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RESEARCH ARTICLE
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EVALUATION OF ANTIDIABETIC POTENTIAL OF BROMOCRIPTINE AND PANDANUS EXTRACTS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS WITH HISTOPATHOLOGICAL EVALUATION OF PANCREATIC TISSUE

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ABSTRACT:

Background: Diabetes is a highly heritable disease worldwide, causing significant damage to vital organs and leading to decreased production of pancreatic enzymes, including amylase and lipase **Objectives:** Pandanus amaryllifolius Roxb (pandan) has been found to enhance insulin sensitivity, reduce insulin resistance, and exhibit potential in treating type 2 diabetes mellitus. This study investigates the antidiabetic properties of pandan, both alone and in combination with Bromocriptine, to produce a synergistic effect in rat models of induced diabetes Study Design: Experimental. Setting: Karachi University.

Methods: The in-vivo testing was done by taking the blood samples of normal and diabetic male rats. Diabetes was induced by streptozotocin and blood glucose level, pancreatic amylase and lipase were assessed.

Results: In this, pandanus as well as Bromocriptine was found to improve blood glucose level and significantly enhance pancreatic amylase and lipase levels compared to control diabetic group. Moreover, diabetic induced group caused substantial damage to pancreatic architecture, which was notably reversed by pandanus and Bromocriptine. In conclusion, Pandanus demonstrates potential in treating diabetes and protecting the pancreas from diabetes-induced damage Conclusion: The studied concluded that pandanus have marked antidiabetic activity and protecting the pancreas from damage.

Key words: Pandanus amaryllifolius, Bromocriptine, antidiabetic, pancreatic amylase, pancreatic lipase.

INTRODUCTION:

Diabetes is a prevalent and insidious health threat in Pakistan, leading to numerous complications. Diabetes mellitus (DM) is a chronic disease, which rapidly increases due to lifestyle changes and some environmental factors. World Health Organization projected that diabetes will be the 7th leading cause of death in 2030 [1]. Diabetes can be found in newborns and the incidence is higher in females than males [1-3]. Plants with medicinal properties have been a cornerstone of traditional medicine in the treatment and management of diabetes. Pandanus amaryllifolius, rich in bioactive compounds, has shown promise in enhancing insulin sensitivity, reducing insulin resistance, and managing type 2 diabetes mellitus [4]. *Pandanus amarylifollius* belongs to Pandanaceae family has many health and remedial benefits. It is used for the treatment of diabetes [5]. It effectively reduces blood glucose levels in streptozotocin induced diabetic rats [6, 7]. Bromocriptine is a sympatholytic D2-dopamine agonist that has been approved for the treatment of type 2 diabetes [8]. Ghasemzadeh et al demonstrated that the Pandanus exhibits several bioactivities i.e. antioxidant and antihyperglycemic activities [9, 10]. In addition, the extracts from roots and leaves of the fragrant pandanus could effectively reduce blood glucose levels by inhibiting α-glucosidase enzyme [11-13]. Millan et al evaluate that the bromocriptine is indicated for the management of type 2 diabetes [14-15]. Luo S et al suggested that bromocriptine has the potential to reverse metabolic disruptions associated with insulin resistance and obesity by reorganizing the hypothalamic circadian system and monoamine neuronal function [16]. Additionally, dopamine agonist treatment exerts its effects by reducing the hypothalamic signals that regulate hepatic glucose production, lipid synthesis, and insulin resistance [17, 18]. Bromocriptine does not have a specific receptor that mediates its action on glucose and lipid metabolism. Rather, its effects are mediated via resetting of dopaminergic and sympathetic tone within the CNS [19]. This study explored the potential synergistic effects of combining *Pandanus amaryllifolius* extracts with bromocriptine, a dopamine D2 receptor agonist that effectively reduced postprandial plasma glucose levels. This study investigates the antidiabetic activity of Pandanus amaryllifolius extracts and Bromocriptine in a rat model of induced diabetes, evaluating the efficacy of different doses of Bromocriptine.

