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GASTRO-PROTECTIVE ROLE AND EFFECT ON GLUCOSE METABOLISM OF TETRAPLEURA TETRAPTERA (AIDAN FRUIT) EXTRACT IN RATS

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ABSTRACT: This study investigated the gastro-protective effect of Tetrapleura tetraptera (Aidan fruit) methanol extract against ethanolinduced gastrointestinal ulcers and changes in rates of glucose metabolism in rats. Extract was prepared from the fruit and subjected to acute toxicity test. In the gastro-protective study, 40 rats assigned to 4 groups of 10 rats each were treated with graded doses of the extract for 14 days. Group 1 was untreated and served as control, group 2 was treated with 20 mg/kg body weight of Omeprazole. Groups 3 and 4 received 400 and 800 mg/kg body weight of the extract, respectively. At the end of treatment, 5 rats from each group were selected and administered 10 ml/kg of 96% ethanol to induce ulcer. After 1 hour, the induced rats were sacrificed under anaesthesia and their stomachs were harvested for macroscopic ulcer assessment. Juice collected from the stomachs were also subjected to pH, pepsin activity and total acidity tests. The remaining 5 rats from each group were also sacrificed and used for the extractible mucus weight study. In the glucose metabolism study, another set of 20 rats assigned to 4 groups of 5 rats each were treated such that while group 1 was the control, group 2 received 50 metformin (50 mg/kg body weight) and groups 3 and 4 received 400 and 800 mg/kg body weight of the extract, respectively. Thirty minutes after treatment, each rat was administered 3 ml of 50% glucose solution via the oral route. Blood glucose levels of the rats were measured at intervals to assess rates of glucose metabolism. Results obtained showed a lethal dose value >5000 mg/kg body weight for the extract. In the ulcer study, the extract significantly inhibited ulcer severity scores in the rats when compared with the control (p<0.05), and also decreased gastric acidity in a dose-dependent pattern, comparing favourably with results obtained for the Omeprazole-treated group. Extractible mucus weights were also significantly higher in the extract-treated groups than the control (p<0.05). Increased rates of glucose metabolism, lower percentage weight gains and increased values of serum antioxidant parameters were also observed in the extract-treated rats when compared with control (p<0.05). Therefore, Aidan fruit may be a safe agent for use in the prevention of ethanol-induced ulcers. The agent may also be of value in strengthening the activity of the pancreas and controlling glucose levels in diabetes mellitus.

KEYWORDS: Aidan fruit, ethanol, extract, gastro-protective, ulcer.

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INTRODUCTION

Globally, the occurrence of gastrointestinal diseases appears to have increased rapidly and currently accounts for the upsurge in disease burden and household expenditures on healthcare (Wang et al., 2019). For example, a 25.82 percent increase in the occurrence of gastric ulcers was reported between 1990 and 2019 and was projected to go higher in the years ahead (Xie et al., 2022). No thanks to increasing levels of Helicobacter pylori infection, stress, andan increase in the use of non-steroidal anti-inflammatory drugs (NSAIDs), which are established causes of gastrointestinal ulcers. These agents/conditions break down the stomach's lining and defense against the acid it produces to digest food, leading to ulcers. Therefore, the use of gastro-protective agents to augment existing natural ones in the control of gastric ulcers is aimed at increasing mucosal protection, healing of mucosal damage, and inhibiting gastrointestinal bleeding (Scally et al., 2018). However, the many side effects associated with the use of these agents, coupled with cost challenges, have limited their use and availability to the high number of persons who currently have ulcers, prompting the search for cheaper, readily available, and safer alternatives. Today, the use of medicinal plants has globally been accepted as effective alternatives and are therefore used in the treatment of disease,s including those affecting the gastrointestinal system such as ulcers. Tetrapleura tetraptera is one of the numerous medicinal plants that are considered useful in the treatment of diseases.

