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PHARMACOLOGICAL EVALUATION OF ANTI-ULCER EFFECTS OF COMBINED DOSES OF *ALOE VERA* AND VITAMIN-E AGAINST INDOMETHACIN INDUCED PEPTIC ULCER MODEL

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Keywords:

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ABSTRACT: Peptic Ulcer Disease (PUD) is one of the most prevalent pathogenic conditions which affects around 5-10% of the global population. *Helicobacter pylori* infection and the use of Non-Steroidal Anti-inflammatory Drugs (NSAIDs) are two of the most common etiological causes in the PUD pathogenesis. First line treatment for PUD involves use of drugs which cause acid suppression or target against the eradication of *Helicobacter pylori* infection. Ulceration generally occurs due to the disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance. Peptic ulcer disease (PUD) may also occur as a result of the regular usage of certain drugs, irregular food habits, stress, alcohol consumption, and so forth. Recent years have shown a significant increase in the consumption of NSAIDs, despite their harmful side effects. However, administration of NSAIDs, along with certain drugs or supplements may reduce the deleterious side effects. This research evaluates the ulcer protective efficacy of the combined dose of *Aloe vera* and vitamin-E in the Indomethacin-induced ulcerated rats. The therapeutic efficacy of the combined dose of drugs was compared with the disease control group and the standard drug Ranitidine. Data indicates that the administration of both individual and combined doses of *Aloe vera* and vitamin E showed a significant decrease ($P<0.001$) in ulcer index, Ulcerated surface (%), gastric volume, total acidity and increase in the gastric pH. The efficacy of the combined dose of drugs was comparable with the control group and standard drug Ranitidine. This research presents the comparative study among the individual and combined doses of *Aloe vera* and vitamin E for their antiulcer efficacy in the Indomethacin administered healthy albino rats. It can help in further studies for their antiulcer efficacy in future work for new antiulcer drugs development.

INTRODUCTION: Ulcer is considered as an inflamed break in the skin or the mucous membrane lining the alimentary tract [1].

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PUD generally comprises gastric and duodenal ulcers and is one of the most prevalent gastrointestinal disorders. Under normal homeostatic conditions, a physiologic equilibrium is maintained between gastric acid secretion and gastric and duodenal mucosal defense systems [2].

However, any disturbance between aggressive and protective factors may result in the mucosal injury and may lead to PUD [3]. The pathophysiology of peptic ulcer involves an imbalance between aggressive factors (acid, pepsin, refluxed bile salts,

ingested drugs and *H. pylori* infection) and defensive factors (mucus, prostaglandin, bicarbonate, phospholipids, nitric oxide and growth factors) [4, 5]. Hence, peptic ulcers are defined as defects in the gastric or duodenal mucosa and submucosa, which extend through the muscularis mucosa [6]. The structural and functional integrity of the gastric and duodenal mucosa maintains the equilibrium between aggressive factors and protective mechanisms. Few of the protective mechanisms involved in maintaining the normal gastric physiology includes: Secretion of mucus by surface epithelial cells [7, 8], discharge of bicarbonate into the surface mucus, to create a buffered surface microenvironment [9], release of acid and pepsin from the gastric pits as 'jets' through the surface mucus layer, entering the lumen directly without contacting surface epithelial cells [10], rapid gastric epithelial redevelopment [10], robust mucosal blood flow, to sweep away hydrogen ions that have back diffused into mucosa from the lumen and to sustain the high cellular metabolic and regenerative activity [11] and mucosal expansion of prostaglandins, which helps in maintaining the mucosal blood flow [12].

An estimated 15,000 death occur each year as a consequence of PUD. In India, PUD is common. In the pharmaceutical industry, antacids and drugs share 6.2 billion rupees and found a 4.3% market share [13]. Several natural products are reported to possess antiulcer activity, but limited clinical data are available to support the use of herbs as gastro-protective agents [14]. However, *Aloe vera* is frequently used in several therapeutic applications because of their high therapeutic efficacy and low toxicity. *Aloe vera* is known to possess anti-ulcer property [15]. *Aloe vera* belongs to the family *Liliaceae* and is usually known as "aloe gel." *Aloe vera is* found all over India. Aloin, isobarbaloin, and emodin are the major chemical constituents of *Aloe vera* [16]. The anti-ulcer activity of the *Aloe vera* is reported in Indomethacin-induced ulcer model. The antiulcer activity of *Aloe vera* is due to it's antioxidant, anti-inflammatory, mucus-secreting, cytoprotective, healing, and immunomodulating properties [17].