MATERIALS AND METHOD:

INVIVO STUDY:

Preparation sample

Pandanus amaryllifolius leaves were collected, washed with tap water, and then chopped into 1-cm pieces. A 100-g sample of the chopped leaves was mixed with 500 ml of ethanol (1:5 ratio) and stored in a dark container at room temperature (25°C ± 2°C) for 3-7 days. The mixture was then filtered using filter paper or membrane filters, and the ethanol was separated from the Pandanus extract using a rotary evaporator, yielding a paste-like sample.

For study, male Wistar rats (200-250 g) were used, allocated into seven groups of 10 animals (n=10) each and maintained in a temperature- and humidity-controlled facility:

Group 1: Normal control group.(normal saline).
Group 2: Diabetic control group (Streptozotocin, 55 mg/kg).
Group 3: Diabetic treated group (Bromocriptine, low dose 1.8 mg/kg)
Group 4: Diabetic treated group (Bromocriptine, high dose 4 mg/kg)
Group 5: Diabetic treated group (*Pandanus amaryllifolius* extract 400 mg/kg)
Group 6: (Diabetic treated group (Bromocriptine, 1.8 + *Pandanus amaryllifolius* extract 200 mg/kg)
Group 7: Diabetic treated group (Standard drug, 200 mg/kg metformin)

After the end of 3 months, cardiac puncture done to collect blood samples from the rats for various biochemical analyses.

Biochemistry Analysis: Blood samples of normal and diabetic male rat was collected (Allain et al,1974) and taken for analysis by centrifugation for 20min. centrifugation was done at 4°C, and stored at−20°C. Blood glucose level, serum amylase, serum lipase level were analysed [20].

Preparation of Pancreatic Tissue for Histological Examination Animal tissues were rinsed with saline and preserved in 10% neutral buffered formalin for histopathological analysis. After fixation, the tissues were embedded in paraffin wax using standard methods. Sections (5 μm) were prepared from the paraffin blocks and mounted on poly-L-lysine-coated glass slides. The sections were then stained with H&E using standard protocols and examined by light microscopy to evaluate histological changes.

ETHICAL APPROVAL

The project was approved by ASRB (Advance study and research board) with (ASRB/No./06788/PHARM) on 17 october 2022.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS 22.0 software (IBM SPSS, USA). A significance level of $p < 0.05$ was adopted for all two-tailed tests. Normally distributed continuous variables with homogeneous variance are presented as mean ± standard deviation (SD) and were compared using one-way analysis of variance (ANOVA).

RESULTS:

EFFECT ON BLOOD GLUCOSE

The random blood glucose of control non-diabetic rat was observed as 70 ± 0.12 and of control diabetic animal was 120 ±0.26 which when Bromocriptine administred it became 116± 302 (low dose); 101± 0.5 (high dose). The blood glucose of diabetic rat was 96 ± 0.12 after pandanus administration. The blood glucose of diabetic rat after administration of combination i.e. pandanus and Bromocriptine was reduced to 96 ± 1.47. The blood glucose of standard treated group was 90± 1.06 (Table 1).

EFFECT ON PANCREATIC AMYLASE

The amylase level of control non-diabetic rat was observed as 393 ± 0.47 and of control diabetic animal was 198 ± 1.96 which when Bromocriptine administred it became 267± 1.02 (low dose); 375± 0.5 (high dose). The amylase level of diabetic rat was 345±1.40 after pandanus administration. The amylase level of diabetic rat after administration of combination i.e. pandanus and Bromocriptine was 401 ± 1.47. The amylase level of standard treated group was 416± 0.6 (Table 1).

EFFECT ON PANCREATIC LIPASE

The pancreatic lipase level of control non-diabetic rat was observed as 126 ± 0.12 and of control diabetic animal was 69 ± 2.06 which when Bromocriptine administred it became 73± 1.02 (low dose); 99± 0.5 (high dose). The lipase level of diabetic rat was 110±0.47 after pandanus administration was observed. The lipase level of diabetic rat after administration of combination i.e. pandanus and Bromocriptine was 112 ± 0.47. The lipase level of standard treated group was 131± 0.26 (Table 1).

