ORIGINAL ARTICLE

In-vivo Assessment of Antipyretic and Anti-inflammatory Activities of *Gymnosporia Royleana* Extract in Mice

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ABSTRACT

Objective: *Despite the presence of multitude of synthetic drugs against fever and inflammation, none has been proven entirely safe. In contrast, the accepted safety of plant derived natural products is inspiring the world. Based on this fact as well as in view of the diversified activities reported from the genus* Gymnosporia*, the present study was designed to evaluate the antipyretic and anti-inflammatory activity of* Gymnosporia royleana (G royleana)*.*

Methods: *The methanolic extract of the aerial parts of* G royleana *was screened for* in-vivo *antipyretic activity using the brewer's yeast-induced pyrexia mice model and for anti-inflammatory activity using the carrageenan-induced paw oedema and xylene-induced ear oedema mice model.*

Results: *In the antipyretic assay,* G royleana *extract showed considerable antipyretic activity in a dose dependent fashion. Statistically significant antipyretic effects ($p < 0.05$) were observed at the end of the second hour of administration for all doses of extract and remained significant until the end of the experiment. The plant extract also displayed promising anti-inflammatory activity, in a dose dependent fashion, in both models of inflammation* ie *carrageenan- and xylene-induced oedema models, when compared to the controls. In the carrageenan-induced oedema model, significant effects ($p < 0.01$) were observed for 300 and 600 mg/kg doses after 60 minutes of xylene administration (*ie *55.51% and 65.88% inhibition of oedema, respectively).*

Conclusion: *The study provided evidence supporting the antipyretic and anti-inflammatory activity of the* G royleana *methanolic extract.*

Keywords: Brewer's yeast, carrageenan, Gymnosporia royleana, xylene-induced oedema

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Evaluación *in vivo* de las Actividades Antipiréticas y Antiinflamatorias del Extracto de *Gymnosporia Royleana* en Ratones

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RESUMEN

Objetivo: *A pesar de la presencia de multitud de fármacos sintéticos en el arsenal contra la fiebre y la inflamación, ninguno ha dado pruebas de ser completamente seguro. En contraste con ello, la seguridad aceptada de los productos naturales derivados de las plantas inspira al mundo. Sobre la base de este hecho, así como en vista de las actividades diversificadas que se reportan con respecto al género* Gymnosporia*, el presente estudio se diseñó con el objeto de evaluar el potencial antipirético y antiinflamatorio de* Gymnosporia royleana *(*G royleana*).*

Métodos: *El extracto de metanol de las partes aéreas de* G royleana *fue tamizado en busca de actividad antipirética* in vivo*, utilizando el modelo de pirexia inducida por levadura de cerveza en ratones, y de actividad antiinflamatoria utilizando modelos de ratones con oedema de las patas inducido mediante carragenina, y oedema de las orejas inducido mediante xileno.*

Resultados: *En el ensayo antipirético, el extracto de* G royleana *mostró una actividad antipirética considerable en forma dependiente de la dosis. Se observó un efecto antipirético estadísticamente significativo ($p < 0.05$) en el transcurso de la segunda hora de administración para todas las dosis de extracto y se mantuvo significativo hasta el final del experimento. El extracto de la planta también mostró una actividad antiinflamatoria prometedora, de una manera dependiente de la dosis, en ambos modelos de inflamación, es decir, modelos de oedema inducido por carragenina y xileno, en comparación con el control. En el modelo de oedema inducido por carragenina, se observó un efecto significativo ($p < 0.01$) para dosis de 300 y 600 mg / kg después de 60 minutos de administración de xileno (es decir, 55.51% y 65.88% de inhibición del oedema, respectivamente).*

Conclusión: *El estudio proporcionó pruebas suficientes sobre el potencial antipirético y anti-inflamatorio del extracto de* G royleana*.*

Palabras clave: Levadura de cerveza, carragenina, Gymnosporia royleana, oedema inducido por xileno

