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Original Article

Antidiabetic Activity of an Ayurvedic Formulation Chaturmukha Rasa: A Mechanism Based Study

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Key Words

acarbose, ά- Amylase Inhibitory Assay, chaturmukha rasa, IC50, α-Glucosidase Inhibitory Assay, streptozotocin

Abstract

Objectives: The objective of this study was to evaluate antidiabetic activity of *Chaturmukha rasa* based on streptozotocin induced diabetes model, alpha amylase inhibitory activity, alpha Glucosidase inhibitory activity and inhibition of sucrase.

Methods: *Chaturmukha rasa* was prepared as per Ayurvedic formulary. Antidiabetic activity was measured in experimentally streptozotocin induced rats. The dose was taken as 45 mg/kg, i.p. The antidiabetic activity of *Chaturmukha rasa* was compared Triphala Kwatha, a marketed formulation. Further In vitro ά- Amylase Inhibitory Assay, In vitro salivary amylase Inhibitory Assay, In vitro α-Glucosidase Inhibitory Assay and In vitro Sucrase Inhibitory Assay was performed with respect to *Chaturmukha rasa*. The IC50 value was calculated for all the above activity.

Results: Streptozotocin with Acarbose showed significant decrease in blood glucose level whereas streptozotocin with Triphala kwatha showed more decrease in blood glucose level than Streptozotocin with Acarbose. The combination of Streptozotocin + Triphala kwatha + *Chaturmukha rasa* showed a significant decrease in blood glucose level on 21st day. In vitro ά- Amylase Inhibitory Assay the *Chaturmukha rasa* showed IC50 value 495.94 µl when compared with Acarbose 427.33 µl, respectively. In the α-Glucosidase Inhibitory Assay *Chaturmukha rasa* showed IC50 value 70.93 µl when compared with Acarbose 102.28 µl, respectively. In vitro Sucrase Inhibitory Assay *Chaturmukha rasa* showed IC50 value 415.4 µl when compared with Acarbose 371.43 µl, respectively.

Conclusion: This study supports that *Chaturmukha rasa* may inhibit diabetes by inhibition of salivary amylase or alpha Glucosidase or sucrase. This may be the mechanism by which *Chaturmukha rasa* inhibits diabetes. Further this study supports the usage of *Chaturmukha rasa* for the management of diabetes.

1. Introduction

Diabetes is considered as a severe health hitch being the third major cause of death all over the world.

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Type 2 diabetes mellitus (T2DM) is a global epidemic with an estimated worldwide prevalence of 6% (246 million people) in 2007, and forecast to rise to 7.3% (380 million) by 2025. Diabetes, if not treated, is conscientious for much harm affecting various organs in the body [1]. Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion and/or insulin action, which results in hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism [2].

One therapeutic approach to decrease Diabetes mellitus is post-prandial hypoglycemia to delay the digestion. The α- amylase and α- Glucosidase enzyme are hydrolyzed complex polysaccharide which is converted into oligosaccharide and disaccharide which are then hydrolyzed by α- Glucosidase enzyme to monosaccharide which are absorbed though the small intestine in to hepatic portal vein [3]. In vitro models are fairly based on a specific process, where in activity of an enzyme on a metabolic reaction or binding to a receptor. In vitro study is of considerable value in identifying the mechanism of action of a test material and more economical [4].

Herbal formulations have a long history of use for the prevention and treatment of diseases. The use of medicinal plants with therapeutical purposes represents a secular tradition in different cultures. These traditional formulations are used by various local tribes for treatment of many diseases. Many of these formulations have been evaluated as per Pharmacopeial standards. But, most of the formulations are still unknown to the scientific world [5]. These herbal preparations also contain mixtures of herb and minerals and are generally referred as herb-minerals preparations. These herb-mineral mixtures provide remedy to a variety of diseases [6]. This study mainly focuses on one such type of herb-mineral mixture known as *Chaturmukha Rasa*.

