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Effect of Oral Administration of Ethyl Acetate Leaf Extract of *Gliricidia sepium* on Blood Glucose and Liver Function Parameters in Wistar Rats

ABSTRACT

Aim: This study was designed to evaluate the effect of oral administration of ethyl acetate leaf extract of Gliricidia sepium on blood glucose and liver function parameters in Wistar rats.

Study Design: Completely randomized controlled experimental study.

Place and Duration of Study: The study was conducted at the Animal House of the Faculty of Pharmaceutical Sciences and the Chemical Pathology Laboratory of the School of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto, between May 2024 and October 2025.
Methodology: Acute oral toxicity was assessed using Lorke's method. Twelve (12) male Wistar rats were randomly divided into four groups of three rats each. In phase one, groups I, II, and III received 10, 100, and 1000 mg/kg body weight of the extract, respectively, while group IV served as control. In phase two, three rats received single oral doses of 1600, 2900, and 5000 mg/kg body weight, respectively. For the sub-chronic toxicity study, forty-eight (48) adult Wistar rats (24 males and 24 females) were randomly assigned into four groups of twelve rats each (6 males and 6 females). Group I received 1 mL of normal saline, while Groups II, III, and IV received 500, 1000, and 1500 mg/kg body weight of the extract, respectively, for 28 consecutive days. After treatment, rats were fasted overnight, anesthetized, and blood samples were collected for analysis of blood glucose and liver function parameters.
Results: The acute toxicity study revealed that the oral LD_{50} of the extract was greater than 5000 mg/kg body weight. Sub-chronic administration of the extract did not produce any significant changes ($p > 0.05$) in blood glucose, AST, ALT, ALP, total protein, total bilirubin, conjugated bilirubin, or GGT levels in treated groups compared with the control group.

Conclusion: The findings suggest that oral administration of ethyl acetate leaf extract of Gliricidia sepium at normal therapeutic doses is relatively safe and does not induce hepatotoxicity or alter glucose metabolism in Wistar rats.

1. INTRODUCTION

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Herbal medicine plays a significant role in the health care of millions of people worldwide through both direct use and as a foundation for the development of conventional medicines (1). Available literature indicates that approximately 60% of the world's population relies on traditional medicine, while about 80% of people in developing countries depend largely on herbal remedies for their primary health care needs (2). The continued patronage of herbal medicine has been attributed to factors such as affordability, ease of access, cultural acceptability, low cost, limited access to conventional health care in some regions, and the perception that herbal remedies have few or no side effects (3).

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Despite their widespread use, there is limited scientific evidence supporting the safety and efficacy of many herbal medicines, which raises concerns among researchers and regulatory authorities (4).

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Gliricidia sepium, a member of the Fabaceae family, is among the commonly used medicinal plants reported to be employed in the management of various ailments by local communities in Nigeria (5).

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Gliricidia sepium is a multipurpose leguminous plant of considerable commercial and medicinal importance. It belongs to the Fabaceae family, one of the largest families of flowering plants, which comprises over 700 genera and approximately 20,000 species, making it the third largest plant family after Orchidaceae and Asteraceae (6). The plant is commonly known as "mother of cocoa," while its Yoruba name in southwestern Nigeria is Agunmaniye (7). It is native to dry regions of Central America and is now widely distributed across the tropics between latitudes 6°S and 19°N of the equator, as well as in many tropical and subtropical regions worldwide (8).

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G. sepium grows well from sea level up to an altitude of 1600 m in areas with mean temperatures ranging from 20°C to 29°C and annual rainfall between 900 mm and 1500 mm, including regions with a dry period of up to five months. It does not tolerate frost or night temperatures below 15°C but is tolerant of waterlogging and can thrive in a wide range of poorly fertile soils (8).

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Several studies have reported that G. sepium leaves possess anti-inflammatory properties, particularly due to their flavonoid content, which can help reduce pain and bleeding. Other studies

have demonstrated their antibacterial and antioxidant activities (8). However, there is limited scientific information on the possible toxicological effects of G. sepium, especially on metabolic and hepatic functions. Therefore, this study aims to provide data on the potential effects of ethyl acetate leaf extract of G. sepium on blood glucose and liver function parameters in both male and female Wistar rats.**2.**

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MATERIAL AND METHODS

2.1 Plant Collection and Identification

The leaves of G. sepium were collected from Osogbo metropolis, Osun State, Nigeria. The plant was identified and authenticated at the Herbarium unit, Department of Botany, Obafemi Awolowo University, Ile-Ife, by comparing with an established Herbarium specimen with Voucher number: IFE/17460, reference number which was kept at the Herbarium (5).

