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ANTI-ULCER ACTIVITY OF POLYHERBAL FORMULATION ACIDEZ IN EXPERIMENTALLY INDUCED GASTRIC ULCERS IN RATS

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ABSTRACT: Aim and Objective: Acid-related health problems mostly affect the quality of life and are a major factor in morbidity and mortality in patients. This study is designed to evaluate the anti-ulcer activity of Polyherbal formulation ACIDEZ against experimentally induced gastric ulcers in rats. **Methodology:** To determine anti-ulcer activity, ulcers were induced by aspirin plus pylorus ligation and acetic acid induced methods. The animals were treated with ACIDEZ (50 mg/kg and 100mg/kg) and the standard drug sucralfate (300mg/kg) for a particular number of days. Anti-ulcer and gastric protection activity was evaluated by ulcer index, gastric secretion, total mucosal content, and pepsin content. The results were expressed as Mean ± S.E.M., and statistical evaluation was done by one-way ANOVA followed by Tukey's multiple comparison tests. **Results and Discussions:** The Polyherbal formulation ACIDEZ was found to be safe upto 2000mg/kg. ACIDEZ demonstrated a dose-dependent decrease in gastric juice volume and total acidity in both models. Both doses of the test formulation exhibited a significant decrease in ulcer index, pepsin content and restoration of mucosal content. **Conclusion:**-The results obtained from our study indicate that Polyherbal formulation ACIDEZ was safe and exhibited anti-ulcer activity, which might be through cytoprotective action.

INTRODUCTION: Acid-related health problems mostly affect the quality of life and major influencer of patient morbidity and mortality. Heartburn, Gastro-esophageal reflux disease and peptic ulcers are the most common gastrointestinal disorders. An ulcer is one of the most common gastrointestinal health issues worldwide, affecting 10% of the population once in their lifetime [1, 2].

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It was also noted that ulcers' chances of relapse or recurrence are more possible. Therefore safe and effective medicine must possess the ability to control gastric acid secretion, delay or avoid the possibilities of recurrence and pain complications and enhance the healing process [3].

Drug-induced, stress-induced, or many reasons why the population relies on acidity relieving medications. But it was already reported that mucosal strength and not gastric acid secretion is a significant factor in ulcer cause and healing process [4]. Hence, the search for an idyllic anti-ulcer drug continues and has also been extended to the traditional system of medicines in search of better gastroprotective action and a decrease in the

incidence of relapse in chronic ulcers. ACIDEZ is a Polyherbal formulation in a tablet dosage form manufactured by ATBU Harita Pharmaceuticals. Pvt. Ltd. Each 500 mg tablet contains *Asparagus racemosus* (Shatavari) 100 mg, Piper longum (Long pepper) 25mg, *Plumbago zeylanica* (Chitrak) 75mg, *Emblica officinalis* (Amla) 100mg, *Glycyrrhiza glabra* (Liquorice) 50 mg and excipients to a quantity sufficient. The formulation content is incorporated to design an effective formulation for gastrointestinal disorders.

The herbal content in ACIDEZ possesses one common mechanism amongst all reported studies, exploring that they might act as cytoprotective and effectively reduce ulcers [5].

Therefore, this study is planned to evaluate and establish scientific pre-clinical data to support the clinical use of ACIDEZ Polyherbal formulation.

MATERIAL AND METHODS: The study's objective was to perform an acute oral toxicity study and evaluate the antiulcer activity of ACIDEZ. IAEC approved the study of ROFEL Shri G.M. Bilakhia College of Pharmacy Vapi with protocol number ROFEL/IAEC/2019/06.

Animals: Wistar rats weighing 150-250 gm were used for the study. They were fed with a standard laboratory diet and water *ad libitum*. Twelve hours of dark-light cycles were maintained.