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INTRODUCTION

Gymnosporia royleana (*G royleana*) is a native plant of northern areas of Pakistan belonging to the genus *Gymnosporia* (*Celastraceae*). The plant is a thorny shrub with stiff branches, and flowers in the months of March to April. Flowers are white in colour and after maturation forms 3-gonous capsule bearing black seeds (1, 2). Northern areas like the valleys of Kaghan, Kashmir, Swat and Buner are its natural habitats (1, 2), where it is used in local traditional medicine for its analgesic, anti-diarrheal, anti-dysenteric, anti-spasmodic, antimicrobial, gastro-protective, anticancer and pediculicidal properties (3, 4). Despite the widespread use of the plant in folk medicine as well as the diverse pharmacological activities of the genus, only (2, 5–10), a very limited number of scientific studies have been conducted on *G royleana* to validate its folklore uses and to investigate novel pharmacological activities (2, 3, 11). Based on the diverse pharmacological activities reported for the genus *Gymnosporia* and the present day need of natural antipyretic and anti-inflammatory drugs having extended safety profiles, we decided to investigate the possible antipyretic and anti-inflammatory properties of the plant.

MATERIAL AND METHODS

Plant collection, identi ication and grinding

The aerial parts of *G royleana* were collected from Mata, in the district of Swat Valley, Khyber Pakhtunkhwa,

Pakistan, and identified by taxonomist, Dr Ghulam Jeelani, Department of Botany, University of Peshawar. A voucher specimen (Bot. 20044/pup) was submitted in the said institution.

The aerial parts of the plant were shaken and washed with distilled water to remove any foreign material and dirt, then placed for several weeks, under shade at room temperature, until sufficiently dried. The material was then chopped and finely powdered using a mechanical grinder (Yigan, Model WF 130).

Extraction

Extraction of chemical constituents from *G royleana* was accomplished using cold maceration technique. Powdered plant material (9.7 kg) was macerated repeatedly in methanol at room temperature with intermittent shaking. The combined filtrate was evaporated using a rotary evaporator (Bucchi Rotavapor R 200), providing the final crude methanol extract (580 g).

Experimental animals

Bagg Albino [inbred research mouse strain] (BALB/c) mice were utilized for conductingthe bioassays. These animals were procured from National Institute of Health (NIH) Islamabad, Pakistan. The animals were acclimatized prior to experimentation, in laboratory conditions (housed in poly-propylene cages with stainless steel grill tops at 25 ± 2 °C with 12 hours light/dark cycle). During the acclimatization period standard diet and tap water *ad libitum* were provided to the animals.

Ethics

In-vivo pharmacological studies were carried out according to standard experimental guidelines and procedures, as approved by the Ethical Committee (05/EC-15/pharm), Department of Pharmacy, University of Peshawar, Pakistan.

Antipyretic activity

The methanolic extract of *G royleana* was evaluated for anti-pyretic activity using the previously described protocol with slight modification (12). The test was performed using BALB/c mice (30–35 gm) of either gender. Mice were deprived of food for 12 hours but water was allowed *ad libitum*. Fever was provoked by injecting 15% *aq* solution of brewer's yeast (Vahine Professional, France) at a dose of 10 mL/kg *sc*. Rectal temperatures of all mice were recorded with a digital thermometer (Hartmann, Germany), before yeast injection, as well as 24 hours after the injection.

Mice that showed less than 0.5 °C increase in body temperature, 24 hours post yeast administration, were excluded from the study, whereas, the rest of the animal subjects were separated into five groups with each group consisting of six animals and treated with N/saline (negative control) and, different concentrations of extract and paracetamol (positive control). Rectal temperatures of mice were continuously recorded after the 1st, 2nd, 3rd, 4th and 5th hours post drug/extract administration. The percentage reduction in body temperature was determined for each group.

Anti-in lammatory activity

Crude methanolic extract of *G royleana* was evaluated for anti-inflammatory activity using the previously reported protocols of xylene induced ear oedema and carrageenan induced paw oedema models.

Carrageenan induced paw oedema model

Bagg Albino (inbred research mouse strain) mice weighing about 25−30 gm, of either gender, were selected to evaluate the anti-inflammatory activity of *G royleana* extracts. The mice were randomly distributed into five groups of six mice each (13). Group 1 was served with N/saline (10 mL/kg; *ip*) and assigned as negative control whereas, Group 2 was served with indomethacin (10 mg/kg; *ip*) and assigned as positive control. Methanolic extract of *G royleana* was injected *via* intraperitoneal route to Groups 3, 4 and 5 at doses of 150, 300 and 600 mg/kg, respectively. After 30 minutes, carrageenan (1%; 0.05 mL) was administered *sc* in the sub-plantar tissue (right hind paw) of each animal.

