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An In vivo Assessment of Diabetes Ameliorating Potentiality of Ethanolic Extract of *Cynodon dactylon* on Alloxan-Induced Diabetic Rat Model

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Abstract

Diabetes is a metabolic disorder. Some plant-derivative products are used to oppose this life-threatening complication. The plant is an assorted source of diverse healing compounds that can be used to ameliorate diabetes. *Cynodon dactylon* is a grass species traditionally used for several medicative objectives from ancient times. It has also been used to ease diabetes. We intended to determine the hypoglycemic outcome of *C. dactylon* extract and ascertain its safety profile. Diabetes was induced in rats via injecting alloxan (150 mg/kg) through the intraperitoneal route. After assessing the blood glucose level, it has been observed that the Ethanolic extract of *C. dactylon* (750 mg/kg) could significantly decrease the blood sugar level compared to the alloxan control group ($p<0.05$). Besides, the extract's hypoglycemic efficiency was comparable with metformin with null statistical significance ($p>0.05$). As a part of the safety profile analysis, we measured SGOT, SGPT, Creatinine, and the lipid profile levels of rats belonging to different groups. It was seen that both metformin and extract of *C. dactylon* improved the pathological conditions induced by diabetes. Furthermore, in healthy individual rats, both metformin and extract of *C. dactylon* did not significantly alter the sound pathological state. Hence, it might be assumed that the extract of *C. dactylon* could be practiced as an excellent alternative therapy to ameliorate diabetes.

Keywords: *Cynodon dactylon*; Diabetes mellitus; Rat; Liver functioning test; Cretinine

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Introduction

Diabetes mellitus is one of the well-known non-communicable disorders confronted as the fourth principal cause of mortality in the most developed countries and highly widespread in many developing countries. This possesses an alarming threat to be met within the 21st century and is already creating hype across the world as there is no permanent cure rather than managing it [1].

Diabetes mellitus (DM) is a chronic metabolic disorder caused by inherited and/or acquired deficiency in the production of insulin by the pancreas' beta cells or by the ineffectiveness of the insulin produced and resulting in high blood glucose levels (hyperglycemia). The chronic hyperglycemia of diabetes is linked with long-term damage, dysfunction, and failure of different organs, especially the kidneys, eyes, nerves, heart, and blood vessels, leading to morbidity and mortality in diabetes [2]. Hyperglycemia comes up with oxidative stress that can lead to

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cellular tissue damage. Diabetes mellitus is connected with the engendering of reactive oxygen species (ROS) and generates oxidative damage, especially to the kidney and the heart [3].

Many oral hypoglycemic agents, like biguanides and sulfonylurea, are available along with insulin for the treatment of diabetes mellitus. Still, they have deleterious effects, and sometimes, they are accountable for being ineffective in chronic diabetic patients [4]. Plants have always been a prominent origin of drugs, and many of the currently accessible drugs have been acquired from them [5]. Permanent cure and optimal control of diabetes are still not possible, but there are various approaches including herbal medicines to treat it and its secondary complications. However, the appropriate selection of herbs might depend on several perspectives, including the stage of advancement of diabetes, types of comorbidities of the patient, accessibility, affordability, and the safety profile of the herbs [6]. Traditional medicine (herbal) is used to treat and manage diabetes in developing countries where the cost of conventional medicines is a burden for the people living below the poverty line [7]. The impact and global urge of natural remedies cannot be disregarded because most of the herbal treatments are seemingly effective, safe, and no high-risk association as there are nominal or almost no side effects [8-10]. Natural healthcare products of organic origin have been regarded as optimistic approaches for managing chronic diseases [11]. Cutting-edge research is presently going on, especially in Asia Pacific regions, to explore traditional medicines scientifically [12,13]. Ethnopharmacological surveys suggest that 1,200 plants have been used in traditional medicines for their hypoglycemic and antidiabetic activities across the world [14,15]. The hypoglycemic and antidiabetic effects of many plants have been evaluated and scientifically proven during studies in animal models of diabetes [16] and diabetic patients [17]. *Cynodon dactylon* (L.) Pers. (Family: Poaceae) is commonly known as “Doob” or “Durva” in Bangladesh. It is a weed having various medicinal properties. Roots, leaves, and rhizomes of the plant have been practiced as folk remedies in many countries, as anticystitis, anti-inflammatory [18], antiviral, hypolipidemic, antihypertensive, anti-hysteric, antipsychotic, and antigonorrheal agent [19]. In India, the plant is used to treat melena, anorexia, burning sensations in the body, pruritis, pregnancy complications, and erysipelas [20], and its leaf juice with a pinch of common salt has been used orally to treat stomachache. It was probably native to East Africa, where it is widely distributed, but now it has scattered throughout the world in temperate and subtropical zones. The plant contains crude proteins, carbohydrates, and mineral constituents, oxides of magnesium, phosphorous, potassium, calcium, sodium, carotene, and sitosterol. Other compounds like vitamin C, palmitic acid, cartone, triterpenoids, ergonovine alkaloids and ergonovinine, etc. In this experimental study, our main goal is to investigate the hypoglycemic and antidiabetic effect of *Cynodon dactylon* in diabetic induced rodents along with side effects analysis.