Acute Oral Toxicity Study: The acute oral toxicity study was determined using OECD 423 toxic class method guideline. As per the guideline mentioned above, three healthy female Wistar rats were orally administered ACIDEZ employed 2000mg/kg considering the available information on mortality is unlikely at the highest starting dose level. Animals were monitored for changes in body weight, clinical symptoms and mortality during the first four hours following the drug's administration and then twice daily for the next 14 days [6].

Aspirin plus Pylorus Ligation (PL) Induced Gastric Ulcer Model [7-8]: 30 Wistar rats were divided into 5 groups of 6 rats in each. The drugs administered daily for 5 days are as follows in **Table 1.**

TABLE 1: STUDY GROUP FOR ASPIRIN + PL-INDUCED GASTRIC ULCER MODEL

Sr. no.	Group	Dose (ml/kg body weight)	No. of animals
1.	Control	10 ml/kg, orally	6
2.	Disease Control(Aspirin + PL)	200 mg/kg, orally	6
3.	Low Dose (Acidez)	50 mg/kg, orally	6
4.	High Dose (Acidez)	100 mg/kg, orally	6
5.	Standard (Sucralfate)	300 mg/kg, orally	6

On the 6th day, after 24 hrs of fasting, pylorus was ligated under anesthesia. 4 hours after ligation, the animals were sacrificed, and the stomach was removed and opened along the greater curvature. The contents were collected in graduated test tubes and centrifuged (1000 rpm. for 10 minutes).

The supernatant was then subjected to biochemical analysis. The antiulcer activity was evaluated using parameters like ulcer scoring, ulcer index, gastric secretion volume, total acidity, gastric wall mucus content and pepsin estimation.

Acetic Acid-Induced Gastric Ulcer Model [9]: The animals mentioned in **Table 2** were grouped in 5 groups containing 6 animals each and were fasted for 24 hrs before the experiment.

Under anesthesia, the abdomen was opened by midline incision below the xiphoid process, and the stomach was exposed. Glacial acetic acid (0.05 ml) was added to the cylindrical mould of 6 mm diameter and placed tightly over the stomach's anterior serosal surface.

This was allowed to remain there for 60 seconds. The acid solution was removed by rinsing the mould with normal saline twice or thrice to avoid damage to the surrounding tissues.

The stomach was then placed back carefully, and the abdominal wall was closed. The drugs were administered daily for 10 days. On the 11th day, the animals were sacrificed using anesthesia, and the stomach was removed.

The stomach was cut open along the greater curvature. The antiulcer activity was evaluated using parameters like ulcer scoring and ulcer index.

TABLE 2: STUDY GROUP FOR ACETIC ACID INDUCED GASTRIC ULCER MODEL

Sr. no.	Group	Dose(ml/kg body weight)	No. of animals
1.	Control	10 ml/kg, orally	6
2.	Disease Control(Acetic acid)	0.05 ml	6
3.	Low Dose (Acidez)	50 mg/kg, orally	6
4.	High Dose (Acidez)	100 mg/kg, orally	6
5.	Standard (Sucralfate)	300 mg/kg, orally	6

RESULTS AND DISCUSSION: The acute oral toxicity study indicated that the ACIDEZ caused no mortality up to the dose of 2000mg/kg in animals. No physical and behavioral changes were observed in the experimental animals.

Aspirin plus Pylorus Ligation (PL) Induced Gastric Ulcer Model:

Ulcer Scoring and Ulcer Index: -A significant decrease in the score of ulcers can be observed in the test and standard treatment groups, respectively. The scored data are as mentioned in **Table 3**.

It can be said that the disease control group that received (Aspirin (200 mg/kg) + PL) had a significantly higher ulcer index than the control group, as no ulcers were found in the control group, and the ulcer index was nil.

A significant decrease ($p<0.05$) in ulcer index can be seen in test groups and standard drug treatment. The observational results also suggested that the high test dose group (ACIDEZ 100 mg/kg) shows a remarkable decrease in ulcer index than the lower test dose group (ACIDEZ 50 mg/kg).