The anti-inflammatory effect was recorded continuously for five hours (at 0, 1, 2, 3, 4 and 5 hours), using plethysmometer (LE 7500 plan lab SL). The percentage of inhibition was calculated from data the data obtained.

Xylene-induced ear oedema in mice

Xylene-induced ear oedema test was performed according to the previously described protocol with slight modification (14). BALB/c mice of either gender (weighing 22–27 gm) were randomly distributed into ten different groups (n = 6). Different doses of methanolic extract of *G royleana* (150 mg/kg, 300 mg/kg and 600 mg/kg), dexamethasone (4 mg/kg) and distilled water (10 mL/kg) were given orally to respective groups of 24 hours fasted mice in duplicated. After 30 minutes of this treatment, inflammation was provoked by applying two drops of xylene topically to the inner surface of the right ear. Xylene was allowed to

act locally for 15 minutes in Groups 1–5. After giving light anaesthesia the animal subjects of Groups 1–5 were sacrificed, followed by cutting of both ears, whereas, animals of Groups 6–10 were sacrificed after one-hour of xylene application. The difference between the ears weights were used for calculating the anti-inflammatory activity of test samples.

Statistical analysis

The data was expressed as mean ± SEM of six animals. For statistical analysis, student *t*-test or analysis of variance (ANOVA) followed by Dunnett's test was applied for multiple comparisons. Effects were considered to be significant at the $p < 0.05$ level. GraphPad Prism 5.0 software was employed for statistical analysis.

RESULTS

Antipyretic activity

Antipyretic effect of the crude extract of *G royleana* has been represented in Table 1 and Fig. 1.

The results showed that crude methanolic extract of *G royleana* possess considerable antipyretic effect when compared with the control. The test samples produced antipyretic effects as early as the first-hour, in a dose dependent manner and achieved peak levels after the third-hour of administration, similar to that of paracetamol. However, the antipyretic effect of any of the doses of crude extract was less than that of paracetamol. Maximum antipyretic effect produced by extract was 78.60% at 600 mg/kg dose, whereas the maximum antipyretic effect produced by paracetamol was 88.20% at 150 mg/kg dose.

Anti-inflammatory activity

Carrageenan induced paw oedema model

The anti-inflammatory potential of *G royleana* extract (at 150, 300 and 600 mg/kg bodyweight) is summarized in Table 2 and Fig 2.

The results revealed that the crude extract of aerial parts *G royleana* ameliorated the phlogistic effect of the carrageenan dose dependently with the most potent effect (62.40% inhibition of oedema) produced at 600 mg/kg dose and was comparable to the anti-inflammatory effects of the standard drug (indomethacin). The maximum anti-inflammatory effect produced by the crude extract was 62.40% at 600 mg/kg dose whereas, indomethacin produced 66.10% inhibition of oedema at 10 mg/kg dose.

The data also suggested that the extract is effective both in the early as well as the late phase of inflammation.

Xylene-induced ear oedema in mice

The results of anti-inflammatory activity of methanolic extract of *G royleana* is presented in Table 3 and Fig 3. The results showed that *G royleana* extract attenuated xylene-induced ear oedema in mice in a dose dependent

