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# ACUTE ORAL TOXICITY OF ANACID TABLET (POLYHERBAL FORMULATION) WITH ITS ANTI OXIDANT AND ULCER PROTECTIVE ACTIVITIES IN PYLORUS LEGATED INDUCED PEPTIC ULCER

**Nilesh Patel[1], Dr. Janmejay Patel[2], Achal Patel[3], Dr. Ankitkumar M. Paneliya[4]***

[1]Associate Professor & Head, Department of Pharmacology, Shree S K Patel College of P'ceutical Education & Research, Ganpat University, At. Kherva – 382711, Dist. Mehsana Gujarat, India.
[2]CEO, Petlad Mahal Arogya Mandal Pharmacy, At. Pipalata -387355, Dist. Kheda, Gujarat, India.
[3]MBBS Student, Pramukh Swami Medical College, Karamsad -388325, Dist. Anand, Gujarat, India.
[4]Associate Professor, Post graduate Department of Rasashastra evam Bhaishajya Kalpana, J. S. Ayurved Mahavidyalaya, Nadiad - 387001, Gujarat, India.

***Corresponding Author: Dr. Ankitkumar M. Paneliya**
Associate Professor, Post graduate Department of Rasashastra evam Bhaishajya Kalpana, J. S. Ayurved Mahavidyalaya, Nadiad - 387001, Gujarat, India.

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## ABSTRACT

**Introduction:** Because of the large and growing use of natural derived substances in all over the world, it is not wise to rely only on tradition or millenarian beliefs; descriptive and pragmatic studies are useful and should be considered to attainment of reliable data regarding toxicity and efficacy of substance. **Aim:** To evaluate Anacid Tablet (AT) (polyherbal formulation) for its acute oral toxicity with antioxidant and ulcer protective activities in Peptic ulcer. **Method:** The certification of IAEC (SKPCPER/IAEC/2016-02/01) as per the CPCSEA was obtained for present study. The acute oral toxicity was done according to OECD guideline AOT-425 to know single dose (2000 mg/kg) toxicity of test drug on swiss albino mice. The effect of AT on peptic ulcer was assessed in Pylorus ligation induced ulcer model in Albino Wister Rats. Various gastric parameters (gastric volume, pH, free & total acidity, ulcer index) and oxidative stress marker level (superoxide dismutase-SOD, catalase, malondialdehyde-MDA) were assessed to know the effect of test drug in comparison with other controlled groups. **Results:** There were no any physical - behavioral changes and mortality were observed in any animal during period of 14 days. The obtained result of test drug treated group in comparison to various control and standard drug treated group shows significant increase in pH and decrease in gastric volume, total acidity, free acidity and ulcer index. Significant effect was observed on antioxidant marker level in test drug treated group as compare to other groups.**Conclusion:** The No-Observed-Adverse-Effect-Level (NOAEL) of Anacid Tablet is 2000 mg/kg as it did not produce any toxic effect at that dose. The Anacid Tablet has significant ulcer protective and antioxidant properties.

**KEYWORDS:** Poly herbal formulation, Anacid Tablet, OECD Guideline, NOAEL, Peptic ulcer, Antioxidant.

## INTRODUCTION

The use of plants for healing purposes predates human history and forms the origin of ample modern medicine. Pharmacological, chemical, toxicity and Clinical studies of these traditional medicines which were derived predominantly from plants were the basis of most early medicines such as aspirin (willow bark), digitoxin (from foxglove), morphine (from the opiumpoppy), quinine (from cinchona bark), and pilocarpine (Jaborandi).[1] Herbal medicine is still the mainstay of about 75 - 80% of the world population mainly in the developing countries for primary health care because of the general belief that herbal drugs are without any side effects besides being cheap and locally available.[2]

Open sores or break in inner surface of stomach, duodenum or esophagus other than erosions called peptic ulcers.[3] The occurrence ratio of duodenal ulcer is higher compare to other types of peptic ulcers and it may converts in tumor due to environmental and diet changes[4]. The main cause of peptic ulcer is H.pylori infection (80%).[5] Other causes are NSAIDS, stress, alcohol, smoking and genetic factors.[6] Occurrence of disease in male is three times higher than female. Treatment cost of peptic ulcer is higher due to requirement of preventive therapy for reoccurrence but now a day's advances in this field expanded other treatment options.[7] Herbal drugs are comparatively safer and treat the disease without or with least side effect or adverse effect.[8]

Considering above facts, this study has been done to develop NOAEL with evaluation of ulcer protective and antioxidant effect of Anacid Tablet (a newly developed polyherbal formulation).