Methods and Materials

Chemicals

The experimental plant *Cynodondactylon* was obtained from Mirpur Botanical Garden, Dhaka, Bangladesh. Metformin, the Active Pharmaceutical Ingredient (API), was obtained as a kind gift from Square Pharmaceuticals Limited, Dhaka, Bangladesh. Alloxan, a cytotoxic glucose analog, was bought from Sigma Aldrich, Germany. Creatinine measurement kits, SGPT, and SGOT kits all were purchased from Plasmatic Laboratory Product Ltd. Humalyzer 3000 (Semi-Automated Clinical Chemistry Analyzer emerged from Medigroup Asia limited, Cambodia) was used during the measurement of biochemical parameters. Glucometer named Alere GI of AlereInc, USA was used in this investigation and was purchased from Shahbag, Dhaka, Bangladesh.

Extraction procedure

The whole part of *C. dactylon*, washed with distilled water, was shade-dried for 24 hours in a drying chamber at 40-500°C and powdered with a mechanical blender (Waring® Commercial Blender). Our plant’s powder was soaked into 200mL of solvent ethanol and shaken on a platform shaker (Lab Companion TM) at 25°C temperature and 150 rpm using a metabolic shaker to get the plant extracts. The soaking was replicated thrice to obtain a complete extraction. The extract obtained was then evaporated and concentrated under reduced pressure (768mmHg to 7mmHg) using a Rotary Evaporator.

Experimental design and animal handling

Healthy adult male Wistar rats weighted above 140 grams were collected from the Department of Pharmacy of Jahangirnagar University, Savar, Dhaka, Bangladesh. At the Institute of Nutrition & Food Science, University of Dhaka, all the rats were kept in 12 ± 1 h light/dark cycle and (25°C) under controlled room temperature. Water and standard pellet *ad libitum* were given to the rats to feed during observation. The rats were kept for acclimatization before the beginning of the study. Afterward, measurements of each rat’s body weight were done-a total of 6 groups consisting of ten rats in each group. An even distribution of rats into different groups was done according to their body weight.

Group 1: Standard control.

Group 2: Alloxan induced control.

Group 3: Alloxan induced diabetic rats treated with metformin (500 mg/kg of body weight)

Group4: Alloxan induced animals receiving the extract of *Cynodon dactylon* (750 mg/kg).

Group 5: Non-diabetic rats receiving (500 mg/kg) metformin.

Group 6: Non-diabetic rat receiving the extract of *Cynodon dactylon* (750 mg/kg).

Before inducing diabetes, the rats were placed in their respective case for 2 weeks for acclimatization. Then the blood sugar level of all the rats was measured. All the rats of groups two, three, and four were injected with alloxan following 150mg/kg via the intraperitoneal route. No alloxan was given to groups one, five, and six. To assure that the rats were induced with diabetes after three days or not, blood glucose levels were measured carefully. The alloxan injected group rats, and the standard group was kept

in normal condition without treating anything. Simultaneously, group 3 and 5 were treated with the drug, following group 4,6 treated with the extract. The treatment continuation was done up to 5 weeks to check the blood glucose level once a week. The administration of drugs and extract was done in the oral route.