Chaturmukha Rasa is an Ayurvedic medicine in tablet form. It is used in the treatment of asthma, anemia, diabetes, digestion power, relieves anemia, diabetes, asthma, abdominal colic, anorexia, hiccups, dyspepsia, epilepsy, gout, herpes, abscess, psychotic disorders and hemorrhoids. It mainly consists of 10 grams fine powder each of: Shuddha Parada (Herbal purified Mercury), Shuddha Gandhaka (Herbal purified Sulphur), Loha Bhasma (Bhasma prepared from Iron), Abhraka Bhasma (Purified and processed Mica), and Kumari (Aloe vera juice extract), Eranda (Castor Ricinus communis leaves). Fine power of above ingredient is ground with juice extract or decoction, made into paste and pills are prepared [7]. Since reports of antidiabetic evaluation of this classical preparation (along with the adjuvant) were not available during extensive literature review, it was thought worthwhile to undertake the antidiabetic assessment in albino rats.

2. Materials and Methods

2.1. Test Drugs

Aloe vera and Ricinus communis were collected from the botanical garden of Columbia Institute of Pharmacy, Raipur, C.G. The plant material were authenticated and voucher specimen of each submitted to pharmacology laboratory of Columbia Institute of Pharmacy. *Chaturmukha Rasa* was prepared in the department of Pharmacognosy (Table 1), Columbia Institute of Pharmacy, Raipur, C.G; and Sops were prepared and documented. All chemical used in this study were of analytical grade.

2.2. Experimental animals

Wistar albino rats of either sex weighing 200 ± 20 g body weight were used for the study. The animals were maintained under ideal husbandry conditions in terms of standard conditions of temperature (23 ± 2℃), relative humidity (50 to 60%), and exposed to 12 h light-and-dark cycles. All animals were exposed to the same environmental conditions and were maintained on standard diet and drinking water ad libitum. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC/2015-16/062) as per the guideline of Committee for the Purpose of Control and Supervision on Experiments on Animals, India.

2.3. Method for induction of diabetes

Several traditional treatment strategies have been

Table 1 Ingredients of Ayurvedic Formulation

Ayurvedic Name	Scientific name	Quantity
Rasa (prada) suddha	Mercury	1 part
Gandhaka suddha	Sulphur	1 part
Loha bhasma	Iron	1 part
Abhra (abhraka) bhasma	Mica	1 part
Kanya (kumara) swarasa	*Aloe barbadensis* Mill.	¼ part,for mardana
Eranda patra	*Ricinus communis* Linn.	Q.S. for avestana

Table2 Dose level for different group for evaluation of toxicity study

Animal	Group	Treatment	Dose	Route
Albino wistar rat	Control group	Triphala kwatha	1 ml	orally
	Standard	Streptozotocin & Marketed drug	45 mg/kg 45 mg/kg	i/p & orally
	Treated group 1	Streptozotocin & Triphala kwatha	45 mg/kg 1ml	i/p & orally
	Treated group 2	Streptozotocin & Triphala Kwatha, Chaturmukha Rasa	45 mg/kg 1 ml	i/p & orally

recommended in the alternative system of medicine for the treatment of diabetes mellitus. Present study was undertaken to evaluate and examine the antidiabetic potential of ayurvedic formulation containing loha bhasma, abharak bhasma, rasa (prada) sudha, gandhaka sudha, in Streptozotocin (45 mg/kg i.p. single dose) induce type-2 diabetes rats.

2.4. Dosing

The animals were divided in to four groups, and keep in their cage for at least five day prior to dosing to allow for acclimatization to conducting the study. The dose for each dose group is mentioned in Table 2.

2.5. In vitro ά- Amylase Inhibitory Assay

2.5.1. Extraction of Wheat alpha amylase

500 g of malted whole wheat flour was added slowly with stirring to 1 liter of 0.2% calcium acetate solution at room temperature and continuously stirred for 2 hours on a stirrer. The suspension was then centrifuged at 4°C at 12000 rpm for 10 minutes. The resultant clear brown supernatant was stored at 2°C to 3°C prior to heat treatment. Since beta amylase interferes with the enzymatic determination of alpha amylase it was inactivated by heating the extract at 70°C for 15 minutes. Alpha amylase is resistant to inactivation by this treatment at pH between 6.5 and 8.0. The pH of the extract was first adjusted to 6.6 with cold 4% ammonium hydroxide. Heat treatment was carried out at 85°C to 90°C and other at 72°C to 74°C using a water bath with continuous stirring. The extract was then cooled to 2°C to 3°C until use [8].