## AIM AND OBJECTIVES

- To evaluate acute oral toxicity of Anacid Tablet on Swiss Albino Mice.
- To evaluate antioxidant and ulcer protective effect of Anacid Tablet against peptic ulcer in pylorus ligation induced ulcer model.

## MATERIALS AND METHODS

**Test Material:** All the GMP standards were maintained during manufacturing of the test drug (Anacid Tablet). The detail of Anacid Tablet is mentioned below;

**Table 1: Ingredients of Anacid Tablet (Each Tablet contains).**

| Sl. No. | Name of ingredient | Quantity |
|---|---|---|
| **1** | Ext. Asparagus racemosus | 40mg |
| **2** | Ext. Hedychium spicatum | 30mg |
| **3** | Ext. Glycyrrhiza glabra | 10mg |
| **4** | Kamdudha Rasa | 100mg |
| **5** | ShankhaBhasma | 60mg |
| **6** | KapardikaBhasma | 60mg |
| **7** | Sutsekhar Rasa | 20 mg |
| **8** | Avipattikar | 80 mg |

**Method:** The present study was performed after obtained permission from IAEC (SKPCPER/IAEC/2016-02/01) as per the CPCSEA, Ministry of Environment, Forest and Climate Change (MoFCC), Government of India.

**(A) Acute oral toxicity:**[9] It was conducted by following OECD guideline AOT-425 to assess single dose toxicity of test drug. All the animals were set aside in standard condition. They were randomized in different groups with irrespective of their gender and acclimatized properly prior to dosing. A limit single oral dose of test drug extract (2000 mg/kg) was administered to each mouse in sequence at 48 h intervals and were observed individually at least once during the first 30 min after dosing, periodically during first 24 h and daily thereafter for total study period for any clinical signs of toxicity or mortality. Body weight of all animals was recorded once in a week. The dosing detail is mentioned below;

**Table 2 Individual animal dosing record of test drug;**

| Expt. Day | Animal No. | Gender | Test drug (mg) | Vehicle Distilled Water (ml) | Volume dose (ml) | Conc. (mg/ml) |
|---|---|---|---|---|---|---|
| 1st day | H | M | 50 | 0.6 | 0.57 | 83.33 |
| 3rd day | B | M | 50 | 0.6 | 0.528 | 83.33 |
| 5th day | T | F | 50 | 0.6 | 0.55 | 83.33 |
| 7th day | HT | F | 52 | 0.6 | 0.58 | 86.66 |
| 9th day | UM | F | 60 | 0.6 | 0.58 | 100 |

H: Head, B: Body, T: Tail, HT: Head & Tail, UM: Unmarked, M: Male, F: Female, Conc.: Concentration, Expt.: Experiment

**(B) Effect on Peptic ulcer:** This study was performed in Pylorus ligation induced ulcer model in Albino Wister Rats. Animals assigned for study were kept in standard condition. They were acclimatized for a minimum period of five days prior to dosing and subjected to randomization.