TABLE 3: EFFECT OF DRUGS ON ULCER SCORING AND ULCER INDEX OF ASPIRIN + PL-INDUCED ULCER

Group	Ulcer score	Ulcer Index
Control (1% CMC)	0 + 0.0	0 + 0.0
Disease control (Aspirin 200 mg/kg)	2.6 + 0.21	1.59 + 0.08
Low dose (50 mg/kg)	0.8 + 0.16	0.29 + 0.09*#
High dose (100mg/kg)	0.5 + 0.22	0.24 + 0.01*#
Standard (Sucralfate 300mg/kg)	0.3 + 0.21	0.17 + 0.008*#

(All values are expressed as Mean ± S.E.M., n=6) analyzed by One way ANOVA followed by Tukey's test. * denotes $p<0.05$ when compared with control. # denotes $p<0.05$ when compared with disease control

Gastric Secretion Volume and Total Acidity: The volume of gastric secretion is mentioned in **Table 4**.

The Low dose test formulation reduced but did not significantly ($P<0.05$) affect the gastric secretion volume. The standard and high-dose test formulation significantly reduce gastric acid secretion. These findings suggest that the gastrointestinal protective action of ACIDEZ may produce dose-dependent anti-secretory activity.

A notable decrease can be seen in total acidity of standard and high dose groups.

TABLE 4: EFFECT ON GASTRIC SECRETION VOLUME AND TOTAL ACIDITYOF ASPIRIN + PL-INDUCED ULCER

Group	Gastric secretion volume (ml)	Total acidity(mEq/I)
Control (1% CMC)	2.4 ± 0.22	29.17± 1.01
Disease control (Aspirin 200 mg/kg)	6.35 ± 0.29*	39.33 ± 1.02*
Low dose (50 mg/kg)	4.06 ± 0.11*	33.00± 1.03*
High dose (100 mg/kg)	2.51 ± 0.20*	22.50± 1.40*#
Standard (Sucralfate 300mg/kg)	2.75 ± 0.21*	20.33 ± 0.88*#

Values are expressed as Mean ± S.E.M., n=6, and analysed by One way ANOVA followed by Tukey's test. * denotes $p<0.05$ when compared with control# denotes $p<0.05$ when compared with disease control

Gastric wall Mucus Content: The standard group restored the gastric wall mucus content in a significant manner.

The low and high dose groups (ACIDEZ 100 mg/kg) restored gastric wall mucus content. Thus, these results mentioned in **Table 5** suggest a significant cytoprotective activity of Polyherbal formulation ACIDEZ, as indicated by mucosal content comparable to a protective effect of Sucralfate [10].

TABLE 5: EFFECT ON GASTRIC WALL MUCUS CONTENT OF ASPIRIN + PL INDUCED ULCER

Group	Gastric wall mucus content µg/gm
Control (1% CMC)	0.22± 0.013
Disease control (Aspirin 200 mg/kg)	0.14± 0.014*
Low dose (50 mg/kg)	0.17 ± 0.014*#
High dose (100 mg/kg)	0.23 ± 0.018*#
Standard(Sucralfate 300mg/kg)	0.26 ± 0.015*#

All values are expressed as Mean ± S.E.M., n=6, and analyzed by One way ANOVA followed by Tukey's test.*denotes p<0.05 when compared with control.# denotes p<0.05 when compared with disease control

Pepsin Estimation: Pepsin is a protein that, along with the gastric juice produced due to pylorus ligation, will digest the mucus that leads to the formation of an ulcer. An increase in pepsin level can be seen in the control group mentioned in **Table 6** compared to the treatment groups. The treatment group, like the low dose, high dose test formulation and standard group, shows a significant decrease in pepsin levels compared to the control and disease control groups.