Vitamin E is a group of eight lipid-soluble compounds comprising four tocopherols and four tocotrienols, and it plays a major role in maintaining the cellular antioxidant defense system. Alpha-tocopherol is the most biologically active and is frequently used as a dietary supplement. Alpha-tocopherol is a naturally occurring antioxidant in biological systems and is present in the cell membrane of various tissues, including the intestine and stomach [18]. The anti-ulcer activity of vitamin E is reported in stress, indomethacin, reserpine, hydrochloric acid, sodium chloride, and ethanol-induced ulcer models [19]. The pretreatment of animals with vitamin E is reported to produce a significant inhibition of gastric lesions. Vitamin E is believed to protect cells from oxidative stress, regulate immune function, and maintain endothelial cell integrity. It is also known to promote the synthesis of prostaglandins and glutathione in tissues of vitamin E treated animals which have been suggested as a possible mechanism of anti-ulcer activity. Vitamin E blocks the free radical chain reaction and thus prevents the ROS-induced injury [20]. The formation of experimental gastric lesions may be reduced through decreasing free radicals and diminishing lipid peroxidation [21]. The mechanisms by which NSAIDs produce acute and chronic gastro-duodenal mucosal injury are partially understood, but it has been suggested that the mechanism underlying the PUD pathogenesis is mediated through lipid peroxidation [22]. In the present study, the antiulcer activity of the combined and individual doses of *Aloe vera*, vitamin E were evaluated in the Indomethacin-induced ulcerated rats.

MATERIALS AND METHODS:

Drugs and Chemicals: *Aloe vera* powder (Neoteric DCBA Ideas, Coimbatore, India), vitamin E (Evion 400 capsules), Indomethacin capsules (Merck & Co., USA), Ranitidine tablets (Sigma-Aldrich Corporation, USA), Sodium hydroxide, Diethyl ether, and Phenolphthalein indicator were obtained from Sigma-Aldrich Corporation, USA.

Experimental Animals: Healthy albino rats (120-140 g) of either sex were randomly selected. The rats were housed in polyvinyl cages of 4 animals per cage and maintained under standard laboratory conditions of relative humidity (50 ± 5%), temperature (28 ± 2 ºC), a 12 h dark and light cycle and received standard pellet diet (Agro Feed,

Calabar) and tap water *ad libitum*. Approval (Approval no.: HIPER/IAEC/05/17/14) was obtained from the Institutional Animal Ethical Committee (IAEC) concerning the regulation of CPCSEA guidelines.

Experimental Protocol: Albino rats were divided into 6 groups containing 6 rats each. Animals were fasted for 24 h with water *ad libitum*, before the start of the experiment.

Normal Control Group (Group I): Rats in this group received distilled water (10 ml/kg, per oral) by oral gavage for 5 days.

Disease Control (Group II): Rats in this group were treated with Indomethacin (20 mg/kg, per oral) by oral gavage for 5 days.

Indomethacin + *Aloe vera* (Group III): Rats were treated with consecutive doses Indomethacin (20 mg/kg, per oral) and *Aloe vera* (150 mg/kg, per oral) by oral gavage for 5 days.

Indomethacin + Vitamin E (Group IV): Rats were treated with consecutive doses Indomethacin (20 mg/kg, per oral) and vitamin E (50 mg/kg, per oral) by oral gavage for 5 days.

Indomethacin + *Aloe vera* + Vitamin E (Group V): Rats were treated with consecutive doses of Indomethacin (20 mg/kg, per oral), *Aloe vera* (150 mg/kg, per oral) and vitamin E (50 mg/kg, per oral) by oral gavage for 5 days.

Indomethacin + Ranitidine (Group VI): Rats were treated with consecutive doses of Indomethacin (20 mg/kg, per oral) and Ranitidine (20 mg/kg, per oral) by oral gavage for 5 days.

On the 5th day, Rats were sacrificed 5 h post treatment and stomach was cut open in the greater curvature, rinsed and examined by a 10X magnifier lens. The number of ulcers formed were recorded and ulcer scores were recorded as 0 = no ulcer, 1 = superficial ulcer, 2 = deep ulcer, 3 = perforation. Ulcer index was measured by the formula [23]:

$$U_I = U_N + U_S + U_P \times 10^{-1}$$

Where; U_I = Ulcer index
U_N = Average number of ulcers per animal
U_S = Average number of severity score and
U_P = Percentage of animals with ulcers.

