JMSCR Vol||05||Issue||03||Page 19680-19688||March 2017

www.jmscr.igmpublication.org
Impact Factor 5.84
Index Copernicus Value: 83.27
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v5i3.213

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Original Article

Effect of Ethanol Extract of Curcuma Longa on Aspirin Induced Gastric Injury
(Gross Morphology and Histopathology Study)

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Abstract
Objective: *To study the Ulcer Index, gross morphology and histopathological pictures of effect of Ethanol extract of curcuma longa (CLE) on Aspirin induced gastric injuries.*
Method: *After animal ethics committee approval, the effect of CLE was studied on ten groups of guinea pigs.*
Group I & 2 were given 1% carboxy methyl cellulose (CMC) & Aspirin 500 mg/kg in 1% CMC respectively. Group 3, 4,5,&,6 were given CLE 20,40, 80 &160mg/kg respectively in 1% CMC followed one hour after by Aspirin. Group 7,8 &9 were given CLE 20, 40 & 80mg/kg respectively. Group-10 received Ranitin 30mg/kg followed one hour after by Aspirin. Ulcer-Index was calculated and analysed statistically and the histopathology study of gastric mucosa was undertaken.
Results: *CLE up to 80mg/kg did not produce any gastric injury. Pretreatment with CLE 80mg/kg & 160mg/kg produced highly significant protection [P<0.001] against Aspirin induced gastric injuries & hemorrhages.*
Conclusion: *Ethanol extract of curcuma longa prevents Aspirin Induced gastric injury.*
Keywords: *Gastric ulcer, Curcuma-longa, Anti-inflammatory, Histopathology.*

INTRODUCTION

Most of the non steroidal anti-inflammatory drugs (NSAIDS) - i.e., aspirin, Diclofenac & Ibuprofen etc, which are the mainstay of therapy for pain and inflammatory conditions are associated with gastric mucosal injuries. The use of selective COX-2 inhibitors, which has less GI Adverse effects is again associated with recent reports of myocardial infarction[1]. But Curcuma longa (Turmeric) though possess significant anti-inflammatory activity [2-8], at the same time also have number of reports suggesting gastro protective effects[9-12]. However, there are conflicting reports regarding the effect of curcuma

longa on gastric mucosa (WHO manographs on medical plants 1999)[2]. Though number of reports suggest gastro protective activities with curcuma longa [9-12], still the following studies have reported ulcerogenic potential with Curcumin { i.e., both intraperitoneal and oral administration of curcumin (100 mg/kg) have reported to induce gastric ulcerations in rats [13,14] & there are conflicting reports regarding the protective action of Curcumin against histamin induced gastric ulceration in guinea pigs. These contradictory reports have possibly prevented any large clinical studies on Curcuma longa extract.

So, the present work was undertaken to re-evaluate the effect of curcuma Longa on NSAID (Aspirin) induced gastric injuries. The test drug preferred is Alcoholic extract of curcuma Longa (CLE) which has most possible Gastro protective actions as evidenced from previous studies with alcoholic extract [8,12]. Because most of the studies with alcoholic extract has demonstrated protective actions in rat models and there are conflicting reports regarding the protective action of curcumin against histamin-induced gastric ulceration in guinea pigs, the present study has included guinea pigs as the experimental animal to see the effects in different species. The dose of CLE preferred is 20 mg/kg to 160 mg/kg, as this is the minimum range in which alcoholic extract of curcuma longa is effective in inhibiting the proliferative phase of inflammation as evidenced by reduction of the weights of the granuloma pouch and inhibition of granuloma tissue formation in cotton pellet tests (the two methods involving proliferative phase of inflammation)[5] . So that we can find if CLE in the dose which have effect in chronic inflammation also have protect-tive effect against Aspirin induced gastric injuries. As analysis of gastric mucosal layer & total acid contents of gastric mucosa were repeatedly done earlier with Curcuma longa and none of the previous research work have demonstrated the histopathological picture of the effect of curcuma longa on NSAIDs induced gastric injuries, so the present study has undertaken both the histopathology and gross morphology study to have a convincing evidence of effect of curcuma longa on NSAIDs induced gastric injury.

MATERIALS & METHODS

The Research project was undertaken with approval of the Institutional Animal Ethics. Committee of S.C.B. Medical College, Cuttack.