**Table 3: Grouping of Animals.**

| Group No. | Group Name | Dose (Oral) | No. of animals |
|---|---|---|---|
| I | Normal control (NC) | Normal saline | 6 |
| II | Disease control (DC) | Normal saline | 6 |
| III | Sham operated control (Sham) | Normal saline | 6 |
| IV | Standard drug (Ranitidine) treated (Std.) | 27 mg/kg | 6 |
| V | Anacid Tablet (AT) | 25 mg/kg | 6 |

One day before surgery, animals were divided in six different groups. Formulation or standard drug dose was given according weight of animal and interpretation of toxicological data. Animals were kept fasted for 24 h and after that, group IV & V were administered orally with standard drug (Ranitidine – 27mg/kg) and Test drug (Anacid Tablet – 25mg/kg) respectively before 1 h of surgery. First animal was anaesthetized then tied on

surgical board. Hairs below xiphoid process were removed and midline incision was made. Then pylorus portion was ligated bylifting it out without damaging any blood supply of stomach. The incision was closed by interrupted suture. Animals were kept for recovery in individual cage. After 24 h, animals were sacrificed by cervical dislocation method. Stomach of animal was isolated and parameters were analyzed.

**Evaluation of gastric parameters**

**Volume of Gastric juice:** The gastric juice was centrifuged at 3000 rpm for 15 min and then it was read from calibration on the centrifuge tubes.

**pH:** After centrifugation, pH of withdrawn liquid from centrifuge tubes was measured by pH strip.

**Free acidity and Total acidity:**[19] 1 ml of collected supernant liquid was taken and diluted up to 10 ml by distilled water. Resulting mixture was titrated using 0.01N NaOH, phenolphthalein and methyl red (2-3 drops of both) as indicator. First end point was taken when yellow color solution turned in orange. The volume of titrant was noted, which gives amount of NaOH required to measure free acidity. Now same solution was kept titrated until pink color obtained and it persisted for more than 30 sec. A tend point amount of titrant was noted down which indicate amount of NaOH required to measure total acidity.

**Ulcer Index:**[11] Calculation and representation of ulcer index is highly complicated and controversial process. Bonny castle (1964) and Robert et al (1968) suggested a method in which the stomach was given grades (0 to 4) as follows:

1. Normal swelling & white spots
2. Red hemorrhagic spots ulcers,
3. Deeper hemorrhagic spots & white spot like ulcers,
4. Hemorrhagic ulcers & other type of ulcers,
5. Perforated stomach due to ulcers.

Ulcer index = % of animals having ulcers × average severity of ulcer (from scale 0 to 4) /Average number of ulcers per stomach.

**(C) Evaluation of oxidative stress markers**

**Superoxide dismutase (SOD) activity**[12]

**Reagents:** 0.0001 M EDTA, 0.003 M Epinephrine, Carbonate buffer (pH 9.7)

The SOD calibration curve was prepared by taking 0.01, 0.1, 1 & 10 U/ml concentration of standard solution. Then solution of 1 ml carbonate buffer, 0.2 ml EDTA, 2 ml epinephrine and 0.5 ml supernant liquid were mixed. Absorbance of resulting solution was taken at 480 nm in spectrophotometer taking solution mixture without supernant as blank. Reading was taken at 30 sec interval for 3 min.

**Catalase activity**[13]

**Reagents:** 50 mM Potassium phosphate buffer (pH 7), 30 mM $H_2O_2$

Solution of 1 ml potassium phosphate buffer, 1 ml hydrogen peroxide and 50 µl sample (supernant) was prepared and absorbance of resulting mixture was taken at 240 nm by UV Visible spectrophotometer taking solution mixture without supernant as blank solution. Reading was taken at 15 sec interval for 2.5 min.

**Lipid peroxidation (LPO-MDA)**[14]

**Reagents:** 0.8 % TBA, 20 % $CH_3COOH$ in 0.27 M HCL (pH 3.5), 4 % W/V SLS, Distilled water In 1 ml of supernant liquid, 0.2 ml of SLS, 1.5 ml 20 % $CH_3COOH$ in 0.27 M HCl& 0.8 % 1.5ml of thiobarbituric acid (TBA) solution was added. Obtained mixture was heated at 85° C for 15 min and centrifuged at 1000 rpm for 15 min. After separation, upper organiclayer was taken and its absorbance was taken in spectrophotometer at 532 nm against blankprepared by omitting sample solution.

**Estimation of total protein**[15]

**Reagents: (A)** NaOH 2 gm, $NaHCO_3$ 10 g, Sodium potassium tartrate 0.1 gm All above reagent added and 500 ml volume was made up with distilled water.