The percentage inhibition of ulceration was calculated as:

$$\text{Percentage inhibition} = (U_I \text{ Control} - U_I \text{ Treated}) / U_I \text{ Control} \times 100$$

Determination of Ulcer Score: The stomachs were released along the greater curvature, rinsed with saline to eliminate gastric contents and blood clots and examined by a 10X magnifier lens to assess the development of ulcers. The numbers of ulcers were counted. Scoring of the ulcer will be made as follows:

Normal colored stomach	0
Red coloration	0.5
Spot ulcer	1
Hemorrhagic streak	1.5
Deep Ulcers	2
Perforation	3

Determination of pH: Aliquot of 1 ml gastric juice was diluted with 1 ml of distilled water, and the pH of the solution was read using pH meter [24].

Determination of Total Acidity: An aliquot of 1ml gastric juice diluted with 1ml of distilled water was taken into a 50 ml conical flask, and two drops of phenolphthalein indicator were added to it. An aliquot was then titrated with 0.01N NaOH until a permanent pink color was observed. The consumed volume of 0.01N NaOH was noted. The total acidity is expressed as mEq/L through the following formula [25, 26].

$$AT = n \times 0.01 \times 36.45 \times 1000$$

Where; n = Volume of NaOH consumed

36.45 = Molecular weight of NaOH
0.01 = Normality of NaOH
1000 = The factor (to be represented in litre).

Determination of Free Acidity: Instead of phenolphthalein indicator, the Topfer's reagent was added an aliquot of gastric juice and then titrated with 0.01N NaOH until the canary yellow colour was observed. The volume of 0.01N NaOH consumed was noted. The formula to determine the total acidity will be used to calculate the free acidity [25].

RESULTS:

Determination of Ulcer Index and Ulcerated Surface (%): In comparison to the disease control,

the individual combined doses of Indomethacin (20 mg/kg, p.o.) + *Aloe vera* (150 mg/kg, p.o.), Indomethacin (20 mg/kg, p.o.) + vitamin-E (50 mg/kg, p.o.), Indomethacin (20 mg/kg, p.o.) + *Aloe vera* (150 mg/kg, p.o.) + vitamin-E (150 mg/kg + 50 mg/kg), and Indomethacin (20 mg/kg, p.o.) + Ranitidine (20 mg/kg) show significant reduction in Ulcer index and Ulcerated surface (%) of Indomethacin induced gastric ulcer in albino rats **Table 1**.

TABLE 1: TABLE SHOWS EFFECT OF COMBINED DOSE OF *ALOE VERA* + VITAMIN-E ULCER INDEX AND ULCERATED SURFACE (%) OF INDOMETHACIN INDUCED GASTRIC ULCER IN ALBINO RATS AND DATA WAS ANALYSES BY ONE WAY ANOVA FOLLOWED BY DUNNETT TEST

Group	Dose (mg/kg)	Ulcer Index	Ulcerated Surface (%)
Group I (Normal control)	Distilled water (10 ml/kg, per oral)	0	0
Group II (Disease control)	Indomethacin (20 mg/kg, per oral)	9.0***	85.2 ± 0.25***
Group III	Indomethacin (20 mg/kg, per oral) and *Aloe vera* (150 mg/kg, per oral)	4.0**	3.2 ± 0.5
Group IV	Indomethacin (20 mg/kg, per oral) and Vitamin E (50 mg/kg, per oral)	4.0**	4.5 ± 0.2*
Group V	Indomethacin (20 mg/kg, per oral), *Aloe vera* (150 mg/kg, per oral) and vitamin E (50 mg/kg, per oral)	3.0*	2.5 ± 0.12**
Group VI (Standard)	Indomethacin (20 mg/kg, per oral) and Ranitidine (20 mg/kg, per oral)	2.0	1.5 ± 0.1