Plant Material and Extract Preparation: The Rhizomes of curcuma Longa, procured from the local market were sundried and made into powders which were subjected to extraction in 90% ethanol by the method of soxhlation (Simultaneous heat evaporation and condensation method)

Animals: The experiments were carried out with sixty numbers of inbred Guinea pigs of either sex weighing between 350 to 700 gms. and maintained in the Animal house of dept. of Pharmacology, S.C.B. Medical College, Cuttack. The guinea pigs were fed standard laboratory diet.

Experimental Gastric Lesions: The gastric injuries were induced by Aspirin suspended in 1% carboxymethyl cellulose (CMC) in water in the concentration of (50mg/ml.) and administered intra gastric in a dose of 500mg/kg, in 36 hours fasted guinea pigs [16].

Experiment Proper: The Guinea pigs were divided into 10 groups of six each. They were kept fasting for 36 hrs, with water ad-libitum before the administration of drugs. In Group - I (normal control group) 4 guinea pigs received 1% carboxy methyl cellulose (CMC) in Distilled Water and 2 guinea pigs is received only water to expose the normal gastric mucosa. Group 2 received Aspirin for experimental gastric lesions. Group - 3, 4, 5 & 6 received ethanol extract of curcuma longa in 1% carboxy methyl cellulose (CLE) in dose of 20, 40, 80 mg and 160 mg/k respectively followed 1 hour after by Aspirin. Group 7, 8 & 9 received CLE alone in dose of 20, 40 & 80 mg/kg respectively. Group 10- received Ranitidine 30mg/kg followed 1 hour after by Aspirin. In the animals in whom aspirin was given it was given as described for experimental gastric

lesions and CLE (ethanol extract of curcuma longa) given in 1% CMC suspension. All the drugs were administered to guinea pigs intra gastric through gavage. After 5 hours of administration of the last drug the guinea pigs were subjected to Euthanasia by high dose Ether Anesthesia, then the stomach was removed in all the animals and exposed by cutting along the greater curvature and examined for gastric injuries. The Ulcer index was calculated and then the tissues were submitted to the pathologists for histopathology study.

The Ulcer Index was calculated by - (Ref.16)

$$\text{Ulcer Index} = \text{where X} = \frac{10}{x} \quad \frac{\text{Total Gastric mucosal area in mm}^2}{\text{Total ulcerated area in mm}^2}$$

in case of **Petechiae** - five of these are considered to be equivalent to 1 mm of Ulcer area.

STATISTICAL ANALYSIS

The mean Ulcer Index and standard deviation of mean in each group of guinea pigs was found out. The Ulcer Index of (CLE + Asp.) treatment groups were compared with Aspirin alone treatment group by analysis of variance (ANOVA)

OBSERVATION

The gross morphology and Histopathology study of normal guinea pig stomach demonstrates uniform gastric mucosa (Photo No.1A & 1B). 1% CMC did not produce any gastric mucosal injuries. Administration of Aspirin in 1% CMC at the dose of 500 mg/kg resulted in gastric mucosal injuries in almost all the animals as evidenced from gross morphology study (Photo No. 2A), the subsequent histopathology study of full cross section of stomach under (5 x 10) magnification demonstrated denudation of gastric mucosal epithelium and deep erosion and further magnification (45x10) showed areas of frank hemorrhages in gastric mucosa (Photo No. 2B & 2C). The details of Ulcer Index with different treatment groups are mentioned in table -1. Pretreatment with CLE 20 mg/kg could not protect the gastric mucosa from Aspirin induced injuries & shows both erosions and hemorrhages of gastric mucosa, (Photo 3A & 3B). CLE 40mg/Kg prior to Aspirin was associated with diminution in Ulcer index which was statistically significant ($p<0.05$) but still there were punctate erosions in gross morphology & Histopathology study (Photo 4A, 4B). Pretreatment of Gastric mucosa with CLE 80mg/kg or 160mg/kg respectively were associated with highly significant protection against Aspirin induced gastric injuries as there were no visible injuries of gastric mucosa(Photo 5A & 6A) with diminution of ulcer index ($p<0.01$ & 0.001) respectively. The histopathology study of guinea pig gastric mucosa who were treated with CLE 80 mg/kg prior to Aspirin showed only hyperemia of gastric mucosa and mild superficial erosion indicating mucosal irritation but no haemorrhage or deep erosion (Photo No.5B). CLE 160 mg /kg treated group prior to Aspirin showed only tiny superficial erosion of surface epithelium in histopathology picture but there was no haemorrhage or hyperemia or deep erosions indicating highly significant protection of gastric mucosa against Aspirin induced gastric erosions and hemorrhages (Photo 6B&6C). The Ulcer Index of CLE 160mg/Kg+Asp treatment group was significantly lower than Ulcer Index of (Ranitin + Aspirin) treatment group ($p<0.05$). Administration of CLE alone in dose of 20mg, 40mg, & 80mg/kg in 1% CMC suspension was not associated with any gastric mucosal injury, rather CLE 80mg /Kg had shown intact gastric epithelial cell layer with increased gastric mucus layer, which has also adhered to the epithelial cell layer (Photo7A&7B).

