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COMPARATIVE ANALYSIS OF VITAMIN-E, LAWSONIA INERMIS & CAMELLIA SINENSIS IN GASTRIC ULCER MODEL

NOOR JAHAN*[1], FARAH ASLAM[1], SONIA KHAN[2], ALMAS NAEEM[1], USAID KHALID[1], MEHJABEEN[3], NOREEN TARIQ[1]

1: Department of Pharmacology, Dow University of Health Sciences, Karachi, Pakistan.

2: Department of Pharmacology, United Medical and Dental College, Karachi, Pakistan

3: Department of Pharmacology, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan

***Correspondence to: Noor Jahan, E-mail address: - noor.jahan@duhs.edu.pk**

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ABSTRACT

Gastric ulcer is growing as a major health problem associated with comorbid conditions like diabetes, arthritis, and NSAIDS ingestion which causes decrease in prostaglandin production, produces oxidative free radicals and inflammation. The medicinally important plants *Lawsonia inermis* & *Camellia sinensis* and Vitamin-E (Tocopherol) have shown some gastroprotective effects. The aim of the present study was to evaluate the comparative effect of three drugs on indomethacin induced gastric mucosal injury in rats. In this experimental study adult albino wistar rats were divided in to six groups A, B, C, D, E & F each group consist of five animals (n=5). Group A (Control group), group B (negative control), group C was given vitamin E (400mg/kg orally), group D was given *L.inermis* seeds extract (500mg/kg orally), group E was given *C. sinensis* leaves extract (500mg/kg orally) and group F as positive control. The treatment protocol was followed by 7 days. From day 5 to 7of protocol Indomethacin was administered 30mg/kg p.o to groups (B, C, D, E & F) of rats to induce the ulceration. Animals were sacrificed and their gastric mucosal changes regarding colour, mucus content and site of ulcers were examined under magnifying lens and histological parameters were observed. Findings suggest that group received Vitamin-E (Group C), *L. inermis* seeds extract (L.S.E) (Group D) &*C. sinensis* leaves extract (C.L.E) (Group E) showed significant protection against gastric ulcer induced by indomethacin in rat model. They have antioxidant & anti-inflammatory effects & can be utilized in near future to provide gastroprotection.

Keywords: *Camellia sinensis*, Gastric ulcer, Indomethacin, *Lawsonia inermis*, Vitamin-E

1. INTRODUCTION

Peptic ulcer disease is still a common illness affecting a considerable number of people in the world. Gastric ulcers are known as erosions of the gastric mucosa that may penetrate and perforate the stomach wall [1]. The development of peptic ulcer is influenced by various aggressive and defensive factors, inadequate dietary habits, cigarette smoking, stress, hereditary predisposition, *Helicobacter pylori* infection , free radicals formation , and decreased prostaglandin synthesis [2]. Moreover, peptic ulcers related to the comorbid conditions, such as diabetic state, arthritis or excessive ingestion of nonsteroidal anti-inflammatory agents, are more severe and are often associated with complications [3]. Although no single mechanism has been elucidated, increased gastric motility, vagal hyperactivity, mast cell degranulation, decreased gastric blood flow and decreased prostaglandin levels have all been associated with gastric ulceration. However, other factors including reactive oxygen species (ROS), inflammatory cytokines and biogenic amines have also been implicated [4]. Non-steroidal Anti-Inflammatory Drugs (NSAIDs) are the most commonly prescribed drugs because of their well-established efficacy in the treatment of pain, fever, inflammation and rheumatic disorders [5]. NSAIDs causes the depletion of PGs, it has proven to be more complicated and involves multiple mechanisms, closely interacting elements such as gastric hypermotility, microcirculatory disturbances, neutrophil-endothelial cell interactions and superoxide radicals generation, in addition to PG deficiency [6]. The high cost of modern antiulcer medication for the population, their unavailability in some rural areas, the multiple side effects that result from their prolonged uses makes this appropriate that research on developing plant-derived antiulcer medication as an alternative strategy for the management of gastroduodenal ulcers were highly recommended [7]. Two medicinally important plants *Lawsonia inermis* & *Camellia sinensis* has been shown to have anti-oxidant and anti-inflammatory actions as well as Vitamin-E (Tocopherol) is a popular and powerful lipid soluble antioxidant that increases secretion of prostaglandins [8]. Vitamin E activates enzyme phospholipase A2 and increases the synthesis and secretion of prostaglandins [9]. These actions are expected to limit gastric mucosal damage. Tocopherol, *L. inermis* seeds &*C. sinensis* leaves exhibit their effects in ameliorating oxidative stress and inflammation, rendering them attractive agents for the prevention of gastric ulcer.