Table. 1: Effect of Bromocriptine, Pandanus and Combination of both on Control Non Diabetic and Diabetic animals

	Control non diabetic	**Control diabetic**	**Control diabetic + Bromocriptine**		**Control diabetic + Pandanus**	**Control diabetic + Bromocriptine + Pandanus**	**Control diabetic + Standard treated**
			Low dose	**High dose**			
Blood glucose mg/dL	70^	220 *	116*^	101*^	100 *^	96 ^	90^
Serum amylase U/L	393^	198*	267	375^	345^	401^	416^
Serum lipase U/L	126^	69 *	73*	102^	99*	110^	130^

Values are given in mean standard deviation is equal to significant at p <0.05
values shown with * are compared with control non diabetic group as significant
values shown with ^ are compared with control diabetic group as significant

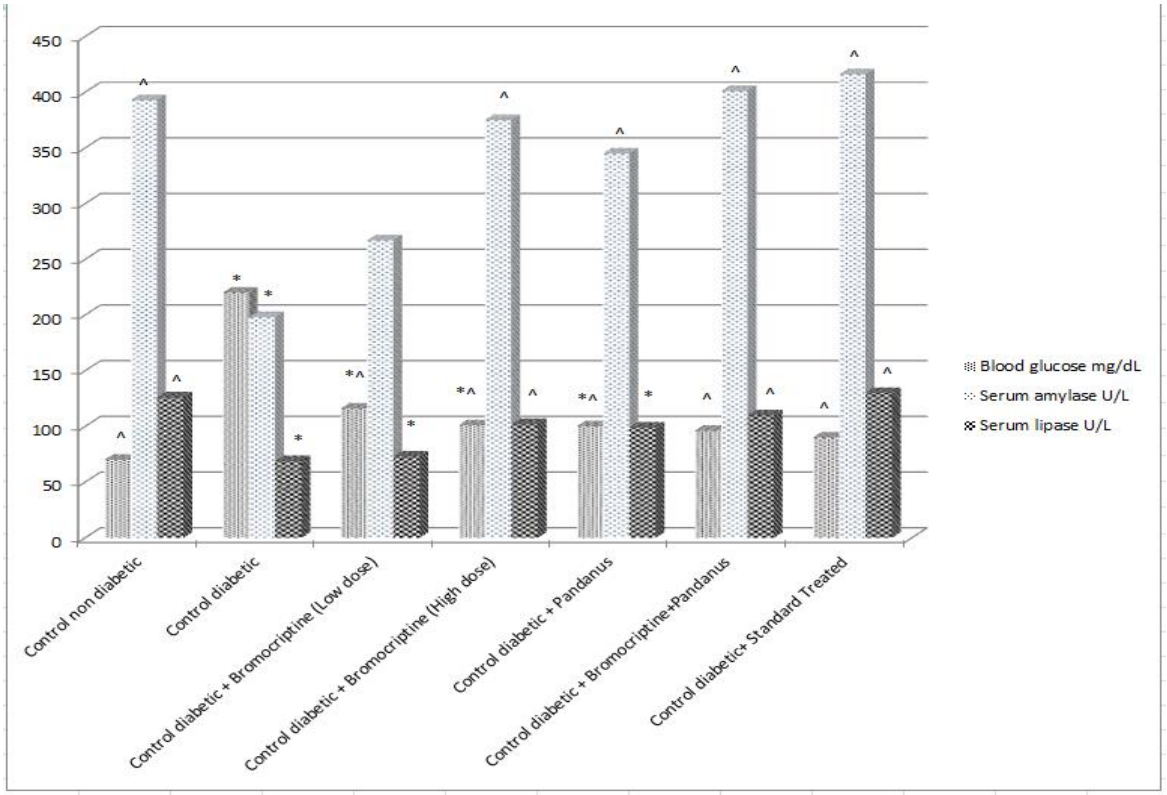


Figure 1: Graph showed the effect of Bromocriptine, *Pandanus amaryllifolius* and standard drug on blood glucose and pancreatic enzymes

HISTOPATHOLOGICAL RESULTS OF PANCREAS

Control Non diabetic Group

Microscopic studies showed that the pancreatic architecture was generally preserved as shown in Figure 2.