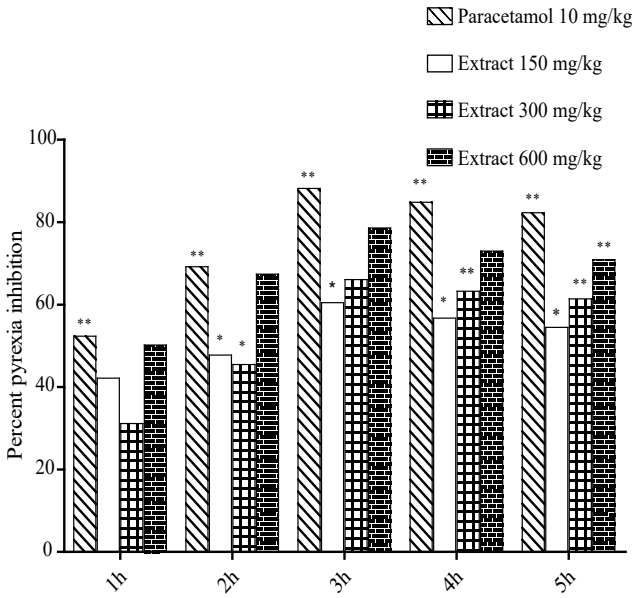

Fig. 1:Antipyretic activity of methanol extract of aerial parts of *G royleana.*

Bars represent mean ± SEM (n = 6). ANOVA followed by Dunnett's test was applied to the data to determine the level of significance in comparison with negative control. *$p < 0.05$, **$p < 0.01$

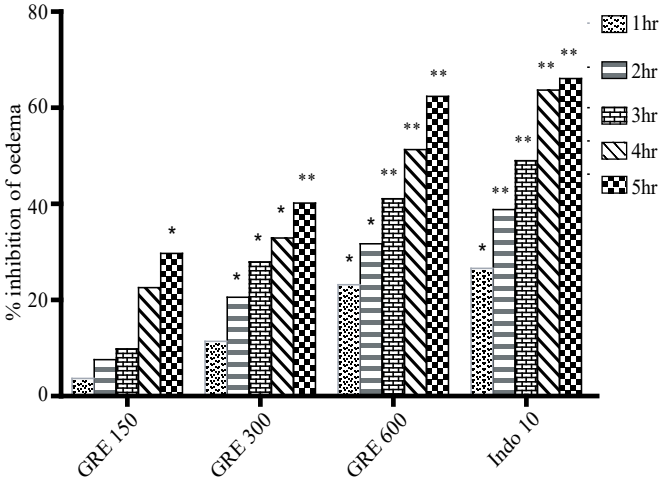

Fig. 2: Anti-inflammatory activity of methanol extract of *Gymnosporia royleana* in Carrageenan-induced oedema model.

Bars represent the per cent inhibition of ear oedema. Values are reported as mean ± SEM (n = 6). ANOVA followed by Dunnett's test was applied to the data to determine the level of significance in comparison with negative control. *$p < 0.05$, **$p < 0.01$.

Table 1: Antipyretic activity of methanolic extract of *Gymnosporia royleana.*

Treatment	Dose (mg/kg)	Rectal temperature (°C) ± SEM						
		Normal	after 24 hours	After drug administration				
				1 hour	2 hours	3 hours	4 hours	5 hours
Saline	10 mL	36.69±0.52	39.71±0.26	38.67±0.31	38.62± 0.44	38.61± 0.21	38. 71±0.33	38.77±0.33
Paracetamol	150	37.05±0.32	39.42±0.32	38.18**±0.26	37.78**±0.36	37.33**±0.39	37.41**±0.44	37.47**±0.47
Extract	150	37.07±0.41	39.75±0.31	38.62±0.23	38.47*±0.34	38.13*±0.22	38.23* ±0.18	38.29*±0.17
	300	37.06±0.56	39.21±0.24	38.54±0.36	38.23*±0.19	37.79**±0.33	37.85**±0.23	37.89**±0.27
	600	37.01±0.46	39.86±0.19	38.43±0.21	37.94*±0.17	37.62**±0.27	37.78**±0.25	37.84**±0.37

Values are reported as mean ± SEM for group of six animals. The data was analysed by ANOVA followed by Dunnett's test. *$p < 0.05$, **$p < 0.01$ in comparison to the control.

Table 2a: Anti-inflammatory activity of methanol extract of aerial parts of *Gymnosporia royleana* in carrageenan induced oedema model.