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2.2 Plant Extraction

The leaves of G. sepium were shade-dried at room temperature over a period of 6 weeks. Then it was pulverized manually using a mortar and pestle into powder, labelled, and stored at room temperature for use. The powdered leaves of a 1000 g portion were weighed and extracted with 5 L of 90% methanol using the maceration method for 48 hours. This was followed by periodic stirring. The resulting crude extract was filtered using Whatman number one filter paper, and the filtrate was concentrated using a dry oven at 40°C for 72 hours to dryness. Some part of crude methanol extract (200 g) was dissolved in distilled water, filtered, and partitioned successively with n-hexane (2 L x 0.5 L), ethyl acetate (3 L x 0.5L), and n-butanol (3 L x 0.5 L). To obtain n-hexane fraction (15.0 g), ethyl acetate fraction (13.0 g), n-butanol fraction (30.0 g), and the residual aqueous fraction (45.0 g), respectively.

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2.3 Experimental Animals

Healthy male and female Wistar rats aged 8–12 weeks (weighing 120–140 g) were obtained from the Animal House, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. The

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animals were housed separately by sex in metal cages and maintained under standard laboratory conditions at a temperature of 25 ± 2 °C with a 12-hour natural light/dark cycle. The rats were allowed to acclimatize for seven (7) days with free access to standard pellet feed and clean drinking water ad libitum. All animal handling and experimental procedures were conducted in accordance with international guidelines for the care and use of laboratory animals (9).

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2.4 Research Design

2.4.1 Acute Oral Toxicity Test

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The acute oral toxicity study was conducted using Lorke's method (10). Nine (9) male Wistar rats were randomly divided into three groups (I, II, and III) of three rats each. Rats in groups I, II, and III received single oral doses of 10 mg/kg, 100 mg/kg, and 1000 mg/kg body weight of the extract, respectively, via intragastric gavage using an oral cannula. The animals were observed for 24 hours for any signs of toxicity.

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Following the absence of toxic effects in phase one, phase two was conducted using three (3) male Wistar rats randomly assigned into three groups (I, II, and III) of one rat each. Single oral doses of 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg body weight of the extract were administered to rats in groups I, II, and III, respectively, via intragastric gavage. The animals were observed continuously for the first 30 minutes post-administration, then hourly for 4 hours, and subsequently once daily for 48 hours for signs of toxicity, including changes in body weight, salivation, tremors, convulsions, diarrhea, skin, fur, eyes, mucous membranes, or mortality (11).

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2.4.2 Sub-chronic Oral Toxicity Test

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The sub-chronic oral toxicity study was conducted using the 28-day repeated-dose toxicity method in accordance with the Organization for Economic Co-operation and Development (OECD) guidelines (9). Forty-eight (48) adult Wistar rats of both sexes were used for the study and randomly assigned into four (4) groups of twelve (12) rats each (6 males and 6 females per group).

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Group I received 1 mL of normal saline, while Groups II, III, and IV received oral doses of 500, 1000, and 1500 mg/kg body weight of the extract, respectively. All treatments were administered once daily

for 28 consecutive days via oral gavage using an oral cannula. Body weights were recorded weekly, and the animals were observed daily for any physiological or behavioral changes.

2.4.2.1 Sample Collection and Processing

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At the end of the 28-day treatment period, the rats were fasted overnight with free access to water. They were then anesthetized using a ketamine–xylazine combination (50:10 mg/kg, respectively). Blood samples were collected by cardiac puncture into fluoride oxalate and lithium heparin bottles for the estimation of blood glucose and liver function parameters.

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Following blood collection, the rats were sacrificed by lumbar dislocation. The liver was carefully excised, rinsed with distilled water, blotted dry, weighed, and fixed in 10% formal saline for macroscopic examination of any gross pathological changes.

2.5 Analytical Method

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Plasma glucose was measured using the enzymatic colorimetric method described by Trinder (12) with a commercially available reagent kit (Agappe Diagnostics Ltd., India). Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined using the method of Reitman and Frankel (13) with reagent kits obtained from Agappe Diagnostics Ltd., India.

Alkaline phosphatase (ALP) activity was estimated based on the method recommended by the German Society for Clinical Chemistry and the Scandinavian Committee on Enzymes (DGKC-SCE) (14) using a reagent kit (Agappe Diagnostics Ltd., India). Plasma gamma-glutamyl transferase (GGT) activity was determined using a kinetic method as described by Reitman and Frankel (13) with a reagent kit (Agappe Diagnostics Ltd., India).

