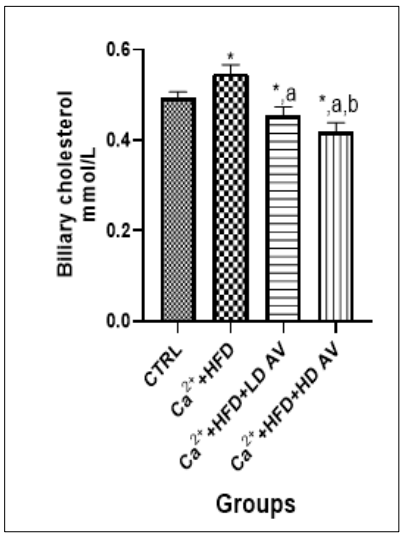
**Figure 3** Biliary unconjugated bilirubin concentration in the different experimental groups

Figure 3. Biliary unconjugated bilirubin concentration in control, Ca2++HFD, Ca2++HFD+LD AV and Ca2++HFD+HD AV groups.

The mean and SEM values were 4.329±0.05216, 5.429±0.06061, 4.657±0.04809, 4.029±0.04206 for control, Ca2++HFD, Ca2++HFD+LD AV and Ca2++HFD+HD AV groups respectively.

The result showed a significant increase group 2 and 3 compared to control and a decrease in group 4 compared to control group. Group 3 and 4 were significantly reduced compared to group 2, as well as group 4 that is significantly decreased compared to group 3.

### 3.2. Biliary cholesterol concentration




Values are expressed as mean ± SEM, N=7; * = p<0.05 vs control; a = p<0.05 vs Ca2++HFD; b = p<0.05 vs Ca2++HFD+LD+ AV

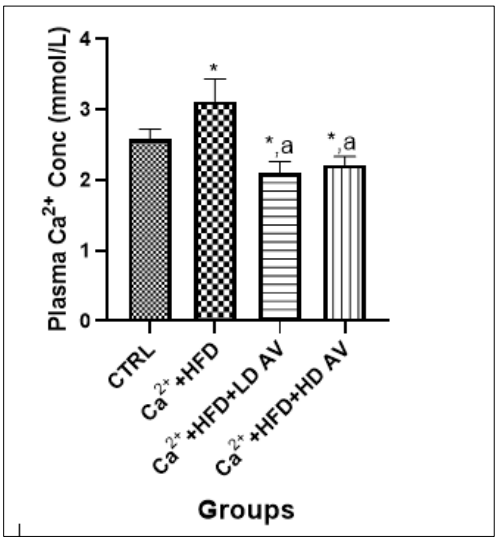
**Figure 4** Biliary cholesterol level in the different experimental groups

Figure 4. Biliary cholesterol level in control, Ca2++HFD, Ca2++HFD+LD AV and Ca2++HFD+HD AV groups.

The mean and SEM values were 0.4943±0.004809, 0.5471±0.007469, 0.4543±0.007190,0.4186±0.007693 for control, Ca2++HFD, Ca2++HFD+LD AV and Ca2++HFD+HD AV groups respectively.

The result showed a significant increase group 2 compared to control and a decrease in group 3 and 4 compared to control and Ca2+ + HFD group. Group 4 was also significantly reduced compared to group 3.

### 3.3. Plasma calcium ion concentrations



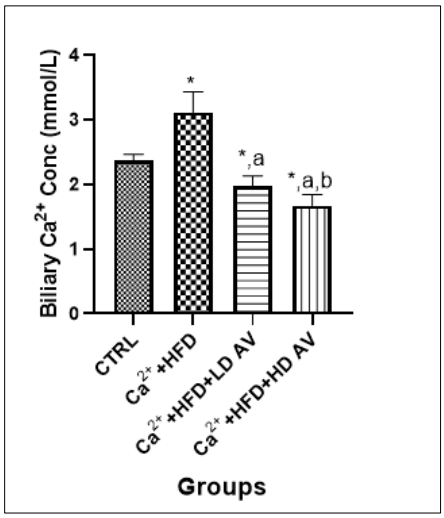

Values are expressed as mean ± SEM, N=7; * = p<0.05 vs control; a = p<0.05 vs Ca2++HFD

**Figure 5** Plasma Calcium ion concentration in the different experimental groups

Figure 5. Plasma Ca2+ ion concentration in control, Ca2++HFD, Ca2++HFD+LD AV and Ca2++HFD+HD AV groups.

The mean and SEM values were 2.586±0.05084, 3.114±0.1204, 2.100±0.06172, 2.214±0.04592 for control, Ca2++HFD, Ca2++HFD+LD AV and Ca2++HFD+HD AV groups respectively.

The result showed a significant increase group 2 compared to control and a decrease in group 3 and 4 compared to control and Ca2+ + HFD group.




Values are expressed as mean ± SEM, N=7; * = p<0.05 vs control; a = p<0.05 vs Ca2++HFD;; b = p<0.05 vs Ca2++HFD+LD AV

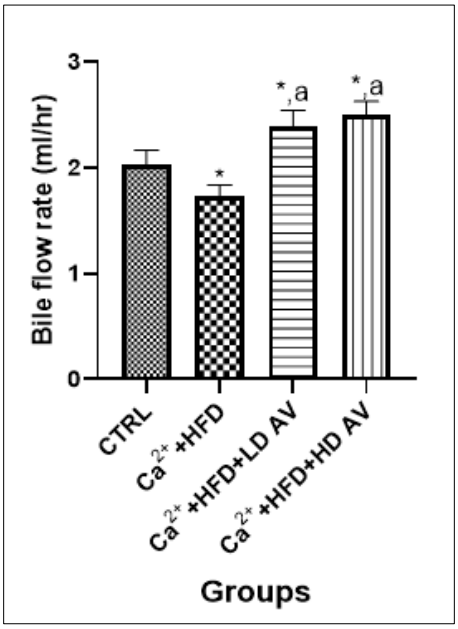
**Figure 6** Biliary Calcium ion concentration in the different experimental groups

Figure 6. Biliary Ca2+ ion concentration in control, Ca2++HFD, Ca2++HFD+LD AV and Ca2++HFD+HD AV groups.