**(B)** 5%$CuSO_4$ in dis.$H_2O$.

**(C)** 10 ml and 0.2 ml of solution A & B taken respectively.

In 0.2 ml of sample, 4 ml of solution C and 0.6 ml distilled water were added and kept aside for 15 min at 37° C. 0.4 ml of Folin-phenol reagent was added in that mixture after 15 min and resulting solution was again incubated for 30 min. After that, absorbance of prepared solutions were taken at 540 nm in spectrophotometer by taking solution without sample as blank. Total protein was obtained in mg/ml of sample from standard albumin calibration curve.

**Statistical Analysis:** Graph Pad Prism computer software was used[16] Result was expressed as Mean ± S.E.M, numbers of rats represented by n. Statistical significance between two means are determined by performing one way analysis of variance (ANOVA) followed by Dunnett's post hoc-test. P value <0.05 was considered significant.

## OBSERVATIONS & RESULT

**(A) Acute oral toxicity:** The animals were observed continuously for behavioural changes, autonomic profiles and other signs of toxicity or mortality up to period of 14 days. The body weight, food intake and water intake were also observed on 1$^{st}$, 7$^{th}$ and 14$^{th}$day. There were no physical and behavioural changes observed in swiss albino mice during observation period. Body weight of all animals did not reveal any significant change as compared to vehicle control group and mortality was Nil.

**Table 4: Individual animal weekly body weight & Mortality record.**

| Animal No. | Gender | Experiment Day, Unit : gm | | | Mortality |
|---|---|---|---|---|---|
| | | 1st | 7th | 14th | |
| H | M | 24 | 25 | 26 | NIL |
| B | M | 22 | 23 | 24 | NIL |
| T | F | 23 | 24 | 25 | NIL |
| HT | F | 25 | 26 | 27 | NIL |
| UM | F | 29 | 30 | 31 | NIL |

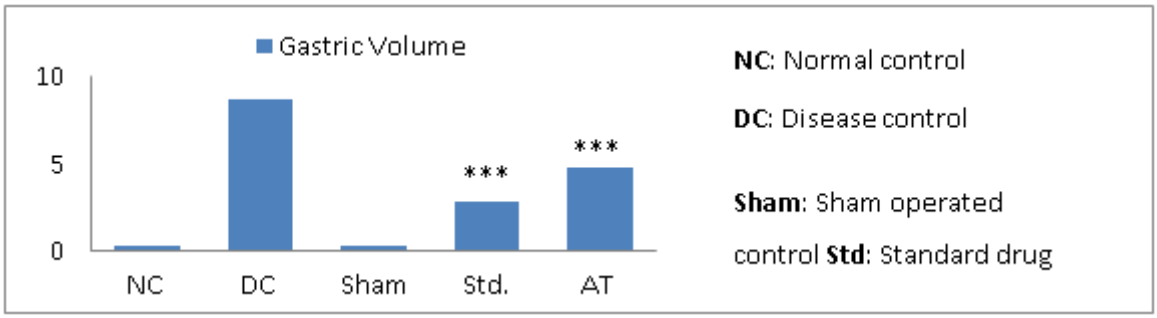
H: Head, B: Body, T: Tail, HT: Head & Tail, UM: Unmarked, M: Male, F: Female

**(B) Effect on Peptic ulcer:** The results of Anacid Tablet on Pylorus Ligation Induced Gastric Ulcer Model are as mentioned below.