TABLE 6: EFFECT OF ON PEPSIN CONTENT OF ASPIRIN + PL-INDUCED ULCER

Group	Pepsin estimation (Mean ± S.E.M.)
Control (1% CMC)	0.14± 0.002
Disease control (Aspirin 200 mg/kg)	0.17± 0.004*
Low dose (50 mg/kg)	0.13± 0.001*#
High dose (100 mg/kg)	0.12 ± 0.001*#
Standard(Sucralfate 300mg/kg)	0.11± 0.003*#

All values are expressed as Mean ± S.E.M., n=6, and analyzed by One way of ANOVA followed by Tukey's test. * denotes p<0.05 when compared with control. # denotes p<0.05 when compared with disease control

The morphological examination results of the Aspirin+PL model are presented in **Fig. 1** below. A morphological examination of the stomach reveals the disease control group has redness; lesions, and more ulcerated areas in the mucosal part of the stomach compared with standard and test groups.

FIG. 1: MORPHOLOGICAL CHANGES SHOWING EFFECT IN ASPIRIN+PL MODEL

Evaluation of Anti Ulcer Activity by Acetic Acid Induced Gastric Ulcer Model:

Ulcer Scoring and Ulcer Index: This model resembles pathological features, relapsing features, and a healing sequence. Acid secretion and pepsin are major determinants in this model of study to determine ulcer healing capacity. A significant decrease in the score of ulcers can be observed in the other three treatment groups, respectively. A significant decrease in their ulcer index can be seen in treatment groups like standard, low dose and high dose test formulations. Hence, it can be concluded that all the treatment groups successfully exhibited anti-ulcer activity in chronic ulcers mentioned in **Table 7.**

TABLE 7: ULCER SCORING AND ULCER INDEXED IN ACETIC ACID-INDUCED GASTRIC ULCER MODEL

Group	Ulcer score	Ulcer Indexed
Control (1% CMC)	0 ±0.0	0 ± 0.0
Disease Control (Acetic acid 0.05 ml)	2.1 ± 0.16	1.382 ± 0.09
Low dose (50 mg/kg)	0.7 ± 0.24	0.225 ± 0.005*#
High dose (100 mg/kg)	0.5 ± 0.22	0.202 ± 0.01*#
Standard (Sucralfate 300mg/kg)	0.3 ± 0.21	0.152 ± 0.006*#

All values are expressed as Mean ± S.E.M., n=6, and analysed by One way ANOVA followed by Tukey's test.* denotes p<0.05 when compared with control# denotes p<0.05 when compared with disease control

The treatment effect in **Fig. 2** demonstrates morphological changes in the Acetic acid induce gastric ulcer model as presented below. The disease control group has the more ulcerated diameter and morphological changes found in the mucosal part of the stomach compared with standard and test groups. In contrast, healing is more intense in standard and test groups.

FIG. 2: MORPHOLOGICAL CHANGES SHOWING EFFECT IN ACETIC ACID-INDUCED ULCER MODEL

G. glabra is one of the elements in ACIDEZ that possibly exerts cytoprotection because it prevents the formation of gastric ulcer lesions in NSAID induced pyloric ligation model.

It seems that ACIDEZ might raise the local level of prostaglandins which promote mucous secretion and cell proliferation in the stomach, leading to the healing of ulcers [11].

During the acute and chronic ulcers, the free radicals medicated injuries and complications are scavenged by *E. officinalis*. Therefore, the ACIDEZ might exhibit antioxidant and free radical scavenging activity and prevent further tissue damage [12].

CONCLUSION: The test formulation was found safe up to 2000mg/kg body weight. The results of the present study exhibit that ACIDEZ has ulcer healing activity observed through a dose-dependent decrease in gastric acid volume and total acidity conferred anti-secretory action.

Furthermore, ACIDEZ treatment offers cytoprotection by inhibiting pepsin and restoring gastric wall mucosal content. Anti-secretory and mucosal defensive action exhibits the cytoprotective nature of ACIDEZ.

Therefore, ACIDEZ exhibited potential in ulcer healing activity in acute and chronic peptic ulcers.

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CONFLICT OF INTEREST: None

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