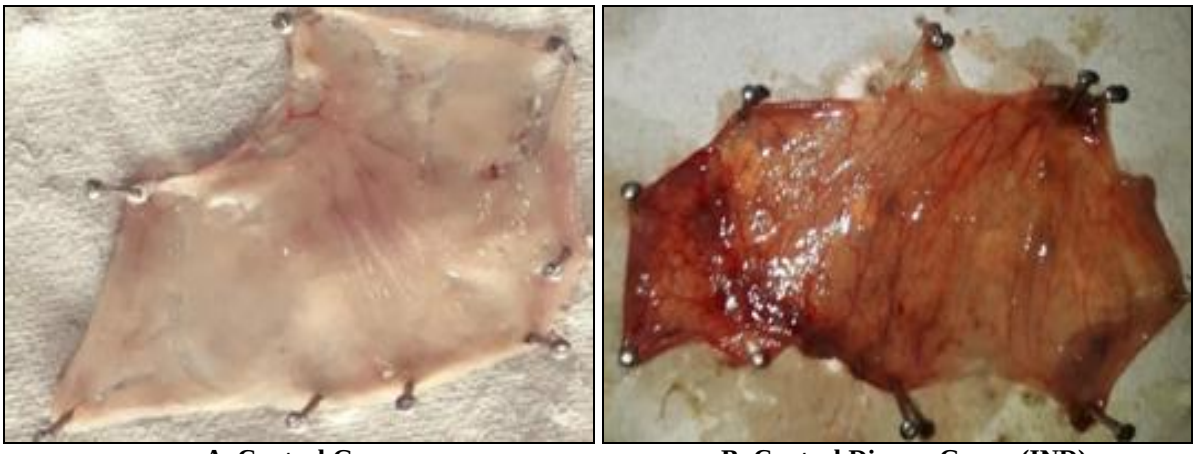
The data were presented as mean ± SEM (6 animals in each group) *P<0.001

Determination of Total Acidity (Meq/L), Total Gastric Volume (ml) and pH: In comparison to the disease control, the individual combined doses of Indomethacin (20 mg/kg, p.o.) + *Aloe vera* (150 mg/kg, p.o.), Indomethacin (20 mg/kg, p.o.) + vitamin-E (50 mg/kg, p.o.), Indomethacin (20 mg/kg, p.o.) + *Aloe vera* (150 mg/kg, p.o.) + vitamin-E (50 mg/kg, p.o.), and Indomethacin (20 mg/kg, p.o.) + Ranitidine (20 mg/kg, p.o.) show significant decrease (P<0.001) in gastric volume, total acidity and increase in the gastric pH **Table 2**.

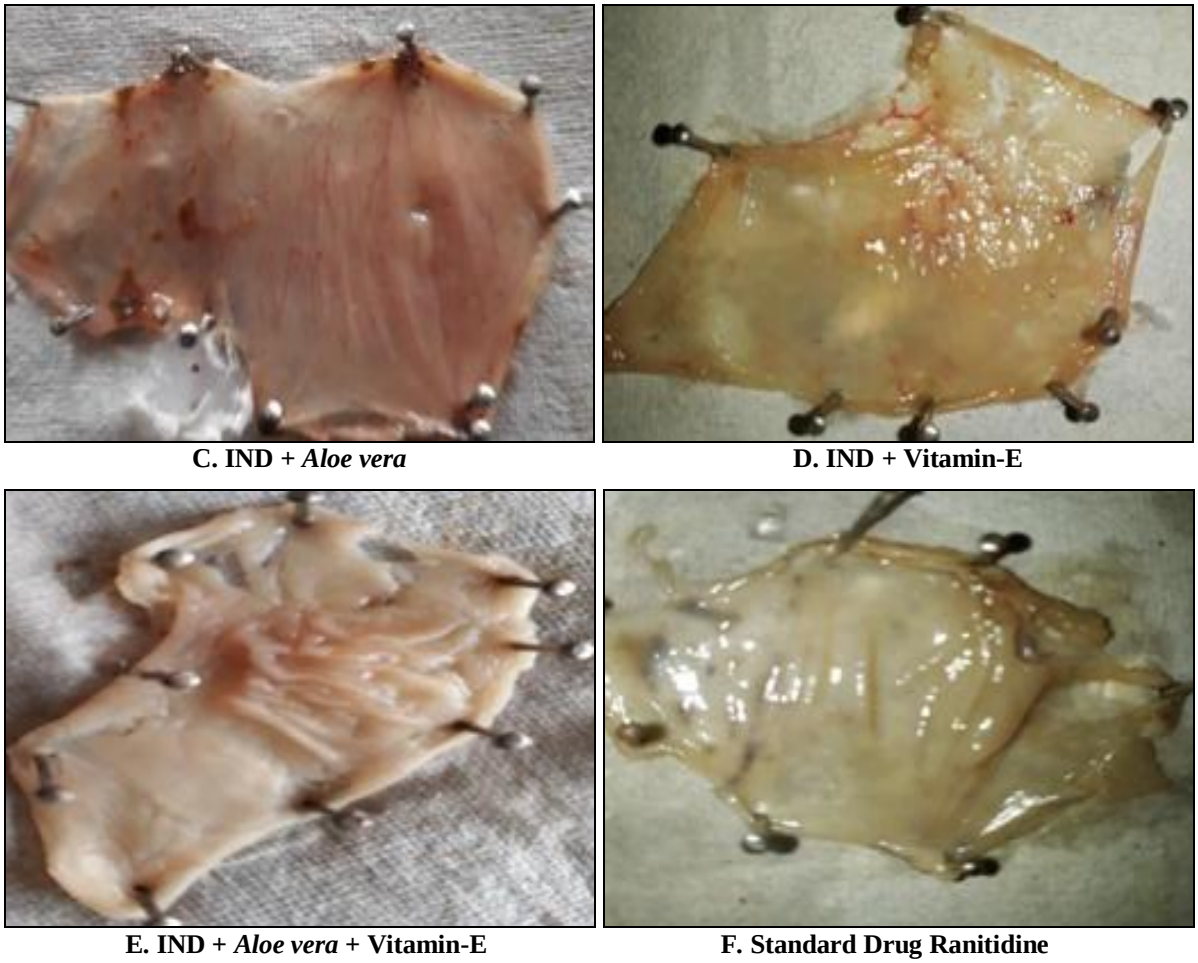
TABLE 2: EFFECTS OF COMBINED DOSE *ALOE VERA* + VITAMIN-E IN ULCEROGENIC ACTIVITY EXPRESSED IN MEAN ± SEM (*P<0.001 *vs.* CONTROL), (***P<0.001 *vs.* DISEASE CONTROL) STATISTICALLY SIGNIFICANT AND DATA WAS ANALYSES BY ONE WAY ANOVA FOLLOWED BY DUNNETT TEST**