Statistical analysis

Mean±SD was used to express all the results of all study parameters obtained from different groups. Statistical significance was found using the "One Way Anova Test" of SPSS 16 software, which analyzed intra-group and inter-group difference where the statistical significance level, 'p' value was set to p>0.05. The variation among the groups was considered significant in terms of results when the 'p' value was greater than 0.05.

Results

Change in body weights

The pre-treatment & post-treatment body weight(gram) of rats belonged to different groups are shown in **Figure 1.**

Change in blood glucose level

The blood glucose level (mmol/dl) of all test group from day 1 to day 42 is expressing below in the mentioned graph in **Figure 2.**

Safety profile study (Liver function test)

The SGOT level of all rats belonged to 6 groups are denoting the condition of liver is shown in below graph **Figure 3.**

The level of SGPT of all rats that belonged to 6 groups is expressing the condition of the condition of the liver are expressing via the blow shown in graph **Figure 4.**

Safety profile study (Kidney functioning test)

The below-mentioned values concerning the level of Creatinine (mg/dl) of rats belonged to 6th group as a requirement of measuring the kidney functioning test are presenting in **Figure 5.**

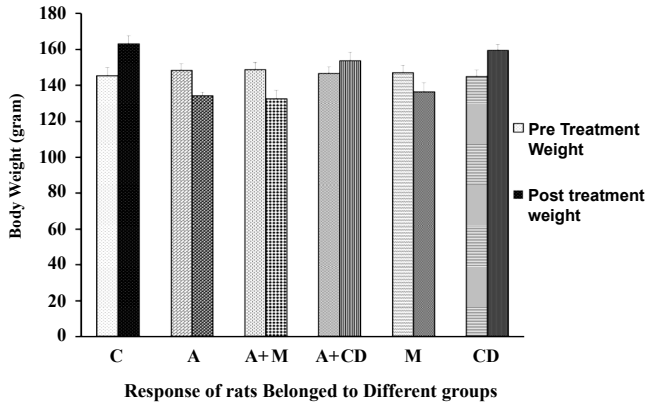

Figure 1 Comparison between the average body weight (mean±standard deviation) of rats belong to 6 groups before starting the experiment and just before sacrifice.

C=Control; A=Alloxan; A+M=Alloxan + Metformin; A+CD=Alloxan + *Cynodon dactylon*; M=Metformin; CD=Cynodon dactylon

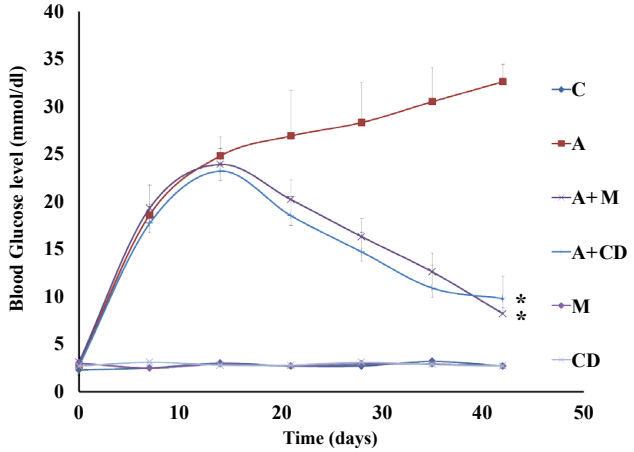

Figure 2 Blood glucose level of six groups from day zero to day forty two. The data were expressed as mean± standard deviation.

*Expresses the significant change

C=Control; A=Alloxan; A+M=Alloxan+Metformin; A+CD=Alloxan+*Cynodon dactylon*; M=Metformin; CD=Cynodon dactylon

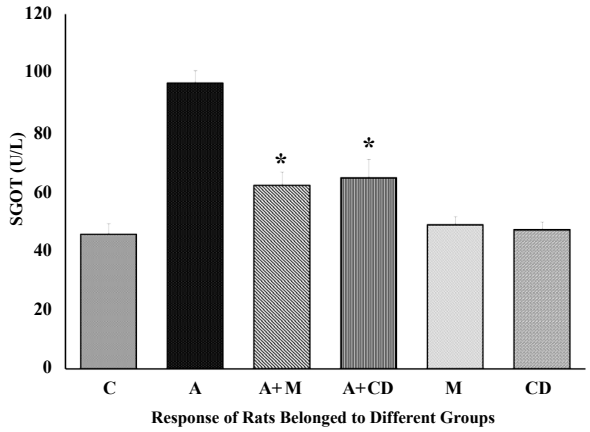

Figure 3 Comparison of SGOT level (U/L)of rats, belonged to 6 groups at day forty two just before sacrifice. *Expressing the significant change