2.5.2. Procedure of wheat alpha amylase inhibitor activity-

The assay mixture containing 200 µl of 0.02 M sodium phosphate buffer, 40 µl of enzyme and the ayurvedic formulation (*chaturmukah rasa*) (1 mg/ml) in concentration range 50-250 µl were incubated for 10 minutes at room temperature followed by addition of 200 µl of starch solution (1%w/v) in all test tubes. The reaction was terminated with the addition of 400 µl DNS (3, 5 dinitrosalicylic acid) reagent and placed in boiling water bath for 5 minutes, cooled and diluted with 15 ml of distilled water and absorbance was measured at 540 nm. The control samples were prepared without any plant extracts. The % inhibition was calculated according to the formula [9].

$$\text{Inhibition (\%)} = \frac{\text{Abs 540 (control) - Abs 540 (extract)}}{\text{Abs 540 (control)}} \times 100$$

The IC50 values were determined from plots of percent inhibition versus log inhibitor concentration and Acarbose was used as the reference alpha amylase inhibitor.

2.6. In vitro α-Glucosidase Inhibitory Assay

2.6.1. Isolation of α-Glucosidase from rat small intestine

The enzyme isolated from before 20 hours of fasting male Wister rats (180-200 gm) was collected after sacrificing the animal under anesthesia and immediately the intestine was cut between the part below duodenum and above cecum, then cleaned with saline and epithelial layer (mucosal tissue) was collected by scraping the luminal surface firmly with the help of spatula. The mucosal scraping were homogenized in phosphate buffered saline (PBS) pH 7.4 containing 1% triton x 10, and then centrifuged at 12000 rpm for 15 min. The supernatant fraction contained rat small intestinal α-Glucosidase. 1- Butanol was added to the supernatant fraction 1:1 proportion and centrifuged at 15000 rpm for 15 min. The aqueous layer was dialyzed overnight against the same buffer. After dialysis, the concentrated enzyme was used as crude α- Glucosidase

enzyme in the study to observe inhibition by different drug test sample. All the preparations were carried out at 4°C. This was used as an enzyme source of sucrase and alpha-Glucosidase [10].

2.6.2. Procedure for α-Glucosidase inhibition activity

The inhibitory studies of the ayurvedic formulation (*chaturmukah rasa*) against rat intestinal alpha-Glucosidase and sucrase were performed according to the method described with few modifications. Briefly, for Glucosidase inhibition assay, the crude enzyme solution was pre-incubated with ayurvedic formulation (*chaturmukah rasa*) (1 mg/ml) at different concentrations (50-250 µl) for 10 minutes at 37°C. The reaction was initiated by the addition of (50 µl) p-nitrophenyl α-D-glucopyranoside as a substrate in phosphate buffer was added to the mixture. The reaction mixture was incubated for 30 minutes at 37°C. The reaction was stopped by adding 3 ml of 0.1 M Na_2CO_3. The activity of the enzyme was determined at 400 nm spectrophotometer. Alpha-Glucosidase activity was determined by measuring release of p-nitrophenyl from p-nitrophenyl α-D-glucopyranoside at 400 nm in using UV-1800 spectrophotometer. The IC50 value was defined as the concentration of alpha-Glucosidase inhibitor to inhibit 50% of its activity under the assay condition [9].

Acarbose was used as positive control. The percentage inhibitory effect of compounds was calculated by the formula:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance(control) - Absorbance(test)}}{\text{Absorbance(control)}} \times 100$$

2.7. In vitro Sucrase Inhibitory Assay

The effect of ayurvedic formulation (chaturmukah rasa) on sucrase activity was assayed according to the method of Honda and Hara with few modifications. The enzyme solution (40 µl) and varying concentrations of the ayurvedic formulation (chaturmukah rasa) (1 mg/ml) at different concentrations (50-250 µl) were incubated together for 10 minutes at 37°C, and the volume was made up to 200 µl with maleate buffer (pH 6.0). The enzyme reaction was started by adding 100 µl sucrose solution (2%w/v). After 30 minutes, the reaction was terminated by adding 400 µL of DNS (3,5- dinitrosalicylic acid) reagent and treating the mixture in a boiling water bath for 5 minutes, cooled and diluted with 15 ml of distilled water and absorbance was measured at 540 nm. The control samples were prepared without any plant extracts [11].