Table -1

Table- 1 shows ULCER INDEX (U.I.) OF - ASPIRIN, CLE + ASP & RANITIN + ASP.
U.I. OF ASP Compared with U.I. of CLE + ASP. &
U.I. of CLE 160 mg/kg + ASP. Compared with U.I. of Ranitin + ASP

Sl. No.	ASP	CLE 20mg/kg. + ASP	CLE 40mg/kg. +ASP	CLE 80mg/kg +ASP	CLE 160mg/kg. +ASP	RANITIN 30mg/ kg+ASP.
1.	0.9	0.72	0.44	0.20	0.13	0.20
2.	0.81	0.51	0.50	0.13	0.07	0.10
3.	0.7	0.8	0.63	0.11	0.00	0.74
4.	1.4	1.2	0.25	0.39	0.04	0.13
5.	0.63	0.68	0.30	0.12	0.09	0.22
6.	1.8	0.90	0.72	0.4	0.02	0.19
MEAN	1.040	0.802	0.473	0.225	0.058	0.263
±SD	0.46	0.234	0.182	0.135	0.048	0.238

ANOVA TABLE

SOURCE OF VARIATION	SS	df	MS	F	P	SIG
BETWEEN GROUPS	4.241	5	0.848196	13.452	< 0.01	**
WITHIN GROUPS	1.892	30	0.063053			
TOTAL	6.132564	35				

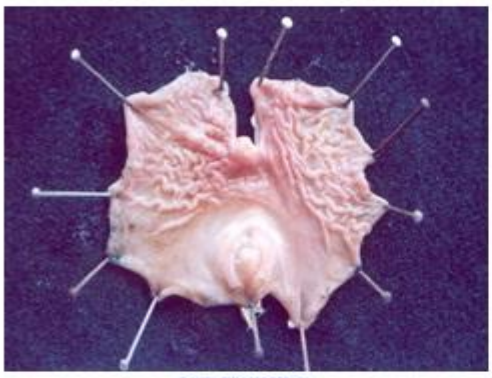
POST HOC TEST (LSD METHOD)

>0.05	<0.05 *	<0.001 **	<0.001 ***	<0.01 **	
				<0.052*	

The number of animals in each group (n) = 6
* - indicates label of statistical significance

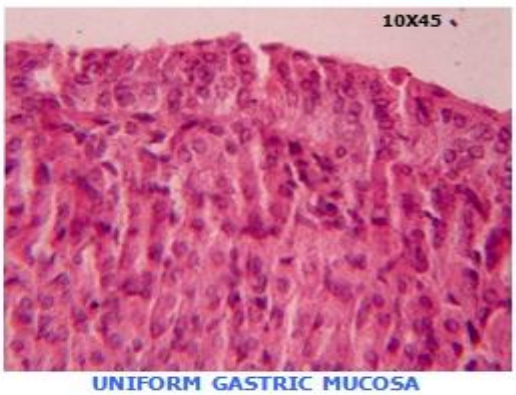
NORMAL GASTRIC MUCOSA

PHOTO NO.1-A
GROSS MORPHOLOGY



NO INJURY

PHOTO NO.1-B
HISTO PATHOLOGY

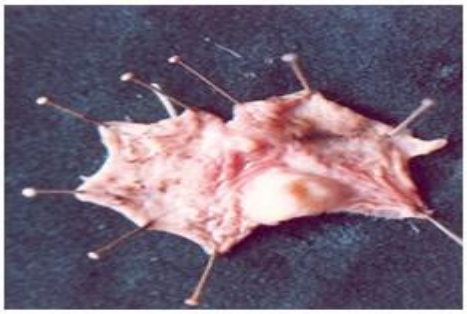


UNIFORM GASTRIC MUCOSA

ASPIRIN 500mg./Kg.