**Table 5: Effect of test drug on various gastric parameters.**

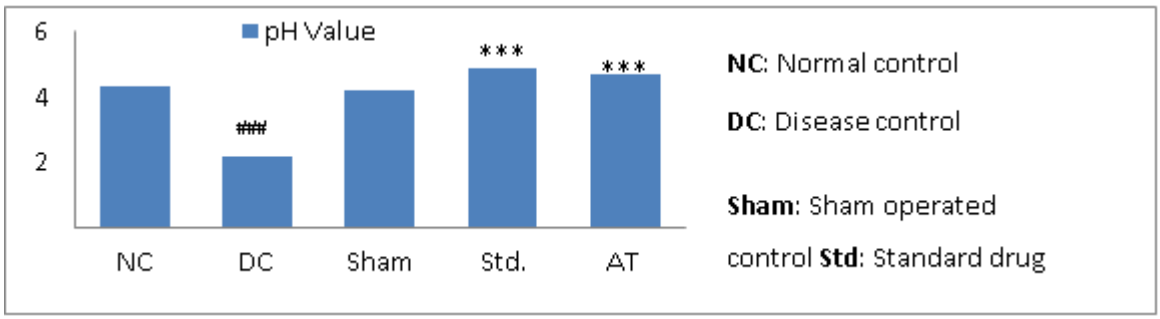
| Group | Dose(Oral) | Gastric volume | pH | Free acidity | Total acidity | Ulcer Index |
|---|---|---|---|---|---|---|
| **I (NC)** | Norma l saline | 0.3±0.0707 | 4.333±0.1667 | -- | -- | 0.0 |
| **II (DC)** | | 8.625±0.4250### | 2.167±0.1667### | 31.33±3.75### | 135.7±8.686### | 213.8±23.75### |
| **III(Sham)** | | 0.3250±0.0853 | 4.167±0.0.1667 | -- | -- | 0.0 |
| **IV(Std.)** | 27mg/kg | 2.875±0.3683*** | 4.833±0.1667*** | 7.00±1.00*** | 48.00±7.234** | 16.25±3.75*** |
| **V (AT)** | 25 mg/kg | 4.525±0.4871*** | 4.667±0.3333*** | 8.0±1.155*** | 45.00±3.055** | 22.50±2.50*** |

###p < 0.001 Vs Normal control, ***p <0.001, **p <0.01, *p <0.05 Vs Disease control.



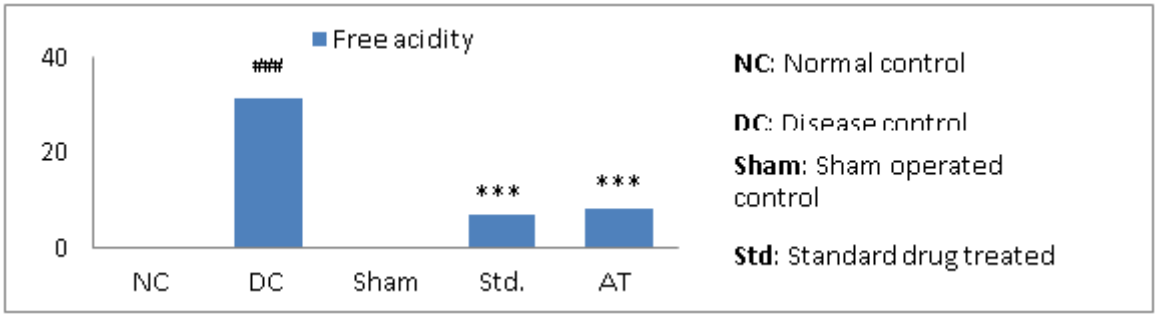

**Graph 1: Gastric volume (Values are expressed as mean ± S.E.M., n=6).**

###p < 0.001 Vs Normal control, ***p < 0.001 Vs Disease control.