The mean and SEM values were 2.371±0.03595, 3.114±0.1204, 1.971±0.06061, 1.671±0.06442 for control, Ca2++HFD, Ca2++HFD+LD AV and Ca2++HFD+HD AV groups respectively.

The result showed a significant increase group 2 compared to control and a decrease in group 3 and 4 compared to control and Ca2+ + HFD group, as well as a decrease in group 4 compared to group 3.

### 3.4. Rate of bile secretion




Values are expressed as mean ± SEM, N=7; * = p<0.05 vs control; a = p<0.05 vs Ca2++HFD

**Figure 7** Biliary flow rate in the different experimental groups

Figure 7. Biliary flow rate in control, Ca2++HFD, Ca2++HFD+LD AV and Ca2++HFD+HD AV groups.

The mean and SEM values were 2.029±0.05216, 1.727±0.04110, 2.386±0.05948, 2.500±0.04880 for control, Ca2++HFD, Ca2++HFD+LD AV and Ca2++HFD+HD AV groups respectively.

The result showed a significant decrease in group 2 compared to control and a increase in group 3 and 4 compared to control and Ca2+ + HFD group.

## 4. Discussion

Effects of administration of HD and LD *Aloe Vera* gel on animals administered calcium and high fat diet parameters which affect gall stones formation were evaluated in albino Wistar rats.

Alterations in bile secretion and composition have been attributed to different biliary diseases and cholelithiasis is one of such disease [28].There is paucity of literature of *Aloe Vera* on factors that affects gallstone formation.

Evaluation of bilirubin concentration reveal the LD and HD *Aloe Vera* extract treated groups caused a significant increase in total bilirubin when compared to the control, With an increased conjugated bilirubin and a reduction in unconjugated bilirubin, more so, the marked increase in unconjugated bilirubin observed in the HD group compared to the LD group shows a dose dependent correlation. Pigmented stones are chiefly made out of bilirubin, which is an element formed because of the ordinary breakdown of erythrocytes [29], and an increase in conjugated bilirubin has been implicated in the Pathogenesis of gall stones formation, particularly the black stone [30].

Evaluation of biliary cholesterol concentration revealed a reduction of total cholesterol in both groups receiving HD and LD *Aloe Vera* gel extract compared to the control, while the HD group caused a significant reduction of total cholesterol compared to the low dose treated group. This connotes that; this effect of *Aloe Vera* was dose dependent. Studies have shown *Aloe Vera* is rich in phytochemicals such as Isoflavones, and flavones reduce blood cholesterol levels through

inhibition of cholesterol synthesis [31,32]. And this is beneficial because an increase in biliary cholesterol has been implicated in the pathogenesis of gallstones, since cholesterol is solely excreted via the bile only [33]. In bile, cholesterol is in equilibrium with bile salts and with phosphatidylcholine. When the cholesterol in the bile becomes too concentrated, it saturates [34]. Abnormalities in lipid metabolism appear to play a pathogenic role in progressive cholelithiasis [35] and Cholesterol stones are mainly causes due to difference in the production of cholesterol or the secretion of bile. The most ideal approach to forestall and eliminate gallstones is to bring down the body's general cholesterol level, which lessens the cholesterol in the gallbladder, and in this way makes a healthy bile-to-cholesterol proportion. When this proportion is restored, the bile acids can disintegrate the cholesterol crystals and stones and prevent cholelithiasis [36].

From the result, there was a reduction in both plasma and biliary calcium ion concentration in the HD and LD treated group, interestingly, the biliary calcium ion was reduced significantly in the HD treated group compared to the LD treated group. This is beneficial in preventing cholelithiasis since biliary calcium has been identified as a key ingredient in the pathophysiology of gall stones where they exist as calcium carbonate crystals and or calcium bilirubinate, nonetheless, majority of pigment stones are made up of 20% calcium as calcium bilirubinate [37].

The current study demonstrates that the rate of biliary secretion was significantly reduced in the ca2+ + HFD group when compared to the control, while the LD AV treated group and HD AV treated group significantly increased compared to the control and ca2+ + HFD. Gallbladder stasis is a prerequisite for formation of gallstones and provides a link between hepatic secretion of supersaturated bile and cholesterol gallstones [38]. Since *Aloe Vera* increased the biliary flow rate, it is suggestive that it attenuates formation of biliary cholesterol due to stasis. But the exact mechanism by which it does this is unclear.

## 5. Conclusion

This study revealed *Aloe Vera* gel extract may attenuate gall bladder stones, by causing a reduction in biliary and plasma calcium ion, increased in biliary flow rate, reduction of total cholesterol concentration. But an increase in total bilirubin, conjugated bilirubin, and reduction in unconjugated bilirubin. Ergo, *Aloe Vera* gel may be beneficial in management of gall stones.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

Authors have declared that no conflicting or competing interests exist.

### *Statement of ethical approval*

Ethical approval was obtained

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