The % inhibition was calculated according to the formula.

$$\text{Inhibition (\%)} = \frac{\text{Absorbance (control) - Absorbance(test)}}{\text{Absorbance (control)}} \times 100$$

2.8. Method for induction of diabetes

Adult male Wistar rats were maintained under controlled laboratory conditions at the temperature of 25 ± 3°C with 60 ± 15% humidity and 12 h dark/light cycle. Male wistar rats (160-240 gm) were maintained on standard chow diet and water ad libitium. Streptozotocin (45 mg/kg) is administered by intravenous injection. Initially blood glucose increases to 150- 200 dl within three h after administration of streptozotocin [9].

3. Result

Acarbose (at a concentrations 250 µl) showed 44.58% inhibitory effects on the α-amylase activity with an IC50 value 427.33 µl (Table 4). *Chaturmukha rasa* (at a concentration 250 µl) exhibited 33.07% of α-amylase inhibitory activity with an IC50 values 495.94 µl.

Acarbose (at a concentrations 250 µl) showed 75.22% inhibitory effects on the alpha-Glucosidase activity with an IC50 value 102.28 (Table 5). *Chaturmukha rasa* (at a concentration 250 µl) exhibited 70.32% of α-amylase inhibitory activity with an IC50 values 70.93 µl.

Acarbose (at a concentrations 250 µl) showed 33.33% inhibitory effects on the sucrose activity with an IC50 value 371.43 µl (Table 6). *Chaturmukha rasa* (at a concentration 250 µl) exhibited 29.28% of α-amylase inhibitory activity

Table 3 antidiabetic study of ayurvedic formulation "*Chaturmukha rasa*" on albino wistar rat.

Group	0 day	24 hour	7 day	14 day	21 Day
Control	89.6 ± 1.6	193 ± 1.5	168.5 ± 1.6	163.1 ± 1.2	143.6 ± 1.7
Stz + Acarbose	87.6 ± 1.4	194 ± 1.9	166.1 ± 1.1	162 ± 1.2	145.3 ± 1.7
Stz + Triphala kwatha	88 ± 1.8	199.3 ± 1.8	168.5 ± 1.7	158.6 ± 2.6	142 ± 1.8
Stz + Triphala kwatha + Chaturmukha Rasa	87.6 ± 1.08	191.6 ± 1.4	170.5 ± 1.5	161.5 ± 1.4	145.1 ± 1.4

Table 4 The percent inhibition of salivary alpha-Amylase by Chaturmukha rasa at varying concentrations

S.No	Concentration (µl/ml)	% Inhibition by Acarbose	IC50 value of Acarbose	% Inhibition by Chaturmukha rasa	IC50 value of Chaturmukha rasa
1	50 µl	34.09	427.33 µl	15.55	495.94 µl
2	100 µl	35.55		19.44	
3	150 µl	36.49		21.62	
4	200 µl	37.41		24.34	
5	250 µl	44.58		33.07	

Table 5 The percent inhibition of alpha-Glucosidase by Chaturmukha rasa at varying concentrations

S.No	Concentration (µl/ml)	% Inhibition by Acarbose	IC50 value of Acarbose	% Inhibition by Chaturmukha rasa	IC50 value of Chaturmukha rasa
1	50 µl	51.97	102.28 µl	47.03	70.93 µl
2	100 µl	60.51		54.23	
3	150 µl	63.82		58.46	
4	200 µl	70.44		66.66	
5	250 µl	75.22		70.32	

Table 6 The percent inhibition of sucrose by Chaturmukha rasa at varying concentrations

S.No	Concentration (µl/ml)	% Inhibition by Acarbose	IC50 value of Acarbose	% Inhibition by Chaturmukha rasa	IC50 value of Chaturmukha rasa
1	50 µl	12.58	371.43 µl	8.33	415.4 µl
2	100 µl	14.28		10.81	
3	150 µl	27.47		19.51	
4	200 µl	31.95		26.66	
5	250 µl	33.33		29.78	