Aidan fruit, scientifically known as *Tetrapleura tetraptera*, is a plant native to West Africa and is widely used in traditional medicine across the region. It is characterized by its distinctive four-winged pods (Akin-Idowu et al., 2011). Traditionally used as a spice and food flavoring, it also serves therapeutic roles in the treatment of gastrointestinal disorders, inflammatory conditions, reproductive issues, and infectious diseases. The fruit contains an array of phytochemicals (Atawodi et al., 2014), and its pharmacological properties include antioxidant, anti-inflammatory, antimicrobial, anti-diabetic, antihypertensive, and anticancer activities (Omonkhua et al., 2014). Aidan fruit is high in dietary fiber, which supports digestive wellbeing, and low in calories, aiding weight management (Karuna et al., 2018). It is richly endowed with anti-inflammatory and antioxidant properties, which protect against chronic illnesses including type 2 diabetes, hypertension, arteriosclerosis, and seizures (Das et al., 2020; Lin et al., 2019). Scientific research has confirmed many health benefits of Aidan fruit, including digestive cleansing, anti-aging, immune support, pain relief during labor, and management of arthritis and rheumatic pain (Dong et al., 2020; Dey and Bhaka, 2012). Phytochemicals present in T. tetraptera include tannins, alkaloids, steroids, flavonoids, triterpenoids, and phytate (Akin-Idowu et al., 2011), and their bioactive properties have been linked to antimicrobial, hypoglycemic, neuromuscular, molluscicidal, trypanocidal, and antiulcerative effects (Adusei et al., 2019; Kuate et al., 2015). Omeprazole, a proton pump inhibitor (PPI), is commonly used to manage acid-related gastrointestinal disorders. However, the gastrointestinal tract remains vulnerable to damage such as inflammation, ulceration, and mucosal erosion, often requiring alternatives or adjuncts to conventional therapy due to potential side effects and limitations of current treatments. Although Aidan fruit is traditionally recognized for its medicinal value, its specific modulatory effect on gastric acid secretion, ulceration, cytoprotection, pepsin activity, and mucus output has not been adequately studied. Therefore, this research was conducted to investigate the gastro-protective role of methanolic extract of Aidan fruit on these parameters in ethanol-induced male Wistar rats.

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MATERIALS AND METHODS

Collection of plant materials and authentication

Aidan fruit used for this study was obtained from a local food stuff market in Okuku, Yala LGA of Cross River State, Nigeria and was authenticated by a botanist in the Department of Physiology, Cross River State University, Igoli-Ogoja. A sample of the material was assigned voucher number 32 and was deposited in a herbarium in the Department of Botany.

Preparation of extract

The collected fruits were chopped into smaller particles and were air-dried on a laboratory bench within 14 days before grinding in a locally fabricated blender driven by a Yamaha motor. Three hundred grams of the pulverized material was macerated in 1.5 liters of 96% ethanol over a 48 hours period with intermittent stirring before filtration with clean handkerchief and also whatman filter paper. The filtrate was dried in a hot air oven at low temperature (40°C) to obtain an oily dark-brown extract which weighed 9.91 g and a percentage extract yield of 3.30%. The extract was preserved in the refrigerator and used from there.

Experimental Animals

A total of seventy-eight (78) male Wistar rats weighing between 120g and 150g were used in the study. The rats were procured from the Animal House of the Department of Physiology, Faculty of Basic Medical Sciences, UNICROSS. Animals were housed in clean cages under standard laboratory conditions (12-hour light/dark cycle, room temperature, good ventilation) and were provided with standard rat feed and tap water *ad libitum* and were acclimatized for seven days prior to the commencement of experiments. Ethical approval for the study was obtained from the Ethics and Approval Committee of the Faculty of Basic Medical Sciences, Okuku Campus, University of Cross River State (UNICROSS).

Determination of the lethal dose (LD50) value of the extract

For this, the modified new Lorke's method used by Ukpai et al. (2023) was adopted. Two phases were involved in the test. In the first phase, 9 rats assigned to three groups (1, 2 and 3) of three animals each and were administered the extract at 10, 100 and 1000 mg/kg body weight dose levels, respectively via the oral route. With no mortality observed, the study proceeded into the second phase which also involved the use of another set of 9 rats also assigned to 3 groups (1, 2 and 3) of 3 rats each, but treated with 1600, 2900 and 5000 mg/kg body weight of the extract, respectively. After treatment, the rats were observed for 24 hours and a further 7 days for toxicity signs and mortalities. The metrical mean of the maximum dose that produced no mortality and the minimum dose that produced 100% mortality was taken as the mean lethal dose (LD_{50}) of the extract.