Sample	Dose (mg/Kg)	Increase in paw oedema size (mm)				
		1 hour	2 hours	3 hours	4 hours	5 hours
Vehicle	–	5.68 ± 1.22	5.83 ± 1.37	5.92 ± 1.16	5.98 ± 1.12	6.17 ± 1.31
Extract	150	5.47 ± 1.13	5.39 ± 1.24	5.34 ± 1.02	4.63 ± 1.13	4.34 ± 1.18*
	300	5.03 ± 0.91	4.63 ± 1.07*	4.27 ± 0.94*	4.01 ± 1.21*	3.69 ± 0.94**
	600	4.36 ± 0.81*	3.98 ± 0.76*	3.49 ± 0.97**	2.91 ± 0.83**	2.32 ± 0.74**
Indomethacin	10	4.17 ± 0.94*	3.57 ± 0.88**	3.02 ± 0.86**	2.17 ± 0.94**	2.09 ± 0.81**

Values are reported as mean ± SEM for group of six animals. The data was analysed by ANOVA followed by Dunnett's test. *$p < 0.05$, **$p < 0.01$ in comparison to the control.

Table 2b: Anti-inflammatory activity of methanol extract of aerial parts of *Gymnosporia royleana* in carrageenan induced oedema model.

Treatment	Dose (mg/Kg)	% inhibition of oedema				
		1 hour	2 hours	3 hours	4 hours	5 hours
Vehicle	–	–	–	–	–	–
Extract	150	3.70	7.60	9.80	22.60	29.70
	300	11.40	20.60	27.90	32.90	40.20
	600	23.20	31.70	41.10	51.30	62.40
Indomethacin	10	26.60	38.80	49.00	63.70	66.10

Values are reported as mean ± SEM for group of six animals. The data was analysed by ANOVA followed by Dunnett's test. *$p < 0.05$, **$p < 0.01$ in comparison to the control.

Table 3: Anti-inflammatory activity of methanol extract of aerial parts of *Gymnosporia royleana* in xylene-induced oedema model.

Sample	*ip* Dose (mg/Kg)	15 minutes		60 minutes	
		Difference (mg)	% inhibition	Difference (mg)	% inhibition
Saline	–	31.43 ± 2.82	–	33.47 ± 2.96	–
Extract	150	21.18 ± 3.09*	32.62	15.09 ± 2.140*	54.91
	300	16.91 ± 2.37*	46.20	14.89 ± 2.87**	55.51
	600	12.59 ± 2.81*	59.95	11.42 ± 3.13**	65.88
Dexamethasone	0.5	9.74 ± 2.18**	69.01	8.12 ± 4.17**	75.74

Values are reported as mean ± SEM for group of six animals. The data was analysed by ANOVA followed by Dunnett's test. *$p < 0.05$, **$p < 0.01$ in comparison to the control.

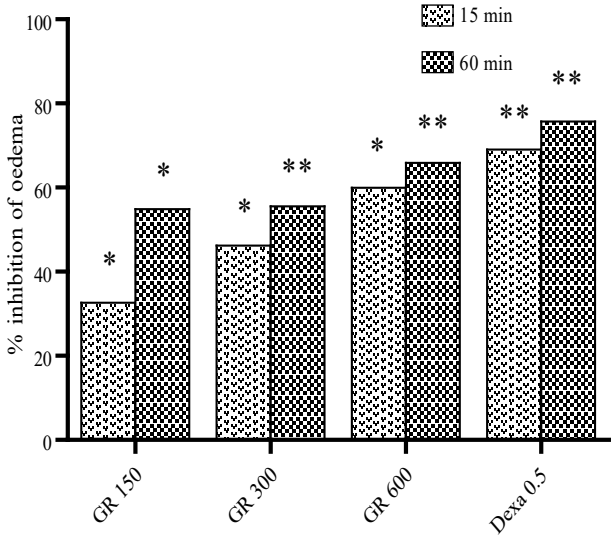

Fig. 3: Effect of methanol extract of *Gymnosporia royleana* in Xylene-induced ear oedema in mice.

Bars represent the percent inhibition of ear oedema. Values are reported as mean ± SEM (n = 6). ANOVA followed by Dunnett's test was applied to the data to determine the level of significance in comparison with negative control. *$p < 0.05$, **$p < 0.01$.

fashion with effects appearing as early as at 15 minutes. The maximum anti-inflammatory effect produced by the extract was 65.88% inhibition of oedema at 600 mg/kg dose. Whereas, the maximum effect displayed by dexa-methasone was 75.74% inhibition of oedema at 0.5 mg/kg dose.

DISCUSSION

Antipyretic activity

Fever basically is an elevated body temperature. The hypothalamus plays a vital role in regulating the body temperature since it controls the set-point at which the body temperature is maintained. This pre-set temperature is achieved through a fine balance of generation and loss of heat. In fever, basically this set-point is elevated (15).