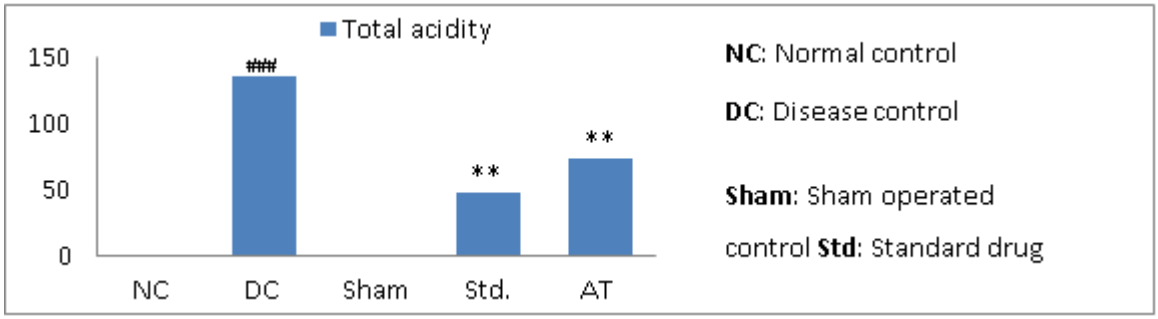

**Graph No. 2 pH of gastric juice (Values are expressed as mean ± S.E.M., n=6).**

###p < 0.001 Vs Normal control, ***p <0.001 Vs Disease control.



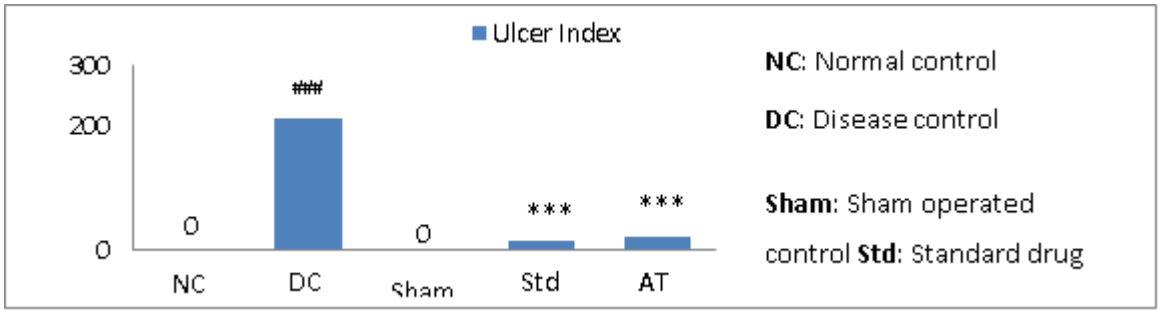

**Graph No. 3 Free acidity (Values are expressed as mean ± S.E.M., n=6).**

###p < 0.001 Vs Normal control, ***p < 0.001 Vs Disease control.




**Graph No. 4 Total acidity (Values are expressed as mean ± S.E.M., n=6).**

###p < 0.001 Vs Normal control, **p < 0.01 Vs Disease control.




**Graph No. 5 Ulcer Index (Values are expressed as mean ± S.E.M., n=6)**

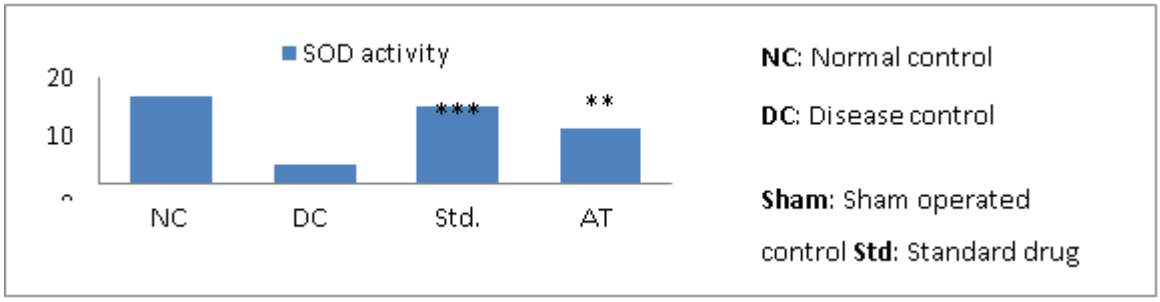
###p < 0.001 Vs Normal control, ***p <0.001 Vs Disease control.

**(C) Effect on oxidative stress markers:** The results of Anacid Tablet on various oxidative stress markers are as mentioned below.