Grouping of animals and experimental design for the gastro-protective studies

The rats were randomly assigned to four (4) groups (n=10) and treated as outlined below:

Group 1 (Normal Control): Received feed and 0.2 ml of normal saline.

Group 2 (Omeprazole Group): Treated with Omeprazole at a dose of 20 mg/kg body weight.

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Group 3 (Low Dose Aidan Fruit): Treated with 400 mg/kg of *T. tetraptera* methanolic extract.

Group 4 (High Dose Aidan Fruit): Treated with 800 mg/kg of *T. tetraptera* methanolic extract.

All treatments were oral and once daily using an oral gavage and lasted for 14 consecutive days before the ulcer and extractible mucus studies were carried out.

Induction of Gastric Ulcer

Five rats were selected from the control and each of the treated groups and used for the ulcer study. The method used by Ibe et al. (2021) was adopted. Each animal was given 1 ml of ethanol (90%) to induce gastric ulcers. After one hour, the rats were sacrificed for blood collection into plain bottles for the determination of serum antioxidant parameters. The stomach of each rat was also harvested, and their juice contents were emptied into a sterile sample bottle, labelled appropriately, and used for the determination of gastric pH, pepsin activity, and total acidity. The stomach was cut along the greater curvature, rinsed in slowly running water, and examined with the aid of a hand lens for ulcer scoring. The scoring formula adopted was:

Normal Stomach = 0

Red Colouration = 0.5

Spot ulcers = 1

Haemorrhagic streaks = 1.5

Ulcers > 3mm < 5mm = 2

Ulcers > 5mm = 3

Determination of ulcer index was done as follows:

$$U_I = (U_N + U_S + U_P) \times 1/10$$

Where U_I = ulcer index; U_N = mean number of ulcers per animal; U_S = mean severity score; U_P = percentage of animals with ulcer.

Percentage ulcer inhibition was calculated using the formula:

PUI = UI (control) - UI (treated) / UI (control) x 100.

Measurement of pepsin activity and total acidity

Gastric juice (1 ml) was taken into a 100ml conical flask, to this 2-3 drops of Topfer's reagent were added and titrated with 0.01N NaOH until all traces of red colour disappear and the colour of the solution turns yellow-orange (end point). The volume of alkali added was noted. This volume corresponds to free acidity. 2-3 drops of phenolphthalein solution was added and the titration was continued until a definite red tinge reappeared. The volume of alkali was noted, which corresponds to total acidity.

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Calculations:

Acidity was calculated by using the formula:

Acidity (mEq/litre) =
$$\frac{Volume\ fo\ NaOH\ x\ Normality\ of\ NaOH\ x\ 100}{0.1}$$

Estimation of pepsin activity

For pepsin estimation, 4 placed test tubes (1) and (2) containing 5ml of 1% bovine albumin in 0.01M Hcl, (3) and (4) containing 10Ml of 0.35 M trichloroacetic acid. The gastric juice was mixed with an equal volume of 0.01 M Hcl warmed to 37°C. 1ml of this mixture was added to each of the test tubes of (1) and (4) incubated for 15 minutes. At the end, the mixed content of tube (1) with (3) were allowed to stand for about 4 minutes. (1) + 3 give test and (2) + (4) give blank. The mixture was filtered. To 2ml of filtrate, 10ml of NaOH was added. Then 1ml of phenol reagent was added and mixed by gentle rotation. After 30 minutes, the absorbance was measured at 680nm. The difference between test and blank give the measures of peptic acidity.

Determination of serum antioxidant parameters

Superoxide dismutase activity was assayed by the method of Arthur and Boyne (1985) as contained in Randox commercial test kit instructions. Catalase activity was by the method of Sinha (1972). Glutathione peroxidase (GPx) activity was determined according to the method of Paglia and Valentine (1967). Reduced glutathione concentration was determined by the method of Exner *et al* (2000), while malondialdehyde was determined in accordance with the methods used by Ibe et al. (2021).