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Plasma albumin concentration was estimated using the bromocresol green method (15) with a reagent kit (Agappe Diagnostics Ltd., India), while total protein concentration was determined by the biuret method (16) using the same manufacturer's kit. Total and conjugated bilirubin concentrations were estimated using the Malloy–Evelyn method (17) with a reagent kit (Agappe Diagnostics Ltd., India).

2.6 Statistical Analysis

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Data obtained from the study were analyzed using the Statistical Package for Social Sciences (SPSS) for Windows, version 23.0 (SPSS Inc., Chicago, IL, USA). Results were expressed as mean ± standard error of the mean (SEM). The Shapiro–Wilk test was used to assess data normality. Group comparisons were performed using two-way analysis of variance (ANOVA), and significant differences were further analyzed using the least significant difference (LSD) post hoc test. Differences were considered statistically significant at $p \leq 0.05$ with a 95% confidence interval**.**

2.7 RESULTS AND DISCUSSION

Table 1 presents the grouping of male Wistar rats used in phases I and II for the determination of the median lethal dose (LD_{50}) of the ethyl acetate leaf extract of Gliricidia sepium. The results showed that neither mortality nor abnormal behaviour was observed in any of the rats during both phases after 24 hours of observation. This finding indicates that the LD_{50} of the extract is greater than 5000 mg/kg body weight.

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Acute toxicity studies are conducted to estimate the median lethal dose (LD_{50}), which is a fundamental parameter in toxicological evaluation of plant extracts. In this study, oral administration of a single dose of the ethyl acetate leaf extract of G. sepium at concentrations up to 5000 mg/kg body weight did not result in mortality or observable behavioural changes. According to the Globally Harmonized System (GHS) for the classification of chemicals, this places the extract in Category 5, indicating relatively low acute toxicity (18, 7, 19).

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A sub-chronic toxicity study was also conducted to assess the effects of repeated exposure. Throughout the experimental period, all rats remained active and responsive to external stimuli. No mortality or clinical signs of local or systemic toxicity were observed. Daily behavioural assessments revealed no abnormalities, and animals in both treated and control groups exhibited normal behaviour. Repeated-dose toxicity studies are essential for identifying potential target organs and alterations in physiological and biochemical parameters. Such studies are required by regulatory agencies to adequately characterize the toxicological profile of test substances (20).

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Figure 1 illustrates the changes in fasting plasma glucose levels in male and female Wistar rats administered ethyl acetate leaf extract of G. sepium at daily doses of 500 mg/kg, 1000 mg/kg, and 1500 mg/kg for groups II, III, and IV, respectively, for 28 days. The results showed no statistically significant differences in plasma glucose levels among the treated groups when compared with the control group. The absence of significant changes in glucose levels suggests that the extract did not interfere with glucose homeostasis or carbohydrate metabolism. The liver plays a central role in glucose metabolism, particularly in gluconeogenesis and glycogenolysis, which are essential for maintaining normal blood glucose levels (21).

Table 2 presents the liver function parameters of male and female Wistar rats administered ethyl acetate leaf extract of G. sepium for 28 days. The mean values of AST, ALT, ALP, total protein, albumin, total bilirubin, conjugated bilirubin, and GGT in groups II, III, and IV were not significantly different from those of the control group. No significant sex-related differences were observed. These findings are consistent with the report of Oduola et al. (7), who observed no hepatotoxic effects following administration of G. sepium extract.

Liver function parameters are vital in toxicological evaluations because the liver is responsible for the metabolism and detoxification of xenobiotics and endogenous compounds essential for organismal survival (21). As the primary site of drug metabolism and albumin synthesis, liver enzymes such as ALT, AST, and ALP are commonly used biomarkers for detecting hepatic injury in toxicity studies (22).

Plate 1 shows the liver histology of Wistar rats administered ethyl acetate leaf extract of G. sepium in the sub-chronic toxicity study. The liver sections from all groups revealed normal hepatic architecture with a well-defined central vein, preserved hepatocyte arrangement, and intact lobular structure. Gross (macroscopic) examination of the liver in both treated and control rats revealed no observable changes in colour, size, or texture. Histopathological examination showed no evidence of cellular injury, cholestasis, or necrosis. These histological findings corroborate the biochemical results and indicate that oral administration of the ethyl acetate leaf extract of G. sepium did not induce significant toxicological or morphological alterations in the liver.