C=Control; A=Alloxan; A+M=Alloxan + Metformin; A+CD=Alloxan + *Cynodon dactylon*; M=Metformin; CD=Cynodon dactylon

Safety profile study (Lipid profile)

The level of Total Cholesterol Level of all rats belonged to 6 groups are expressing in below, **Figure 6.**

Safety profile study (Lipid profile)

The level of HDL level (mg/dl) of all rats belonged to 6 groups are presenting in below drawn-graph, **Figure 7.**

Safety profile study (Lipid profile)

The below mention values regarding the level of LDL level (mg/dl) of rats belonged to 6 groups are presenting in below, **Figure 8.**

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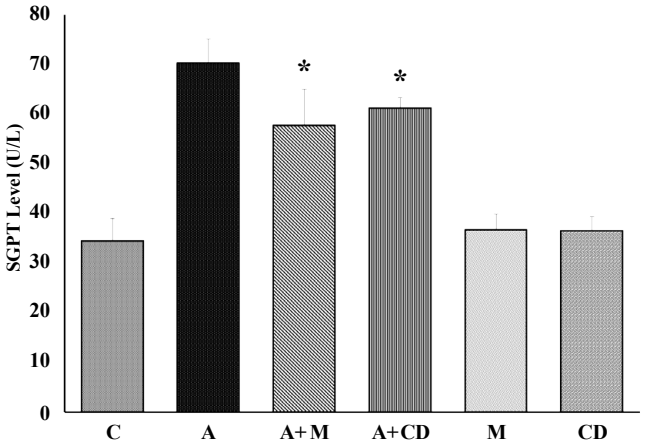

Figure 4 Comparison of SGPT level (U/L)of rats, belonged to 6 groups at day fortytwo just before sacrifice.
C=Control; A=Alloxan; A+M=Alloxan+Metformin; A+CD=Alloxan+*Cynodon dactylon*; M=Metformin; CD=Cynodon dactylon
*Expressing the significant change

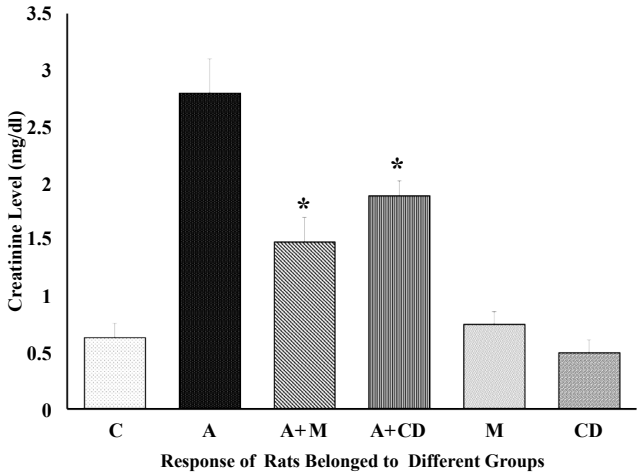

Figure 5 Comparison of SGOT level (U/L)of rats, belonged to 6 groups at day fortytwo just before sacrifice.
C=Control; A=Alloxan; A+M=Alloxan+Metformin; A+CD=Alloxan+*Cynodon dactylon*; M=Metformin; CD=Cynodon dactylon
*Expressing the significant change