Measurement of extractible mucus weight

This was achieved by scraping the mucosal surface with a spatula after opening the stomach along the greater curvature. Mucus was collected into a pre-weighed bottle with 4 ml normal saline. Mucus output was calculated as the weight difference.

Oral glucose tolerance test (OGTT)

Following 14 days of extract administration, 5 rats from each group underwent OGTT. After fasting, baseline glucose was measured using a single-touch Accu-Chek glucometer on blood obtained from the rats by tail snip. Each rat thereafter received 3 ml of 50% glucose solution via the oral route, and blood glucose concentrations of the rats were determined after 30, 60, 90, and 120 minutes of the administration.

Statistical Analysis

Data were expressed as Mean \pm SEM. Statistical significance was determined using one-way analysis of variance (ANOVA) followed by appropriate post hoc tests. Differences were considered significant at p<0.05. Analysis was conducted using GraphPad Prism version 5.

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RESULTS

Acute toxicity test (LD50)

The methanolic extract of *Tetrapleura tetraptera* produced no observable signs of toxicity such as weakness, agitation, reduced locomotor activity, and death, even at very high doses. Therefore, the LD₅₀ was adjudged to be greater than 10,000 mg/kg body weight, indicating that the extract is safe at therapeutic doses.

Effects of extract against ethanol-induced ulcerations

At all doses administered, the extract demonstrated significant protective effects against ethanol-induced ulcers, as ulcer indices were significantly lowered in all treated groups when compared with control (p<0.05). Percentage inhibitions against ulcer were also significantly high and compared favourably with the protective effect offered by Omeprazole (Table 1). The extract also significantly lowered gastric acidity in the treated rats as gastric pH and total acidity were all lower in the treated rats than control, while extractible mucus weights were higher in treated rats than control (Table 2).

Table 1: Effects of extract against ethanol-induced ulcers in rats

Group	Treatment	Number of ulcers per animal (UN)	Ulcer severity score (US)	Percentage of animals with ulcers (UP)	Ulcer index (UI)	Percentage ulcer inhibition
1	Control	7.00±0.58°	15.83±1.01°	100.00±0.00 ^b	12.28±0.16°	0.00 ± 0.00^{a}
2	Omeprazole (20 mg/kg)	$3.33{\pm}0.33^{ab}$	5.67±0.44 ^a	93.33±1.67 ^a	10.23±0.22 ^{ab}	16.67±1.83 ^{bc}
3	Aidan fruit extract (low dose)	4.33 ± 0.33^{b}	8.50 ± 0.58^{b}	95.00 ± 0.00^{a}	10.78 ± 0.09^{b}	12.19±0. 72 ^b
4	Aidan fruit extract (high dose)	2.67 ± 0.33^{a}	5.50 ± 0.76^{a}	91.67±1.67 ^a	9.98±0.25 ^a	18.70±2.03°

Table 2: Effects of extract on stomach acidity and extractible mucus weight in rats

Group	Treatment	pН	Total acidity (mmol/L)	Pepsin activity	Extractible mucus
				(µmol	weight (g)
				tyrosine/ml)	
1	Control	3.01 ± 0.05^{a}	88.06 ± 1.60^{b}	16.61 ± 0.44^{b}	0.04 ± 0.00^{a}
2	Omeprazole (20 mg/kg)	3.50 ± 0.08^{c}	79.00 ± 2.81^a	13.90 ± 0.44^{a}	0.05 ± 0.00^{b}
3	Aidan fruit extract (low dose)	3.22 ± 0.05^{b}	82.94±1.60 ^{ab}	14.98±0.18 ^a	0.05 ± 0.00^{b}
4	Aidan fruit extract (high dose)	3.39±0.02 ^{bc}	77.23±1.13 ^a	14.63±0.30 ^a	0.05±0.00 ^b

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Effect of extract on glucose tolerance levels of treated rats

Like the group treated with metformin, the extract-treated rats had significantly lower blood glucose concentration following oral glucose administration when compared with the control (p<0.05). The treated rats also had faster glucose metabolism rates when compared with control, and at low and high doses were able to bring blood sugar levels in the rats down to 104.67±2.40 mg/dl and 89.00±7.09 mg/dl, respectively (table 4), representing percentage glucose falls of 16.93±0.88% and 25.79±4.43% respectively (table 5). Serum insulin concentrations in the extract-treated rats were also significantly higher than the control value, and compared favourably with the mean serum insulin concentration of the group treated with metformin (Table 6). Percentage weight gain was significantly lower in the extract-treated rats than in the control (Table 7).