It is well-known that exogenous as well as a number of endogenous pyrogens including tumour necrosis factor-α (TNF-α), prostaglandins (PGs), interleukins (IL-1β, IL-6, IL-8) and macrophage protein-1 triggers the development of fever. Furthermore, prostaglandin synthesis is also stimulated by TNF-α and phospholipase A_2. In yeast model of pyrexia, brewer's yeast induces both TNF-α and PG synthesis. The role of prostaglandin E_2 as the final mediator of pyrexia at hypothalamic level (preoptic area of anterior hypothalamus) is currently a well-established concept. Antipyretic drugs like paracetamol as well as other nonsteroidal anti-inflammatory drugs (NSAIDs) decrease the elevated body temperature by inhibiting interleukin-1β production peripherally as well as by blocking the formation of PGE_2 at the central level through inhibition of the enzyme, cycloxygenase, hence lowering the body's thermoregulatory set-point (16).

From the results of our experiment, it is assumed that one of the possible antipyretic mechanism of *G royleana* extract could be through the inhibition of PGs. This conclusion is also strengthened by the profound analgesic as well as anti-inflammatory activities exhibited by the extract, where also, inhibition of PGs is the key mechanism.

Previously reported phytochemical studies of *G royleana* extract has revealed the plant to possess flavonoids and others (2). Since flavonoids are known to inhibit phospholipase A_2 and cyclooxygenase enzymes resulting in blockade of PGs formation (17), the antipyretic activity of the plant might be partly due to this class of phytochemicals.

Anti-inflammatory activity

Carrageenan-induced paw oedema model is an extensively employed protocol for evaluating the anti-inflammatory properties of crude extracts as well as purified compounds and is additionally employed as a routine and simple animal model for the assessment of pain at inflammation sites while causing least damage or injury to the inflamed paw (18).

It is believed that carrageenan-induced paw oedema involves two phases; the early and the late phases. The early phase is commonly observed during the initial 1.5–3 hours, which is not suppressed by anti-inflammatory drugs like aspirin or diclofenac and involves the liberation of histamine, serotonin (5-HT) and bradykinin, whilst the late phase (4.5–6 hours) is characterized by the infiltration of polymorphonuclear (PMN) leucocytes as well as the persistent generation of prostaglandins (13). Moreover, the liberation of reactive oxygen species (ROS) and other free radicals from neutrophils, pro-inflammatory cytokines such as tumour necrosis factor (TNF)-α and interleukin-1β (IL-1) as well as nitric oxide (NO) also accounts for the delayed or late phase of carrageenan-induced inflammation (18–20). The late phase is inhibited by NSAIDs like aspirin.

Xylene-induced ear oedema is also a widely used model of acute inflammation. In this test the application of xylene to the lower surface of the ear is believed to provoke neurogenic oedema mediated through the release of substance P, a peptide related to the peripheral as well as central nervous system. The peripheral release of substance P and other neuropeptides from sensory neurons then act on target cells, including mast cells, leukocytes as well as endothelial

cell causing the release histamine, serotonin, prostaglandins, throm-boxanes, leukotrienes, cytokines and NO together with vasodilatation and extravasation of plasma, leading to the development of neurogenic inflammation and subsequent oedema formation of the ear (20–22).

From our results it is evident that the crude methanolic extract of *G royleana* possesses considerable anti-inflammatory activity and inhibits the early as well as the late phase of inflammation and also suppresses neurogenous inflammation, thus reflecting its inhibitory actions on multiple inflammatory and pro-inflammatory mediators.

Several phytochemicals have exhibited anti-inflammatory activity in a variety of experimental models, the most prominent of which are phenolics, terpenoids and alkaloids (17).

Since these phytochemicals have also been detected in *G royleana* in significant quantity, the anti-inflammatory activity of the crude methanol extract could be accredited to these phytochemicals (2).

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Author's contributions:
HK and IK designed the experiments, HK, AA, and AW performed the experiments, NK and AS executed statistical analysis of data whereas, HJ and IR wrote the paper.

AUTHOR'S NOTE

Authors declare that there is no conflict of interest.

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