Vitamin E effects on various disorders have been extensively studied either in humans or experimental animals. It was shown to be protective against many diseases like cardiovascular [10], cerebrovascular [11], liver disease [12], cancer [13], and diabetes [14]. Both α-tocopherol and tocotrienol, two chemical compounds with a similar structure of Vitamin-E reduced gastric lesion index in experimental animals that were exposed to ulcerogens [15, 16]. Alpha-tocopherol administration caused a marked reduction in mucosal damage and ulcer index with the increased adherent mucin content, suggesting the gastroprotective role of the drug [17]. Vitamin E supplementations (PVE or α-TF) at the dose of 60 mg/kg body weight for 28 days was able to reduce the formation of gastric lesions significantly compared to the stress control group by reducing the plasma ACTH and corticosterone levels [18]. Vitamin E has a protective effect on gastric mucosal injury by increasing mucus secretion and by increasing secretion of prostaglandins [19].

L.inermis is an important medicinal plant, native of North Africa and south-west Asia commonly known as Heena. Henna has been reported to have many different healing effects, antibacterial effects specially for gram positive bacteria, anti-tumoral effects in rat, antifungal activity against dermatophytes and wound healing [20]. Research studies have demonstrated that the ethanolic, aqueous and chloroform extract of *L.inermis* leaves in pylorus ligatated and NSAIDs induced ulceration in the rats possess significant antiulcer properties by decreasing the gastric volume, total acidity, free acidity, and ulcer index in a dose dependent manner [21]. The extracts of *L. inermis* mainly contain active constituents including carbohydrates, tannins, gums, glycosides, phenolic compounds and sterols which were mainly responsible for gastroprotective potential of *L. inermis* by significant antioxidant property. The ethanolic extract of heena seeds were also found to have an antioxidant property using ABTS, DPPH & FRAP assays [22].

C.sinensis a tea plant considered as a potent medicine for the maintenance of health since ancient times, commonly known as green tea. Green tea that are made from their dried fresh leaves have been shown to exhibit multiple health benefits [23]. GT polyphenols, especially catechins, are well known antioxidative agents that have the potential to prevent GI diseases [24]. GT has been shown to reduce inflammation associated with Crohn's disease and ulcerative colitis, a type of inflammatory Bowl disease (IBD) [25]. GT is effective in reducing cholesterol and lipid absorption in the GI tract [26]. It also appears to protect liver from damaging effects of toxic

substances and is also effective against the development of liver tumors [24]. Recently it is observed that GT consumption enhanced cellular energy metabolism and antioxidant defense mechanism in the liver, kidney, and small intestine [27] and prevented Gentamicin and Cisplatin-induced nephrotoxicity and oxidative damage in the kidney ofrats [28].

There are many models that can be used to explore the protective effects of medicinal compounds on gastric lesion development. The commonly used models are nonsteroidal anti-inflammatory drugs (NSAIDs)-induced [29], ethanol-induced [15], stress induced [18], Helicobacter pylori-induced [30], acid-induced [31], and pyloric ligation induced [32]. These models induce gastric ulcer via different mechanisms. The current study is conducted to evaluate the gastroprotective effects of the Vitamin E, *L.inermis* & *C.sinensis* against indomethacin-induced gastric ulcers in rat model, with theultimate aim of providing information regarding the putative antiulcer properties of these drugs.

2. MATERIALS AND METHOD

2.1. Extract Preparation: Freshly collected 500 gm. leaves of *C. sinensis* & seeds of *L. inermis* were dried and ground and then soaked in 2000ml ethanol for 15 days with daily stirring. After 15 days both the extracts i.e. *Lawsonia inermis* seeds extract (*L*.S.E.) & *Camellia sinensis* leaves extract (*C*.L.E.) were filtered and dried on rotary evaporator. The thick crude extracts were obtained that was used for further studies.