Control diabetic Group

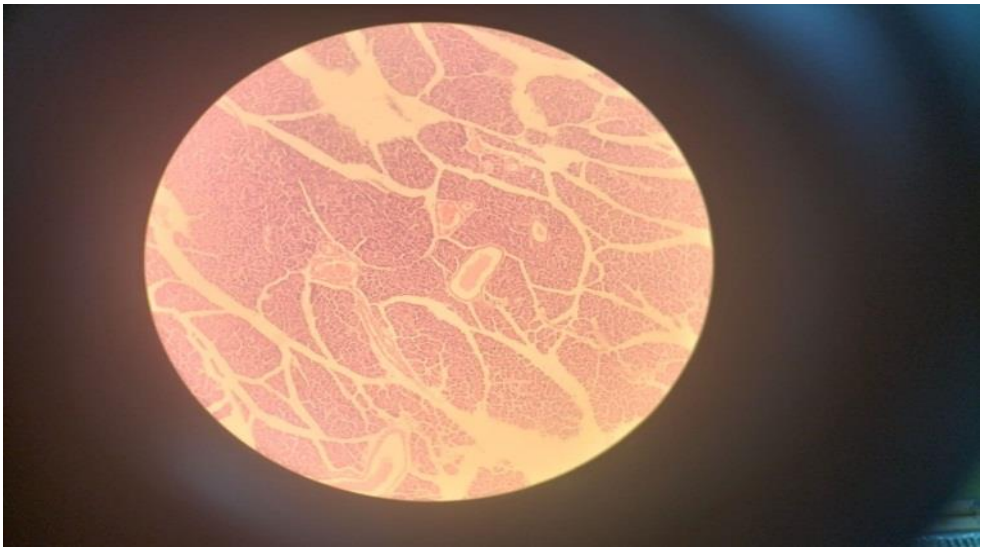
Microscopic studies showed that the decrease in the size of pancreatic islet cells was observed, whereas the size reduction was consistently observed across different microscopic fields as shown in Figure 3.

Diabetic treated Group (Bromocriptine)

The pancreatic section of the diabetic treated Group with Bromocriptine exhibited a normal pancreatic architecture, but a slight decrease in the number andsize of pancreatic islet cells was observed with mild vacuolization of islets of Langerhans as shown in Figure 4.

Diabetic treated Group (Bromocriptine & Pandanus)

The pancreatic section of the diabetic treated Group with Bromocriptine and pandanus exhibited a normal pancreatic architecture. Islets cells secretions and exocrine cell of pancreas were regular as showed in fig 5.



2: 10X Photomicrograph of pancreas showing normal pancreatic cells. No significant change in control non-diabetic rat

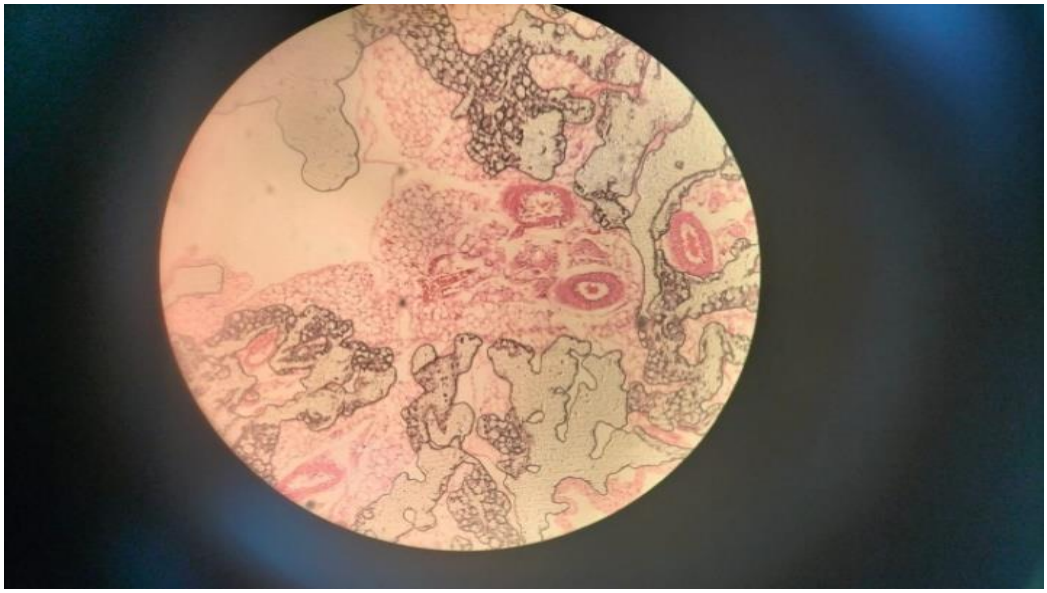


Figure 3: 10X Photomicrograph of pancreas of STZ treated group showing shrinkage of islets of langerhens