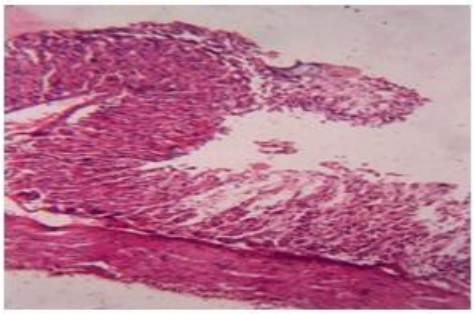
GASTRIC MUCOSAL INJURIES

PHOTO NO.2-A
GROSS MORPHOLOGY



ULCERATION

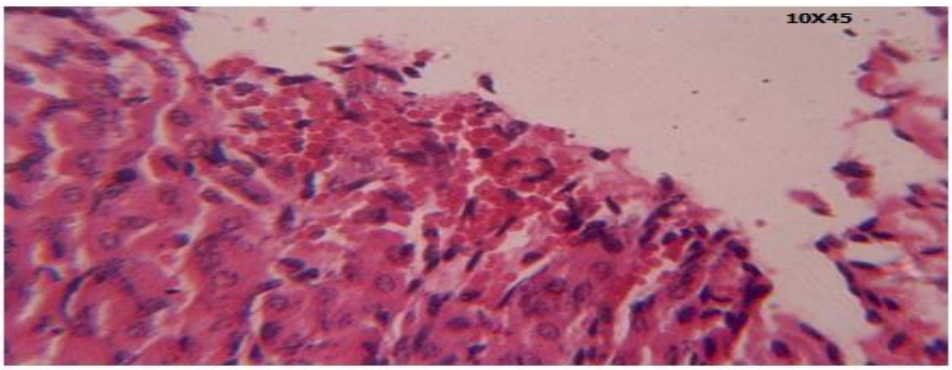
PHOTO NO.2-B
HISTO PATHOLOGY



DEEP EROSION

ASPIRIN 500 mg./Kg.

PHOTO NO.2-C
GASTRIC MUCOSAL HAEMORRHAGE



CLE 20 mg./Kg. + ASP.

PHOTO NO.3-A
GROSS MORPHOLOGY

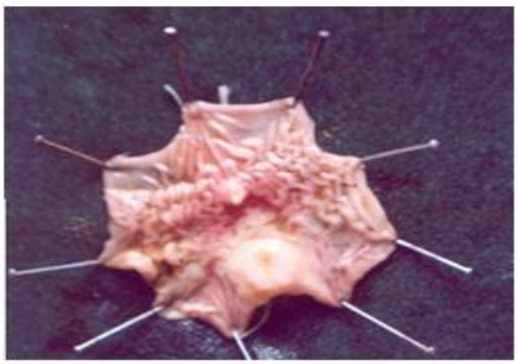
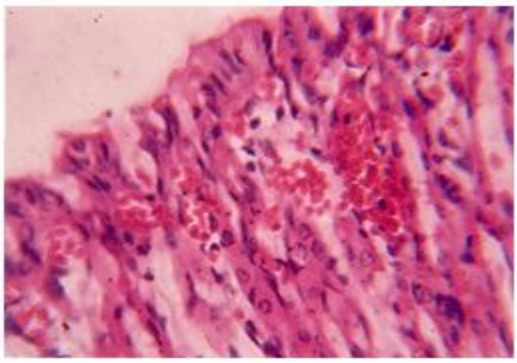