2.2. Drugs and chemicals: Indomethacin, Vitamin E, Omeprazole was purchased from a Pharmacy in Karachi, Pakistan. *L. inermis* (seeds) & *C. sinensis* (leaves) were freshly collected.

2.3. Experimental Animals: Albino wistar male rats of comparable weight (150 ± 25 g) were obtained from the Animal House of Dow University of Health & Sciences. The animals were maintained at 25 ± 2 ºC and 50-55% relative humidity with 12h light and dark cycles. The animals were provided with standard animal feed and water ad libitum. The animals were transported to the experiment site at pharmacology laboratory of Dow College of Pharmacy. The animals were acclimatized to the laboratory conditions prior to experimentation

2.4. Experimental Grouping: A total of 30 wistar rats were used in the study and randomly divided into six groups (A, B, C, D, E & F). Each group consisted of five animals (n = 5); group A served as the normal control, B as the negative control, C, D & E as the treated group and F as the positive control.

Group A=Normal control (n = 5): They received normal saline (1 ml/kg) orally.

Group B=Indomethacin (n = 5): They received oral dose of indomethacin (30mg/kg) for 3 days.

Group C=Vitamin-E + Indomethacin (n = 5): They received oral vitamin E (400mg/kg) once daily.

Group D= *L*.S.E. + Indomethacin (n = 5): They received oral *L*.S.E. (500mg/kg) once daily.

Group E=*C*.L.E. + Indomethacin (n = 5): They received oral *C*.L.E. (500mg/kg) once daily.

Group F=Omeprazole + Indomethacin (n = 5): They received oral omeprazole (20mg/kg) once daily.

2.5. Ulcer Induction: The protocol was followed by 7 days. Ulcer was induced by indomethacin (30mg/kg/ bwt) oral administration in groups B, C, D, E & F of rats. Indomethacin was administered for last 3 days of the protocol. Gastric ulceration was induced by 24 hours later of the last dose of indomethacin. Rats were fasted for 24 h prior to the sacrificing but allowed free access to water, except for the last hour prior to the sample collection.

2.7. Sample Collection: At the end of the treatments, the rats in each group were sacrificed under mild ether anesthesia and the stomach was isolated and washed in normal saline. Each stomach was studied for ulceration and mucosal damage using magnifying lens.

2.8. Sample Analysis: The stomach was opened along the greater curvature and rinsed with saline water. Gastric mucosa was scraped for evaluation of gastric injury. Gross gastric mucosal lesions were examined using a magnified lens. Gastric lesions severity was measured as described by Wilhelmi and Menasse-Gdynia [33] using 1 to 5 scoring system (severity factor 1 = 1 or 2 minutes, sporadic, punctuate lesion; 2 = several small lesions; 3 = one extensive lesion or multiple moderate sized lesions; 4= several large lesions; 5 = several large lesions with stomach perforation). The Ulcer index for each group was taken as the mean lesion score of all the rats in that group [34]. The percentage ulcer inhibition (% UI) was calculated by the equation of Hano et al. [35].

%UI = [(UI of ulcer control − UI of treated) / (UI of ulcer control)] × 100%.

After ulcer scoring stomach of rats were collected from each group and placed in 10% formalin for accessing histological parameters.

2.9. Data Analysis: Data was analyzed by one-way analysis of variance (ANOVA) which was performed using SPSS version 21 software. The post hoc test was followed by the Tukey and the significance level was set at $p< 0.05$.

3. RESULT & DISCUSSION

3.1. Gross observations of stomach: In control group A the colour of the gastric mucosa was greyish pink which is normal. But in group B which is indomethacin treated positive control had reddish yellow

mucosa, group C, D & E which received pretreatment of Vitamin-E, *L*.S.E. & *C*.L.E. respectively, shows mucosal colour ranging from dull pink to pink. Vitamin-E treated group showed no epithelial erosions with mild inflammation whereas in *L*.S.E. & *C*.L.S. treated groups have mild epithelial erosion, no hemorrhagic spots only mild inflammation was observed. Omeprazole pretreated standard group showed almost equal result as the control group A. Hemorrhagic spots were also visualize in group B& some in group E in which group B show mild gastric mucosal lesions. Pyloric part of the stomach of rats show change in colour and mucosal injury. Swelling of stomach especially at fundus and body part is significantly present in indomethacin treated group as compare to treated group (Fig. 1-2).