Table 4: Oral glucose tolerance test results in mg/dl

Groups	Treatment	Pre-test blood sugar level (mg/dl)	30 minutes post-glucose administration , blood sugar level (mg/dl)	60 minutes post-glucose administration blood sugar level (mg/dl)	90 minutes post-glucose administration blood sugar level (mg/dl)	120 minutes post-glucose administration blood sugar level (mg/dl)
1	Control	86.33±2.33 ^a	150.33 ± 2.85^d	141.67±4.26°	132.67±3.71°	129.33 ± 2.96^d
2	Metformin	81.67±1.45 ^a	106.33 ± 0.88^a	$86.33{\pm}1.20^a$	77.33 ± 1.45^{a}	73.67 ± 1.45^a
3	Aidan fruit extract (low dose)	84.67±3.28 ^a	126.00±2.65 ^b	145.33±4.91°	119.00±3.79°	104.67±2.40°
4	Aidan fruit extract (high dose)	80.00±2.08 ^a	119.67 ± 2.60^{b}	108.33 ± 10.04^{b}	96.67±7.75 ^b	89.00±7.09 ^b

Table 5: Effect of extract on blood glucose concentration

Groups	Treatment	Percentage rise in blood sugar level 30 minutes after glucose administration	in blood sugar
1	Control	74.34±4.95°	13.98±0.80 ^a
2	Metformin	30.27 ± 1.96^a	30.69 ± 1.82^{b}
3	Aidan fruit extract (low dose)	49.02 ± 2.59^{b}	16.93 ± 0.88^a
4	Aidan fruit extract (high dose)	49.62±1.24 ^b	25.79±4.43 ^b

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Table 6: Effect of extract on serum insulin concentration

Groups	Treatment	Serum insulin concentration (pg/ml)
1	Control	2.59±0.01 ^a
2	Metformin	4.08 ± 0.33^{c}
3	Aidan fruit extract (low dose)	3.20 ± 0.10^{b}
4	Aidan fruit extract (high dose)	3.22 ± 0.04^{b}

Table 7: Effect of extract on body weight changes in rats

Groups	Treatment	Initial body weight (g)	Final body weight (g)	Percentage weight gain
1	Control	82.00±4.14	97.25±7.69	18.60±0.96
2	Metformin	95.75±3.72	106.50±6.09	11.23±0.82*
3	Aidan fruit extract (low dose)	96.00 ± 5.80	105.00±7.31	9.38±0.55*
4	Aidan fruit extract (high dose)	104.00±4.69	113.50±3.77	9.13±0.69*

Effect of extract on serum antioxidant parameters

Table 8: Effect on serum antioxidant parameters

Group	Treatment	SOD (U/L)	Catalase (U/L)	MDA (mMol/L)	GPx (U/L)	GSH (mg/dl)
1	Control	37.80±2.00 ^a	24.33±0.79 ^a	0.49±0.01°	35.87±0.75 ^a	9.01 ± 0.18^{a}
2	Aidan fruit extract (low dose)	39.87±0.70 ^a	25.87±0.50 ^{ab}	0.38±0.01 ^b	39.67±0.49 ^b	9.42±0.16 ^a
3	Aidan fruit extract (high dose)	41.53±0.62 ^a	27.13±0.44 ^b	0.34±0.01ª	39.50±0.68 ^b	10.07±0.15 ^b

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DISCUSSION

This study investigated the gastro-protective role of methanolic extract of *Tetrapleura* tetraptera (Aidan fruit) on ethanol-induced gastric ulceration and various gastrointestinal and metabolic parameters in male Wistar rats. The results revealed that the extract, particularly at high doses, exerted beneficial effects comparable to the standard anti-ulcer drug, Omeprazole. The observed increase in ulcer inhibition and reduction in ulcer severity and ulcer index in Aidan-treated groups is indicative of its potent gastro-protective activity. This aligns with previous findings by Ajani et al. (2018), who reported that Aidan fruit possesses anti-ulcer and cytoprotective properties. Notably, the high-dose group exhibited even greater ulcer inhibition than the Omeprazole group, suggesting superior efficacy at higher concentrations.