PHOTO NO.3-B
HISTO PATHOLOGY



GASTRIC MUCOSAL HAEMORRHAGE
AND DEEP EROSIONS

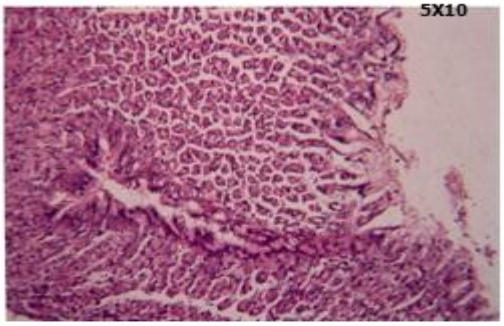
CLE 40mg./Kg. + ASP

PHOTO NO.4-A
GROSS MORPHOLOGY

ONLY PUNCTATE HAEMORRHAGE

NO FRANK HAEMORRHAGE

PHOTO NO.4-b
HISTO PATHOLOGY



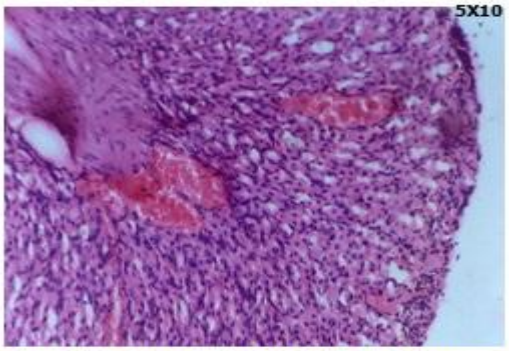
SUPERFICIAL EROSION

CLE 80mg./Kg. + ASP.

PHOTO NO.5-A
GROSS MORPHOLOGY

NO VISIBLE INJURIES

PHOTO NO.5-B
HISTO PATHOLOGY



ONLY HYPEREMIA + SUPERFICIAL EROSIONS

NO FRANK HAEMORRHAGE OR DEEP EROSIONS

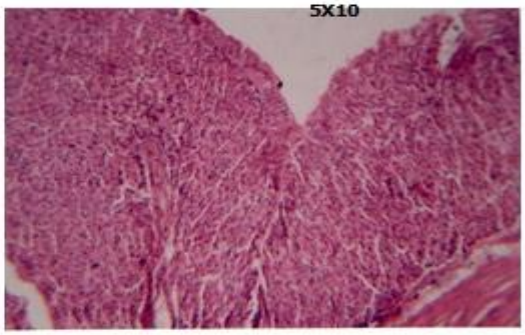
GASTRIC MUCOSAL PROTECTION

CLE 160 mg./Kg. + ASP.