3.2. Severity of gastric lesions: Ulcer index was calculated which show significant difference between the control group A, negative control group B, treated group C, D & E and positive control group F. The highest ulcer index in group B is 4.2 ±0.83. Pretreatment with Vitamin-E, *L*.S.E.& *C*.L.E. resulted in1.8, 2.2 & 2.8 ulcer index in group C, D& E respectively (Table 1; Figure 3-4).

3.3. Histopathological parameters of gastric cells: Mucus was present in the glandular cells of the group A, C, D,E & F and was completely absent in indomethacin treated group B(Fig.5-9), whereas mucus was shown to be present in 2 specimen of group C which was pretreated with Vitamin-E and 4 specimen of group D which was *L*.S.E. pretreated & 3 specimen of group E which was pretreated with *C*.L.E.

Figure 1: Gross features of normal control group

Figure 2: Gross features of indomethacin treated group showing hemorrhagic spots

Table 1: Effect of Vitamin-E, L.S.E. & C.L.E. on ulcer parameters in rats

Groups	Drugs	Ulcer Index	% Gastric Mucosal Injury	% Ulcer Inhibition
A	Normal Saline	$0.03^{a} \pm 0.02$	0.80	99.2
B	Indomethacin	$4.2^{b} \pm 0.83$	100	0.0
C	Vitamin-E + Indomethacin	$1.8^{c} \pm 0.44$	42.9	57.1
D	*L. inermis*.+ Indomethacin	$2.2^{c} \pm 0.83$	52.3	47.7
E	*C. sinesis* + Indomethacin	$2.8^{c} \pm 0.83$	66.7	33.3
F	Omeparazole+ Indomethacin	$0.6^{a} \pm 0.54$	14.3	85.7

Data expressed in the form of mean ± SEM, values with different superscripts were have significant difference ($p<0.05$) n= 5;a=Normal Control and Positive control; b=Negative Control;c=Treated Groups

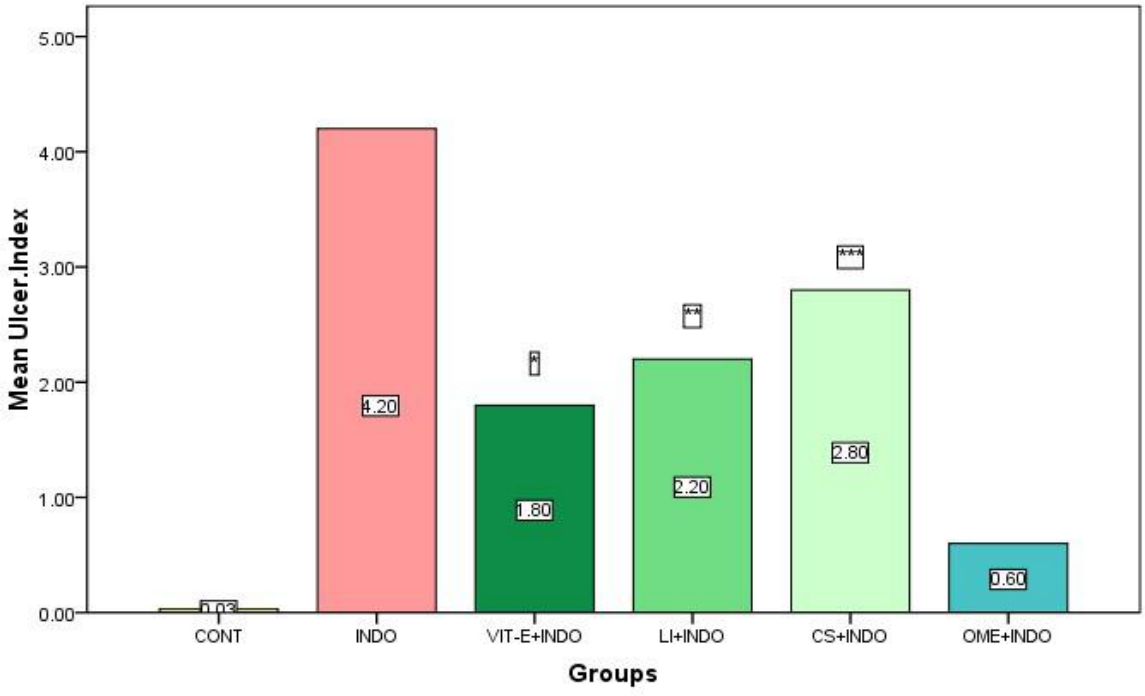

Figure 3: Ulcer Index compared between control & treated. n= 5; denotes significant difference ($p<0.05$)