.Conclusion

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The median lethal dose (LD_{50}) of the ethyl acetate leaf extract of Gliricidia sepium was found to be greater than 5000 mg/kg body weight in Wistar rats, indicating that the extract possesses relatively low acute toxicity. Sub-chronic oral administration of the extract at doses of 500, 1000, and 1500 mg/kg for 28 days did not produce adverse effects on blood glucose levels or liver function indices in both male and female Wistar rats. Furthermore, histopathological evaluation revealed no adverse morphological changes in the liver. These findings suggest that the ethyl acetate leaf extract of G. sepium is relatively safe at the tested doses and duration

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Table 1: Acute toxicity profile (LD_{50}) of *G. sepium* ethyl acetate leaves extract in male Wistar rats.

Dose (mg/kg)	Number of rats used	Observation period	Behavioral changes	Mortality
10	3	24 hours	None	0/3
100	3	24 hours	None	0/3
1000	3	24 hours	None	0/3
1600	2	24 hours	None	0/2
2900	2	24 hours	None	0/2
5000	2	24 hours	None	0/2

Therefore the $LD_{50\ is\ >}$5000 mg/kg body weight

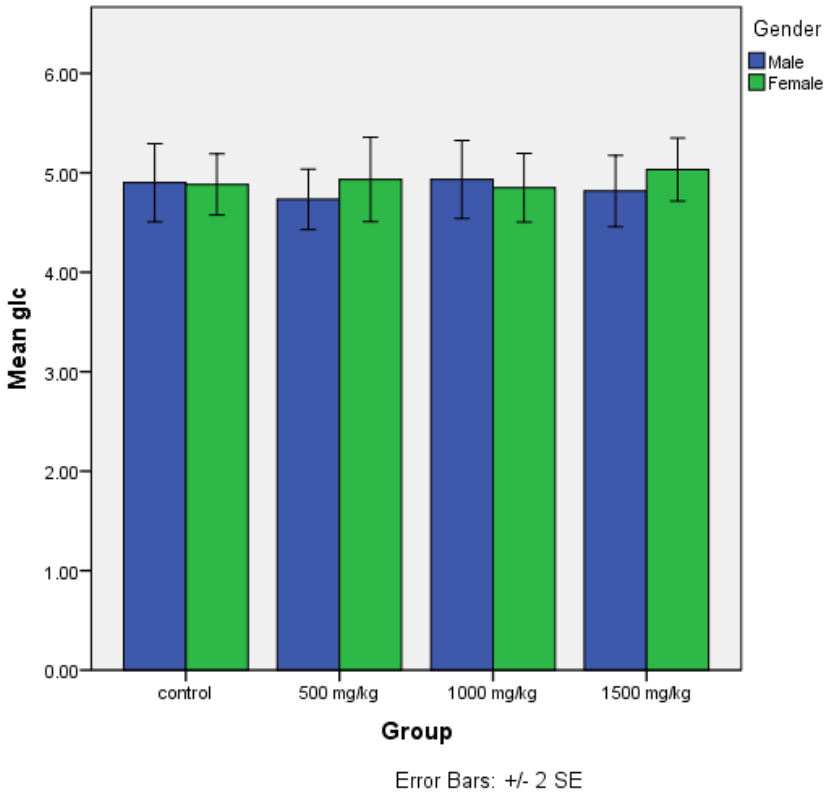

Fig. 1: Glucose Value in mmol/L of Wistar Rats Exposed to *G. sepium* Ethyl Acetate Leaf Extract in Sub-chronic Oral Toxicity Study

Key: Bars represent mean ± SEM, **glc**= glucose, control=group I, 500 mg/kg=group II, 1000 mg/kg= group III, 1500 mg/kg= group IV

Table 2: Liver Function Parameters in Wistar Rats Exposed to *G. sepium* Ethyl Acetate Leaf Extract in sub-chronic Oral Toxicity Study