Gastric acid secretion is a tightly regulated physiological process involving several hormonal and neural mechanisms. Gastrin stimulates parietal cells via the H⁺/K⁺ ATPase proton pump to secrete hydrochloric acid (HCl), contributing to ulcer formation when in excess (Schubert and Peura, 2008). The extract's ability to significantly increase gastric pH and reduce total acidity supports its anti-secretory role, similar to that of Omeprazole, a proton pump inhibitor that elevates gastric pH and inhibits gastric acid output (Shin and Kim, 2023). These findings suggest that *T. tetraptera* may modulate acid secretion through a mechanism that attenuates HCl production, thereby preserving mucosal integrity. Furthermore, the extract enhanced mucus production and reduced pepsin activity, both of which are critical for mucosal protection. Mucus forms a physical barrier against gastric acid and pepsin, and increased mucus output signifies enhanced cytoprotection. Suppression of pepsin activity implies less proteolytic damage to the mucosa, reinforcing the protective effects of the extract.

The oral glucose tolerance test (OGTT) showed that T. tetraptera significantly blunted the rise in plasma glucose following a glucose load and improved glucose clearance over time. The high-dose extract group demonstrated a pronounced glucose-lowering effect comparable to that of Metformin, suggesting potential anti-diabetic properties. These findings are in agreement with earlier reports of the hypoglycemic effects of Aidan fruit, possibly via enhanced insulin secretion or improved insulin sensitivity (Ugwu et al., 2020; Okafor et al., 2018). Elevated insulin levels in the treated groups further support this hypothesis. The observed insulinotropic effect may be attributed to the presence of bioactive compounds such as flavonoids and alkaloids, known for their role in pancreatic β -cell stimulation and insulin release.

In the context of oxidative stress, the extract significantly improved the activities of antioxidant enzymes, including catalase (CAT) and glutathione peroxidase (GPx), and reduced levels of malondialdehyde (MDA) and lactate dehydrogenase (LDH), markers of lipid peroxidation and tissue damage, respectively. These results highlight the antioxidant potential of *T. tetraptera* in protecting gastric tissues against ethanol-induced oxidative injury. This is consistent with previous studies that attributed the protective effects of Aidan fruit to its rich polyphenolic and antioxidant content (Atawodi *et al.*, 2014; Panche *et al.*, 2016). Moreover, the extract did not induce significant weight loss or toxicity in the experimental animals, further reinforcing its safety at therapeutic doses. The acute toxicity study showed no mortality at doses up to 10,000 mg/kg, indicating a high margin of safety.

The findings suggest that *Tetrapleura tetraptera* methanolic extract offers multi-targeted protection against gastric ulceration through modulation of gastric acidity, enhancement of mucosal defense, attenuation of oxidative stress, and regulation of glucose metabolism. These

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effects are likely mediated by its phytoconstituents, including flavonoids, saponins, and tannins, which are known for their antioxidant, anti-inflammatory, and gastro-protective activities.

CONCLUSION

This study demonstrates that the methanolic extract of *Tetrapleura tetraptera* (Aidan fruit) possesses significant gastro-protective, anti-ulcer, antioxidant, and glucose-regulatory properties in ethanol-induced male Wistar rats. The extract effectively reduced gastric acidity and ulceration, enhanced mucosal defense, improved antioxidant enzyme activity, and modulated plasma glucose levels. These findings support the traditional use of Aidan fruit in managing gastrointestinal disorders and suggest its potential as a natural therapeutic agent for ulcer-related conditions.

DECLARATION OF CONFLICT OF INTEREST

Authors hereby declare that there is no conflict of interest.

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