**Table 6: Effect of test drug on oxidative stress markers.**

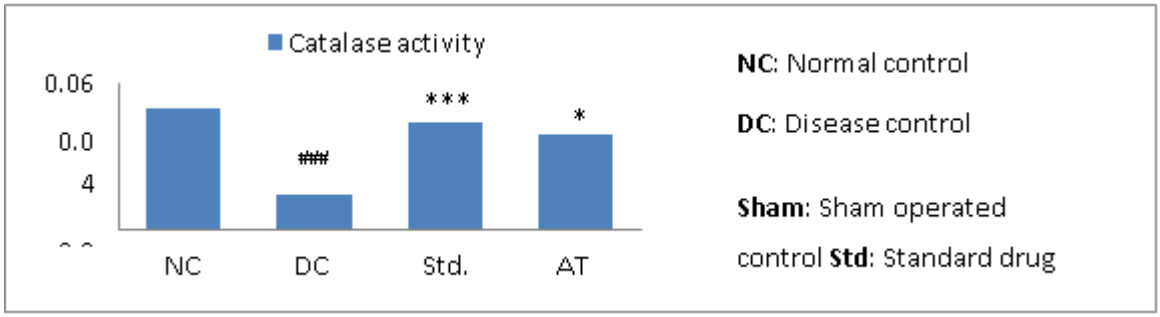
| Group | Dose (Oral) | SOD activity Units/mg protein | Catalase activityUnit/min /mg tissue protein | LPO MDA mmoles/mg tissue peotein |
|---|---|---|---|---|
| **I (NC)** | Normal saline | 16.28±1.071 | 0.04983±0.0008 | 17.55±1.360 |
| **II (DC)** | | 3.724±0.3645### | 0.0146±0.0011### | 59.41±2.883### |
| **IV (Std.)** | 27 mg/kg | 14.37±0.2256*** | 0.04361±0.00063*** | 19.04±0.7666*** |
| **V (AT)** | 25 mg/kg | 10.45±0.7096** | 0.03896±0.0016* | 21.50±1.524*** |

###p < 0.001 Vs Normal control, ***p < 0.001, **p < 0.01, *p < 0.05 Vs Disease control.



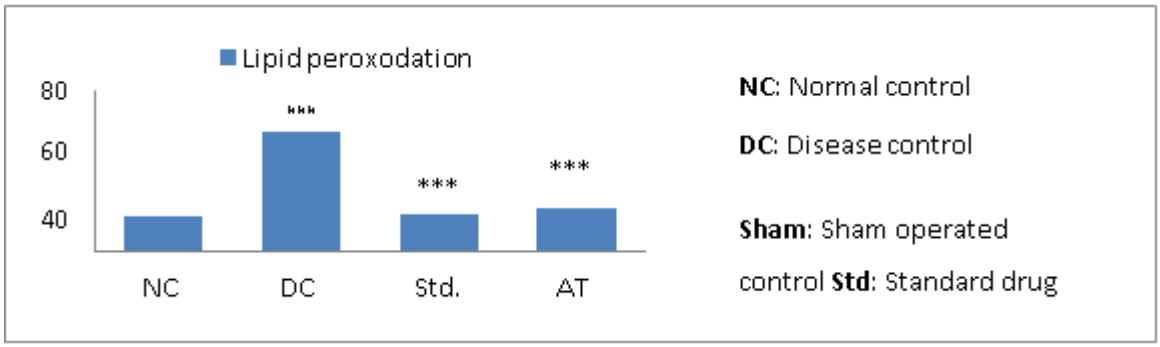

**Graph No. 6 SOD activity (Values are expressed as Mean ± S.E.M.)**

###p < 0.001 Vs Normal control, ***p < 0.001, **p < 0.01 Vs Disease control.




**Graph No. 7 Catalase activity (Values are expressed as Mean ± S.E.M.)**

###p < 0.001 Vs Normal control, ***p < 0.001, *p < 0.05 Vs Disease control.




**Graph No. 8: LPO-MDA (Values are expressed as Mean ± S.E.M.).**

###p < 0.001 Vs Normal control,***p < 0.001 Vs Disease control.