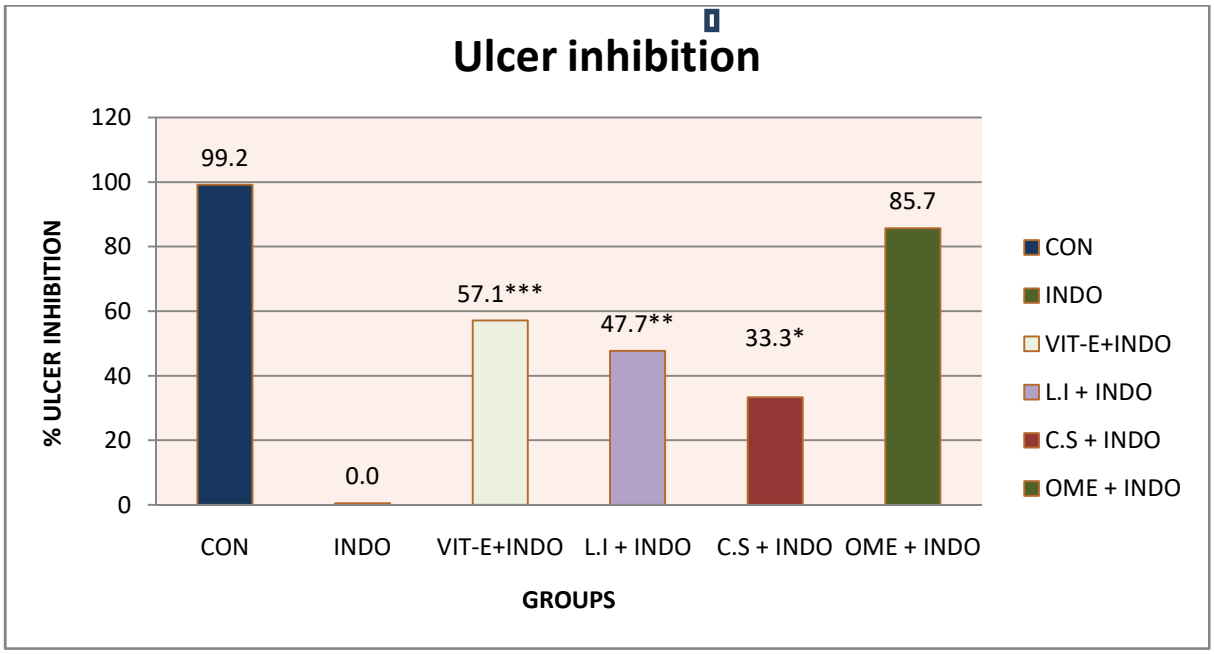

Figure 4: Percentage of Ulcer Inhibition in rats. n= 5;* denotes significant difference (p<0.05)

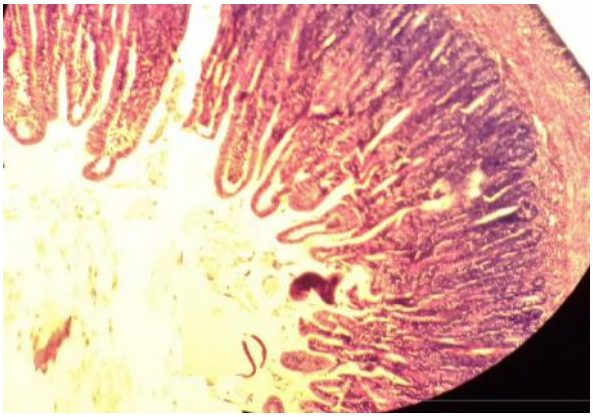

Figure 5: Histopathology (4X) of Indomethacin treatedgroup showing epithelial damage