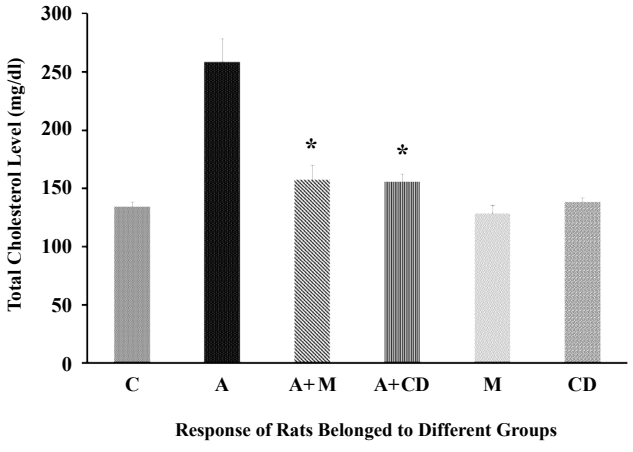

Figure 6 Comparison of Total Cholesterol Level (mg/dl)of rats, belonged to 6 groups at day fortytwo just before sacrifice.
C=Control; A=Alloxan; A+M=Alloxan+Metformin; A+CD=Alloxan+*Cynodon dactylon*; M=Metformin; CD=Cynodon dactylon
*Expressing the significant change.

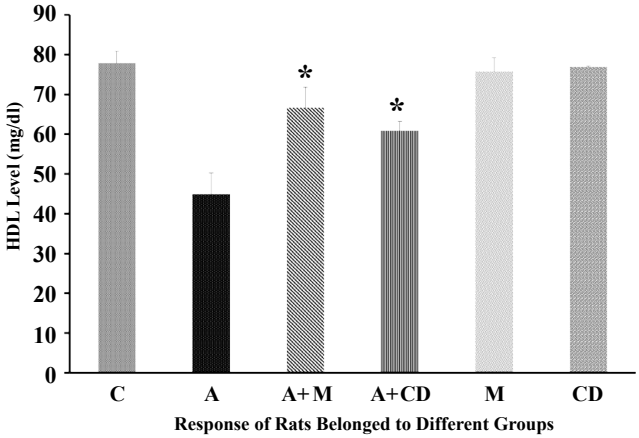

Figure 7 Comparison of HDL level (mg/dl)of rats, belonged to 6 groups at day forty two just before sacrifice.
C=Control; A=Alloxan; A+M=Alloxan+Metformin; A+CD=Alloxan+*Cynodon dactylon*; M=Metformin; CD=Cynodon dactylon
*Expressing the significant change

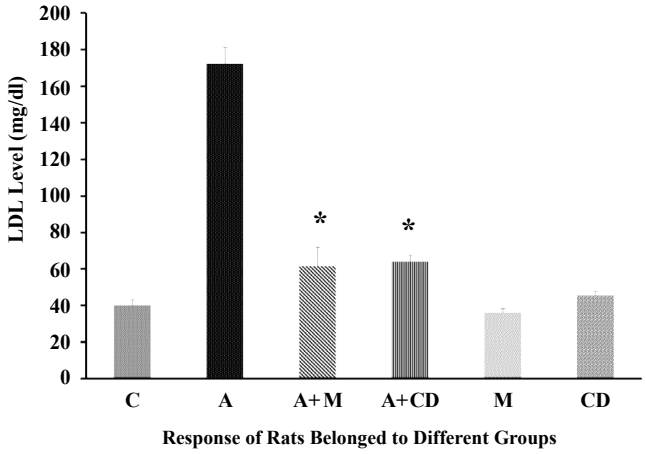

Figure 8 Comparison of LDL level (mg/dl)of rats, belonged to 6 groups at day fortytwo just before sacrifice.
C=Control; A=Alloxan; A+M=Alloxan+Metformin; A+CD=Alloxan+*Cynodon dactylon*; M=Metformin; CD=Cynodon dactylon
*Expressing the significant change

Safety profile study (Lipid profile)

The below mention values regarding the level of Triglyceride level (mg/dl) of rats belonged to 6 groups are given below **Figure 9.**