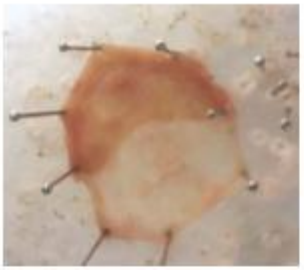
## DISCUSSION

In this age of globalization, assessing the transferability of treatments between different stream is not a relevant goal for clinical research, while are the assessment of efficacy and safety should be based on the regular patterns of mainstream clinical medicine. This study can consider as a pioneer step for the establishment of safety profile and efficacy of Anacid Tablet.

The acute oral toxicity study was done on Swiss Albino Mice for 14 days to rule out any toxic effect of Anacid Tablet at the single dose of 2000 mg/kg. Individual animal weekly body weight was recorded and found to be increasing during the observation period [**Table 4**]. Animal daily observation was recorded and found to be same and mortality rate was Nil [**Table 4**]. There were no physical and behavioral changes observed in animals during the observation period. This study reveals that Anacid Tablet which is indicated as antacid have no oral toxicity effect on Swiss albino mice. So, it can be used safely for therapeutic purposes.

### 6.3 Photographs

Effect of Anacid Tablet on pylorus ligation induced gastric ulcer model.

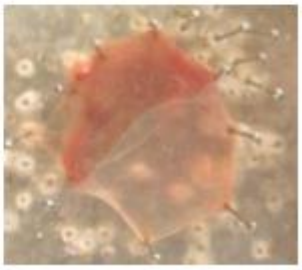


Normal control group

Disease control group

Anacid Tablet treated group

Sham control group



Standard control group

The Anacid Tablet is combination of eight ingredients. Among them *Asparagus racemosus* (*Shatavari*)[17] and *Glycyrrhiza glabra* (*Yastimadhu*) are proven to have anti secretary and ulcer protective activity.[18,19] *Hedychium spicatum* (*Shati*) showed protection against histamine-induced gastric ulcer.[20] *Shankha Bhasma* is alkaline in nature and has been proved for its anti ulcer effect. It is indicated in various pathological conditions like hyper acidity (*Amlapitta*), loss of appetite (*Agnimandhya*), dysentery (*Grahani*) and duodenal ulcer (*Parinama Shula*).[21] *Kamadudha Rasa* and *Sutashekhar Rasa* are proven formulation for having antacid properties. [22] Evidences of antacid property of *Kapardika Bhasma* is also available as well.[23]*Avipattikar churna* soothes the stomach tissues and promotes normal level of acid during the digestive process. It also helps to direct *Apana vata* downwards helping to promote post-meal esophageal comfort and healthy elimination.

The ulcer protective effect of test drug was performed in Pylorus ligation induced ulcer model in Albino Wister Rats. The statistically significant increase in pH and decrease in gastric volume, total acidity, free acidity and ulcer index was found in test drug treated group in compression to various control and standard drug treated group [**Table 5**] which proves potential ulcer protective and antacid effect of this combination.

During normal metabolic process reactive oxygen species are generated and its accumulation is controlled by specific enzymes like superoxide dismutase, catalase and glutathione peroxidase. Any disturbance in enzyme activity leads to accumulation of free radicals which can cause peptic ulcer. The antiulcer and healing mechanism can be obtained by antioxidant activity of any medicinal plant or herbal formulation. The results of test drug on oxidative stress markers favors its anti oxidant properties [**Table 6**].

## CONCLUSION

The No-Observed-Adverse-Effect-Level (NOAEL) of

Anacid Tablet is 2000 mg/kg as it does not have any toxic effect at same dose. The obtained results suggest that, tested polyherbal formulation (Anacid Tablet) has potential antiulcer and anti oxidant effect.

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1. Petalad Mahala Arogya Mandal Pharmacy, At.Po. Pipalata, Dist. Kheda, Gujarat, India.
2. Shree S.K. Patel College of Pharmaceutical Education And Research, Ganpat University, Ganpat Vidyanagar-384012, Gujarat, India.
3. J. S. Ayurved Mahavidyalaya, College Road, Nadiad - 387001, Gujarat, India.

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