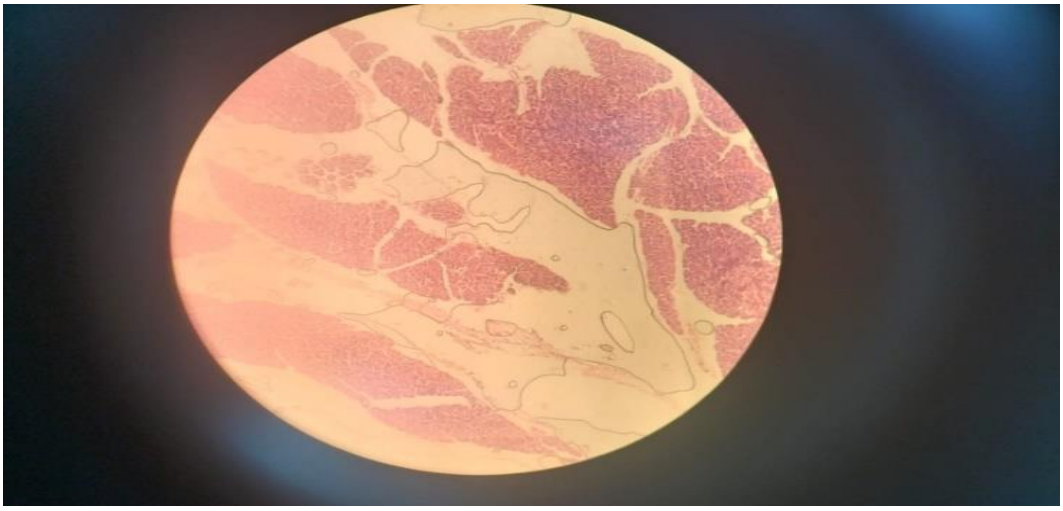


Figure 4: 10X Photomicrograph of pancreas of STZ treated group with Bromocriptine with mild vacuolization of islets of Langerhans

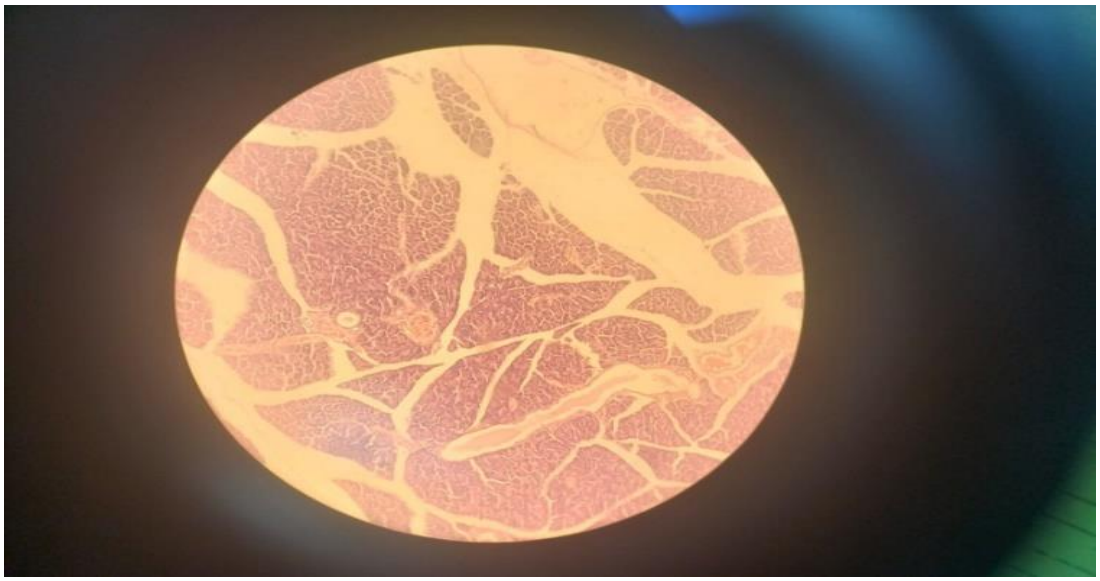


Figure 5: 10X Photomicrograph of pancreas of STZ treated group treated with Bromocriptine and pandanus looks normal with mild vacuolization