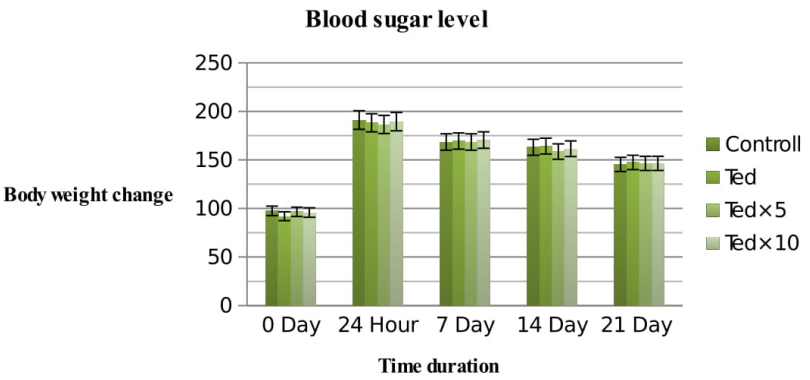

Figure 1 antidiabetic study of ayurvedic formulation "chaturmukha rasa" on albino wistar rat

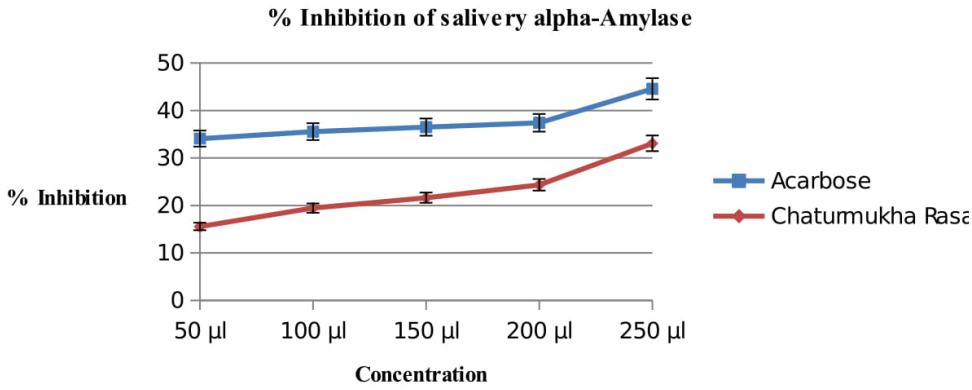

Figure 2 The percent inhibition of salivary alpha-Amylase by Chaturmukha rasa at varying concentrations

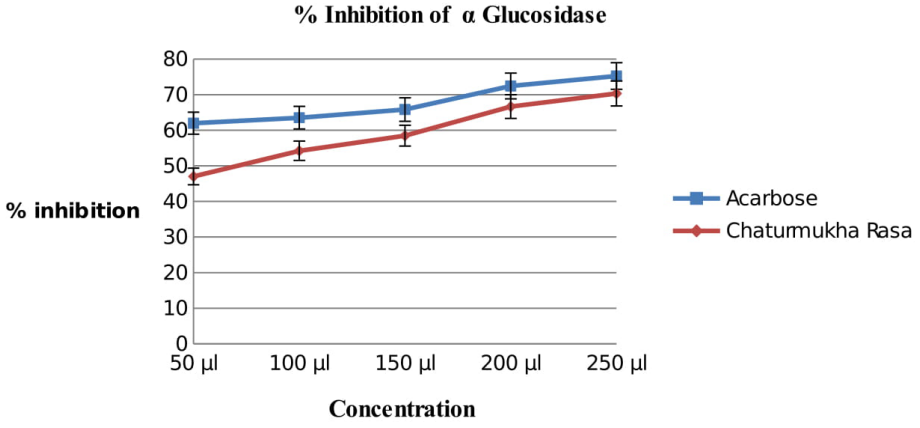

Figure 3 The percent inhibition of alpha-Glucosidase by Chaturmukha rasa at varying concentrations