PHOTO NO.6-A
GROSS MORPHOLOGY

NO GASTRIC MUCOSAL INJURY

PHOTO NO.6-B
HISTO PATHOLOGY



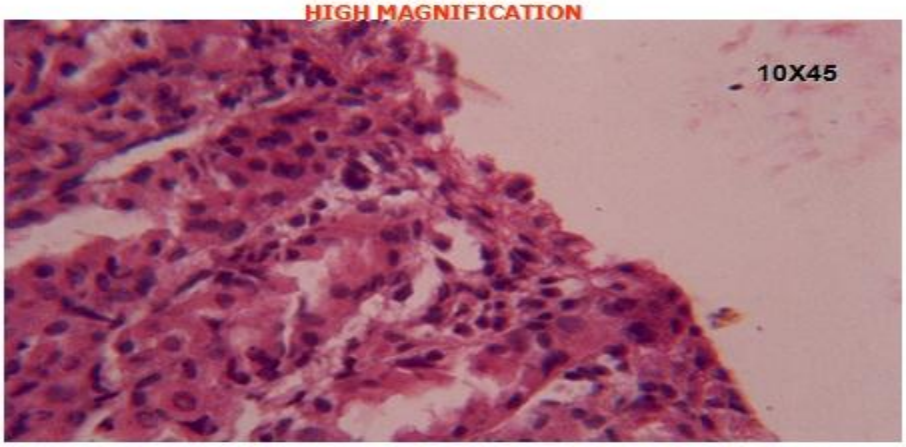
NO HYPEREMIA + NO EROSION

CLE 160mg. / Kg. + ASP

GASTRIC MUCOSAL PROTECTION

PHOTO NO.6-C

HIGH MAGNIFICATION



MINIMAL SUPERFICIAL EROSION OF SURFACE EPITHELIUM
BUT
NO HAEMORRHAGE & NO MUCOSAL CONGESTION

GASTRIC MUCOSAL PROTECTION

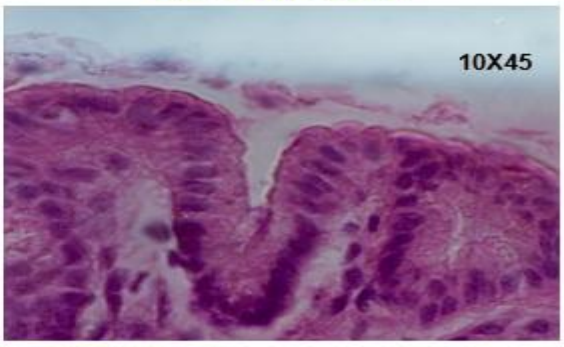
CLE 80 mg./Kg.

PHOTO NO.7-A

GROSS MORPHOLOGY

PHOTO NO.7-B

HISTO PATHOLOGY



NO GASTRIC MUCOSAL INJURY
INCREASED MUCOSAL LAYER

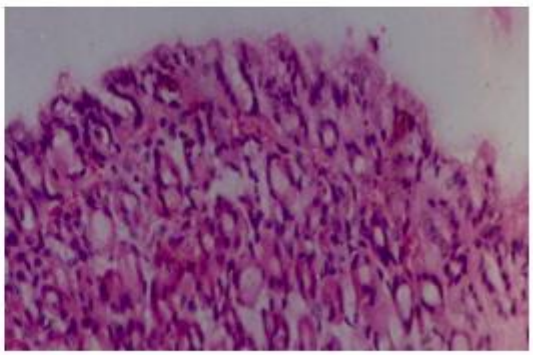
RANITIN 30 mg./Kg + ASP

PHOTO NO.8-A

GROSS MORPHOLOGY

PHOTO NO.8-B

HISTO PATHOLOGY



MILD EROSIONS
GASTRIC MUCOSAL PROTECTION

‘

DISCUSSION

As evidenced from the present study - Ethanol Extract of curcuma longa up to 80 mg/kg in 1% CMC suspension did not produce any significant gastric mucosal injury, rather there is increased gastric mucosal layer in histopathology study. This increase in gastric mucosal layer was already reported by various authors by chemical analysis [9,10,12]. It is now further substantiated by histopathology picture in this study & this mucosal layer has adhered to the surface epithelium like a protective covering (Photo 7A & 7B). Aspirin 500mg/kg in 1% CMC suspension - produced both superficial and deep gastric mucosal erosions and frank gastric mucosal hemorrhages). CLE 40mg/kg to 160mg/kg significantly reduced the Aspirin induced Gastric mucosal erosions established both by evaluation of Ulcer Index and Histopathology studies. Another important finding which came out of histopathology study in this experiment was significant reduction in gastric mucosal haemorrhage in guinea pigs who were treated with CLE 80mg/kg and 160mg/kg prior to Aspirin. This finding of prevention of Aspirin induced gastric mucosal haemorrhage by curcuma longa was not demonstrated in any previous studies but is of significant clinical importance as the dangerous presentation of NSAID induced gastric injury is hematemesis and melena. Previous studies with 100mg/kg of curcumin has demonstrated ulcerogenic potential[14], but as 1/5th of this dose i.e., (20 mg /kg of curcumin) possess significant anti-inflammatory activity[5] , so this does not prohibit to use curcumin as an anti-inflammatory agent. Again ethanolic extract of Curcuma longa even up to 160mg/kg do not have ulcerogenic potential rather prevents Aspirin induced Gastric injuries. Alcoholic extract also possess significant anti-inflammatory activity against granuloma pouch model in dose of 100mg/kg and against cotton pellet granuloma model in dose of 25-100 mg/kg, the two methods involved in proliferative phase(chronic phase) of inflammation [5]. A preliminary clinical study on 10 patients have reported ulcer healing potentials with curcuma longa[15].

So on the basis of the present study evaluating all the three parameters (Ulcer Index, Gross morphology and Histopathology) we can conclude that alcoholic extract of curcuma longa in the dose of 40 to 160mg/kg in 1% Carboxy Methyl Cellulose suspension will protect the gastric mucosa from any NSAID induced gastric injuries and may also reduce gastric mucosal bleeding. Further study in this regard may demonstrate the molecular mechanisms of this action and future use of Curcuma Longa Extract.

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