Group	Total Gastric Vol. (ml)	pH	Total Acidity (mEq/L)
Control	3.75 ± 0.33	4.25 ± 0.33	3.25 ± 16
Disease control group (Indomethacin)	5.88 ± 0.45	2.5 ± 0.23	5.5 ± 0.21***
Indomethacin + *Aloe vera*	3.02 ± 0.22*	4.6 ± 0.35	3.9 ± 0.42*
Indomethacin + Vitamin-E	3.95 ± 0.1*	4.01 ± 0.1	4.65 ± 0.2*
Indomethacin + *Aloe vera* + Vitamin-E	2.2 ± 0.25**	5.0 ± 0.35**	3.4 ± 0.1**
Indomethacin + Ranitidine	1.85 ± 0.42**	3.5 ± 0.25**	3.22 ± 0.35

The data were presented as mean ± SEM (6 animals in each group) *P<0.001



A. Control Group **B. Control Disease Group (IND)**



C. IND + *Aloe vera* **D. IND + Vitamin-E**

E. IND + *Aloe vera* + Vitamin-E **F. Standard Drug Ranitidine**

FIG. 1: RATS ISOLATED STOMACHS WERE DIRECTLY EXAMINED THROUGH 10X MAGNIFIER LENS TO OBSERVE DIFFERENT ULCEROGENIC ACTIVITY

DISCUSSION: Non-steroidal anti-inflammatory drugs like indomethacin are known to induce numerous punctiform and filiform gastric ulcers during anti-inflammatory therapy, and hence, indomethacin-induced model was used in the present study. One of the major mechanisms underlying the ulcerogenecity of indomethacin involves the inhibition of prostaglandin synthesis. Prostaglandins promote mucus secretion, bicarbonate secretion, inhibit acid secretion, alter mucosal blood flow and hence provide protection against agents that cause acute mucosal damage [26].

Thus, inhibition of prostaglandin synthesis by non-steroidal anti-inflammatory drugs like Indomethacin consequences in increased susceptibility to gastric mucosal lesions and mucosal injury, as observed in disease control. Individual doses of both *Aloe vera* and vitamin E are reported to reduce the aggressive factors and maintain normal homeostatic conditions in the stomach [23].

CONCLUSION: The present study reveals that the therapeutic efficacy of the combined doses of *Aloe vera* and vitamin E was far better than their doses in the treatment of PUD. The combined dose of *Aloe vera* (150 mg/kg) + vitamin-E (50 mg/kg) significantly protected the mucosa from being damaged by Indomethacin, thus signifying that combination may act as an excellent candidate for future studies on peptic ulcer.

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CONFLICT OF INTEREST: Nil

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