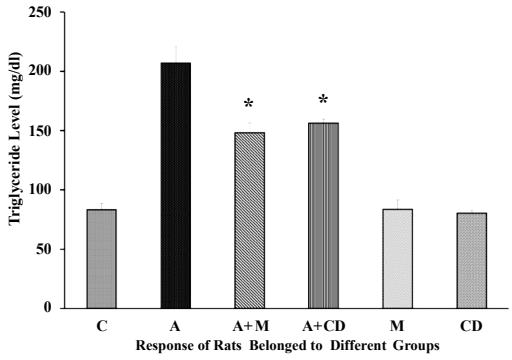

Figure 9 Comparison of Triglyceride level (mg/dl)of rats, belonged to 6 groups at day fortytwo justbefore sacrifice.
C=Control; A=Alloxan; A+M=Alloxan+Metformin; A+CD=Alloxan+*Cynodon dactylon*; M=Metformin; CD=*Cynodon dactylon*
*Expressing the significant change

Discussion

The present investigation examines the antidiabetic efficacy of *Cynodon dactylon*. Alloxan is renowned for generating DM in animal models due to its relative affordability and availability [21-23]. In the current study, the average body weight of rats belonged to alloxan control is reduced. Therefore, statistically significant variations between body weights were derived from positive and negative control groups. The rodents' bodyweight after administration of the test extract provided non-significant data with a negative control group, indicating the extract's efficacy. The same data was also developed for the administration of test extracts to healthy rats. Similar findings were obtained when *DilleniaIndica* (L.) [24], and *Sigesbeckiaorientalis* [25]

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was administered. The test extract exerted better results in body weight than metformin, *Calpurnia aurea* [26], and *Sedum adenotrichum* [27]. Metformin and plant extract both decreased blood glucose levels in alloxan-induced diabetic rats. Still, metformin provided better results than test extract as the test extract contains a smaller number of active components. When metformin and test extract were administered separately in healthy rats, no substantial difference was observed. While there is a statistically significant difference between the positive and negative control groups and the difference between the negative control and treatment groups, the former is much greater than the latter. This indicates increasing dose/an increase in dose may generate similar data to that of negative controls. Administration of *Dillenia indica* [24], *Calpurnia aurea* [26], *Zizyphus mauritiana* [28], *Aloe megalacantha Baker* [29], *Sigesbeckia orientalis* [25] and *Sedum adenotrichum* [30] produced a similar result. Like metformin, when administered to diabetic rats, the test extract significantly decreased the SGOT and SGPT levels but did not affect the healthy rats. While there is a statistical difference between the positive and negative control groups and the difference between the negative control and treatment groups, the former is much greater than the latter.

The test extract also improved the renal function by reducing the amount of serum creatinine, just as *Dillenia indica* [24], *Zizyphus mauritiana*, [28] and *Sedum adenotrichum* [27]. In this work, the enhancement in kidney function of the sample may be due to its anti-diabetic properties, leading to an increase in the impaired metabolic condition in specimens and renal tubule regenerative potential [30]. Lipid abnormality is considered one of the severe complications in diabetes, manifested predominantly by high serum TC, TG, and low HDL [31,32]. Hypertriglyceridemia and hypercholesterolemia are the most common lipid abnormalities in diabetes [33]. Test extracts have greatly increased HDL levels like *Dillenia indica* (L.) [24]. In diabetic rats, triglyceride, LDL, and cholesterol levels were significantly elevated. Similar to metformin, the test extract decreased the amount effectively. The baseline for the negative control, metformin, and test extract groups were the same. While there is a statistical difference between the positive and negative control groups and the difference between the negative control and treatment groups, the former is much greater than the latter. This indicates that an increase in dose may generate similar data to that of negative controls. For *Sedum adenotrichum* [27], *Aloe megalacantha Baker* [29], *Sigesbeckia orientalis* [25] and *Zizyphus mauritiana* [28], similar results have been observed. It was found that *C. dactylon* was more potent than *Calpurnia aurea* in the treatment of hyperlipidemia [26]. And so, *C. dactylon* has been observed to normalize the functions of vital organs affected by alloxan-induced diabetes

Conclusion

According to our findings, it might be supposed that the *Cynodon dactylon* extracts impart a similar but insignificantly weaker effect than metformin. Besides, in alloxan-induced diabetic rats, it improved pathological parameters like SGOT, SGPT and Creatinine and conferring an anti-diabetic effect. These parameters are also found unchanged when non-diabetic rats were fed *Cynodon dactylon* with a comparable dose. We, therefore, presume that this herbal medicine can be introduced for disease management of type I diabetes mellitus.

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Conflicts of Interest

None

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