DISCUSSION: The incidence of diabetes mellitus is rising rapidly worldwide, frequently giving rise to severe metabolic disturbances and life-threatening complications [21]. Fletcher et al stated that insulin resistance is a critical risk factor for the development of impaired glucose tolerance and type 2 diabetes. Individuals with insulin resistance often exhibit a cluster of risk factors, including hyperinsulinemia, atherogenic dyslipidemia and glucose intolerance, which are also commonly observed in people with type 2 diabetes [22]. The secondary outcomes of this study will examine the impact on cardiovascular disease and its associated risk factors, as well as changes in glycemic control, insulin dynamics, obesity, physical activity, nutrient intake, quality of life and the incidence of adverse events [23, 24]. Reaven supported the view that the concurrent presence of insulin resistance and hyperinsulinemia sets the stage for the emergence of a constellation of metabolic abnormalities, characterized by impaired glucose tolerance, elevated plasma triglycerides, reduced high-density lipoprotein cholesterol, and hypertension [25].

Cincotta et al demonstrated that Bromocriptine has a positive impact on metabolic parameters, reducing insulin resistance and glucose intolerance and improving hyperglycemia in obese individuals

with type 2 diabetes [26]. Additionally, research by Lous et al revealed that bromocriptine significantly improved insulin's ability to suppress hepatic glucose production during hyperinsulinemic-euglycemic clamp conditions [27]. Vicchi et al studied that the discovery of dopamine receptors' role in glucose regulation paved the way for the FDA's approval of bromocriptine as a treatment for adults with type 2 diabetes, aiming to enhance glycemic control [28]. Ellen Bakke et al stated that bromocriptine is a robust diabetic treatment and resilient to genetically induced obesity, diabetes, and circadian disruption [29]. Sasidharan et al worked on the phytochemical screening indicated that the presence of flavonoids, alkaloids, saponin and tannin in Pandanus amaryfollius. The antidiabetic effect of *P. amaryfollius* observed in the present study may be due to the presence of these phytochemicals [30]. Peungvicha et al observed that the repeated administration of the extract of pandanus at different doses produced a significant hypoglycemic effect in diabetic rats [31]. Hypoglycemic activity-guided fraction led to the isolation of the known compound, 4-hydroxybenzoic acid, from Pandanus plant that showed a hypoglycemic effect in normal rats after the oral administration [32]. Sasidharan et al demonstrated that there was significant decrease in the blood glucose level of the plant-treated groups compared to the diabetic control. Histologically the pancreas of the treated groups indicated significant regeneration of the β-cells when compared to the diabetic control [30].

According to the findings of the study, administration of pandan leaf ethanol extract and bromocriptine for 12 weeks significantly reduced plasma glucose level as shown in table 1. The study's findings revealed a significant decrease in pancreatic enzyme levels, with amylase (198.30±1.96 p*^ mean±SEM) and lipase (69±2.06,p*^ mean±SEM) showing substantial reductions in the diabetic treated group. When Bromocriptine alone was given to diabetic and non diabetic group, in both groups serum amylase level increased. p-value (0.0001) gives significant difference in diabetic and non diabetic groups. When combination of Bromocriptine and pandanus was given in diabetic group serum amylase level was not much altered near to standard treated group So it is analysed that in diabetic animals combination caused elevation inpancreatic enzymes in diabetic treated group. Histopathological examination of pancreatic tissue revealed that pancreatic tissue damage in the positive control group (Fig. 3) was characterized by lymphocyte infiltration and a decrease in the size and number of islet cells. Notably, these changes were significantly mitigated in both the bromocriptine and pandanus treated groups (Fig.4, 5).

CONCLUSION:

This study revealed that the combination of pandan leaf and Bromocriptine exhibits substantial antidiabetic effects. Our findings proposed a promising, natural therapeutic strategy for diabetes management, potentially serving as a complementary or alternative approach to conventional treatments.

CONFLICT OF INTEREST:

No conflict of interest associated with this work.

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