Groups	Gender	N	AST (U/L)	ALT (U/L)	ALP (U/L)	TP (gldl)	AB (g/dl)	TB (mg/dl)	CB (mg/dl)	GGT U/L
I	Male	6	25.00±0.73	14.33±0.49	59.00±3.18	6.45±0.48	3.05±0.17	0.59±0.04	0.05±0.01	2.22±0.16
	Female	6	24.50±0.67	13.67±0.42	57.17±1.79	6.77±0.25	3.17±0.09	0.61±0.04	0.06±0.01	1.65±0.14
II	Male	6	25.33±0.76	14.50±0.43	58.00±2.89	6.50±0.12	3.17±0.07	0.60±0.03	0.06±0.01	2.07±0.21
	Female	6	24.50±0.43	14.00±0.6	55.33±2.24	7.13±0.31	3.15±0.08	0.65±0.06	0.05±0.01	2.13±0.09
III	Male	6	25.33±0.56	14.50±0.53	58.33±2.17	7.52±0.49	3.20±0.14	0.56±0.05	0.04±0.00	1.95±0.13
	Female	6	24.50±0.76376	14.00±0.36	54.83±1.85	6.68±0.42	2.95±0.09	0.62±0.10	0.05±0.01	1.88±0.07
IV	Male	6	24.00±0.58	14.17±0.42	54.33±2.33	6.92±0.14	2.93±0.04	0.51±0.07	0.05±0.00	2.00±0.08
	Female	6	24.50±0.67	13.83±0.60	54.67±2.03	7.20±0.19	3.13±0.07	0.5817±0.09	0.05±0.01	1.97±0.06
F Value			0.465	0.221	0.244	1.874	1.782	0.504	1.152	2.431
P			0.708	0.882	0.865	0.150	0.166	0.682	0.340	0.079

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Key: Value are expressed as mean ± SEM, n= number of Wistar rats per group, AST= aspartate amino transferases, ALT= alanine amino transferases, ALP= alkaline phosphatases, TP= total protein, TB= total bilirubin, CB= conjugated bilirubin, GGT= gamma glutamyl amino transferases, Group I= Control, Group II = 500 mg/kg, Group III = 1000 mg/kg, Group IV= 1500 mg/kg.

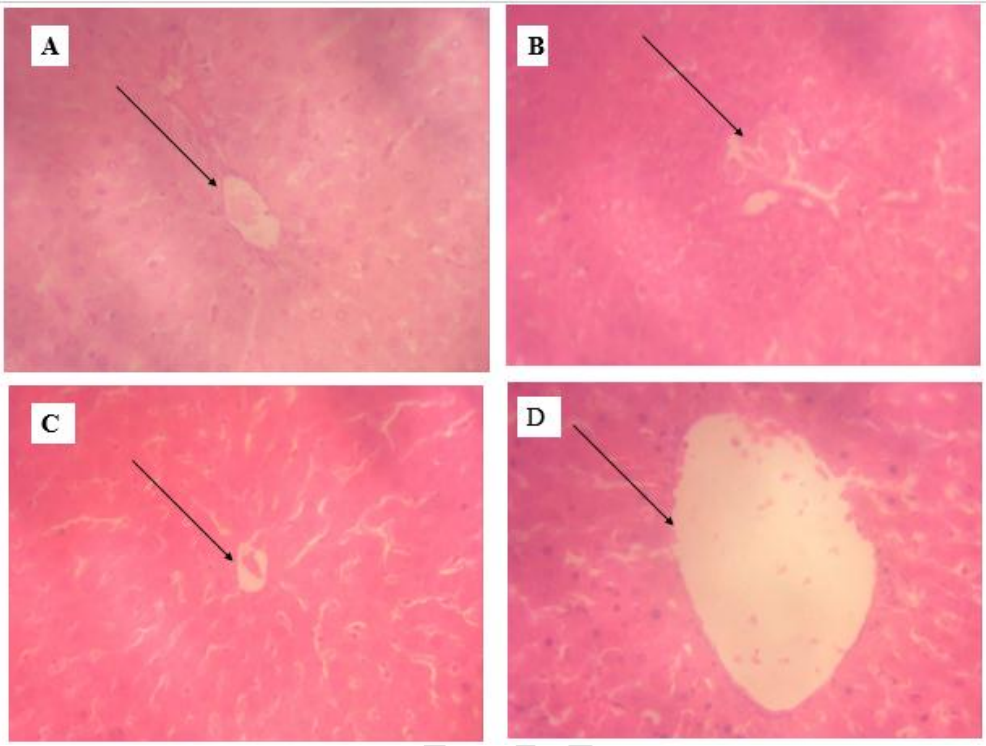

Plate 1: Photomicrographs of liver tissue sections of Wistar rats administered *Gliricidia. sepium* ethyl acetate leaves extract in sub- chronic oral toxicity study H and E, X400.

Key: A, B, C, and D represent Group I (control), Group II (500 mg/kg), Group III (1000 mg/kg) and Group IV (1500 mg/kg) respectively. Normal central vein (black arrow)

Ethical approval: This research was approved by the Health and Biomedical Research Ethics Committee (HBREC) of Usmanu Danfodiyo University, Sokoto (UDUSOK). Ethical approval registration number: NHREC/UDU-HREC/25/06/2023 (Appendix 1).

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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