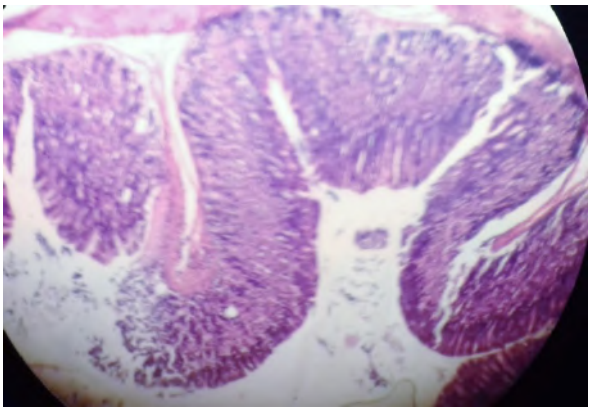

Figure 6: Histopathology (4X) of Vit-E treated group

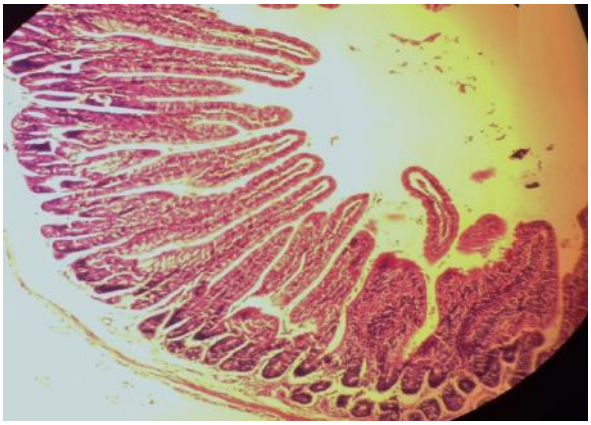

Figure 7: Histopathology (4X) of L. inermis treated group

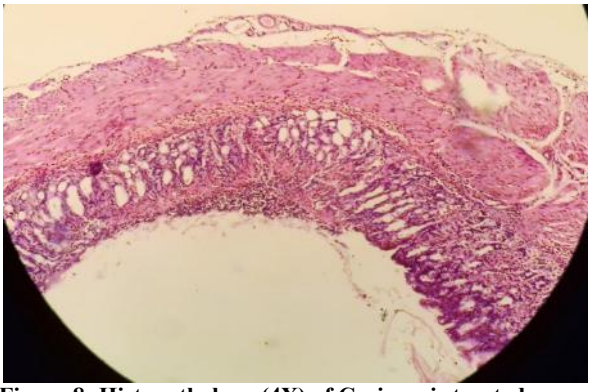

Figure 8: Histopathology (4X) of C. sinensis treated group

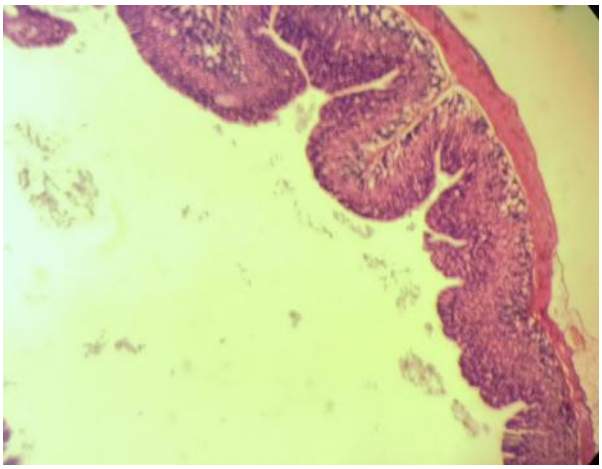

Figure 9: Histopathology (4X) of Omeprazole treated group with normal epithelial cells

4. CONCLUSION

The results concluded from the study that indomethacin induces marked gastric mucosal damage with highest ulcer index, whereas Vitamin-E, L. inermis & C. sinensis shown protective effect on gastric ulcer that may due to their antioxidant & anti-inflammatory properties. L. inermis, is a historical plant commonly known as Henna, apart from its role in cosmetics, shows evident gastroprotective effect in the comparative analysis. Lawsonia inermis, Camellia sinensis & Vitamin-E may be valuable in near future for patients

suffering from the peptic ulcer. Further experimental and clinical studies are needed for confirmation of mechanism.

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