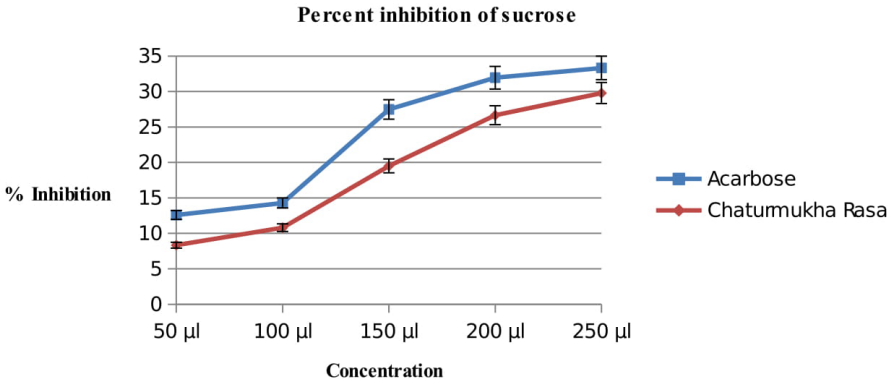

Figure 4 The percent inhibition of sucrose by Chaturmukha rasa at varying concentrations

with an IC50 values 415.4 µl.

In vitro Antidiabetic activity was performed using the following combinations: STZ + Acarbose, STZ + Triphala Kwatha and STZ + Triphala kwatha + *Chaturmukha Rasa* (Table 3). With the first combinations there was a variable increase and decrease in blood pressure. After administration of the combinations the 0th day blood glucose level was 87.6 ± 1.4 and 21st day blood glucose level was 145.3 ± 1.7. The second combination showed blood glucose level on 0th day 88 ± 1.8 and on 21st day blood glucose level as 142 ± 1.8. The third combination showed 0th day blood glucose level as 87.6 ± 1.08and on 21st day the blood glucose level was 145.1 ± 1.4.

4. Discussion

Many herbal herbo metallic formulations have been reported to have antidiabetic activities. These therapies are used in Ayurveda for the cure of diabetes. In this study, In vitro antidiabetic activity of *Chaturmukha rasa* against Streptozotocin induced diabetes was evaluated (Table 3). The herbo metallic formulation was compared with allopathic marketed drug (Acarbose) and herbal drugs (Triphala kwatha). *Chaturmukha rasa* showed antidiabetic effect which may be due to inhibition of enzyme such as alpha amylase or alpha Glucosidase.

We compared IC50 values of α-amylase inhibitory effects of Acarbose and *Chaturmukha Rasa*. Acarbose with 44.58% inhibitory effects at concentration of 250 µl showed IC50 (Table 4) value of 427.33µl, whereas *Chaturmukha rasa* (at a concentration 250 µl) exhibited 33.07% of α-amylase inhibitory activity with an IC50 values 495.94 µl.

We also compared IC50 values alpha-Glucosidase Inhibitory effect of Acarbose and *Chaturmukha Rasa*. Acarbose with 75.22% inhibitory effects at concentration of 250 µl (Table 5) showed IC50 value of 102.28, whereas *Chaturmukha rasa* (at a concentration 250 µl) exhibited 70.32% of alpha-Glucosidase inhibitory activity with an IC50 values 70.93 µl.

IC50 values sucrose Inhibitory effect of Acarbose and *Chaturmukha Rasa*. Acarbose with 33.33% inhibitory effects at concentration of 250 µl showed IC50 value of 371.43 (Table 6), whereas *Chaturmukha rasa* (at a concentration 250 µl) exhibited 29.28% of sucrose inhibitory activity with an IC50 values 415.4 µl.

With the inhibition of alpha amylase, alpha Glucosidase and sucrose the herbo-metalic formulation offers a prospective therapeutic approach for the management of diabetes. This formulation also showed lowering of blood glucose level with streptozotocin induced diabetes model. As a whole this formulation may be used for management and treatment of diabetes.

5. Conclusion

The result of the study indicates that *Chaturmukha rasa* may be used as drug for treatment and management of diabetes. This study also confirms that the antidiabetic activity of this drug may be due to inhibition of enzyme system such as alpha amylase and alpha Glucosidase which are generally used by the body for the metabolism of glucose. The may be its mechanism of action and a newer thrust area for the researchers to established its mechanism at molecular level.

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Conflict of interest

The authors declares no conflict of interest.

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