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Acute oral toxicity study of Acideem Plus Tablet: An Ayurvedic polyherbal formulation

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Abstract

Objective: This study aimed to evaluate the acute oral toxicity of Acideem Plus Tablet in female wistar rats.

Materials and Methods: 12 rats were divided into four groups of three at random. Group 1 acted as the control and merely received distilled water. Acideem Plus Tablet, doses of 2000 mg/kg body weight, was given to groups 2 and 3, while group 4 received doses of 5000 mg/kg body weight. For 14 days, the rats were monitored for any indications of toxicity. At the end of the study period, all rats underwent necropsy, and gross pathology was documented.

Results: According to the results, neither the rats in the treatment group nor the control group displayed any clinical indications of toxicity or mortality over the course of the 14-day observation period. The LD_{50} value was found to be greater than 5000 mg/kg body weight.

Conclusions: The acute oral toxicity study of Acideem Plus Tablet in wistar rats concluded no adverse effect at doses up to 5000 mg/kg body weight. These results demonstrated the safety of the Acideem Plus Tablet's oral administration.

Keywords: Acideem Plus; Acute oral toxicity; LD50 value; GERD; Acid Peptic Diseases

1. Introduction

The term "gastro-oesophageal reflux disease" (GERD) often refers to a chronic, relapsing disorder where symptoms and/or difficulties are brought on by the reflux of stomach contents into the oesophagus and beyond. Heartburn and regurgitation, which are the main symptoms associated with gastro-oesophageal reflux, are common in the general population [1]. One of the most often diagnosed digestive conditions in the US, with a prevalence of 20%, it has a negative impact on quality of life. It imposes a heavy financial burden in the form of direct and indirect costs [2]. Proton pump inhibitors (PPIs) are a crucial and individualized treatment for GERD. Due to a mechanically or physiologically inadequate lower oesophageal sphincter, PPIs do not stop reflux even though they alter the pH of the reflux rate [3]. PPIs could have adverse consequences. Many patients choose to utilise conventional therapy because GERD is chronic and progressive [4]. According to the World Health Organization, the use of herbal treatments has grown two- to three-fold globally compared to the use of conventional medications [5]. Since ancient times, India has been using Ayurvedic medicine as a part of its traditional medical system. Due to ingrained beliefs that ayurvedic medicines are safe, many of them are utilised in clinical settings to treat a wide range of illnesses without having undergone any safety or toxicological studies. Though there have been reports of negative effects from various ayurvedic and herbal medications

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[6]. The lack of safety/toxicity data for these medications limits their acceptance and popularity on a global scale. Therefore, the safety and toxicity of Ayurvedic medicines and polyherbal formulations must be thoroughly documented [7].

Acute toxicity is usually defined as the unfavourable changes occurring immediately or within a short period of time after being exposed to a substance or substances once or for a short period of time or as unfavourable changes occurring after the administration of a single dose of a substance or multiple doses given within 24 hours [8]. An adverse effect is "any effect that results in functional impairment and/or biochemical lesions that may affect the performance of the whole organism or that reduce the organ's ability to respond to an additional challenge" [9]. A study by our group reported that Acideem Plus Tablet, a polyherbal formulation, exhibits safety and efficacy in treating Acid Peptic Diseases [10]. Acideem Plus Tablet is a polyherbal formulation consisting of Amala (*Emblica officinalis*), Mulethi (*Glycyrrhiza glabra*), Guduchi (*Tinospora cordifolia*), Sunthi (*Zingiber officinale*), Bael Ext (*Aegle marmelos*), Shankha Bhasma, Kapardik Bhasma, Shuddha Suvarngairik. Amla (*Emblica officinalis*) helps in reducing heartburn and regurgitation [11]. Mulethi (*Glycyrrhiza glabra*) was reported as having anti-H. Pylori effects [12]. Guduchi (*Tinospora cordifolia*) stem showed clear antiulcer activity in an in vivo study where a decrease in ulcer index and acid content volume have been reported [13]. The rhizome of the well-known herb sunthi (*Zingiber officinale*), which has anti-oxidant, anti-ulcer [14], anti-inflammatory, antitumor [15], carminative, diaphoretic, digestive and gastroprotective properties [16], contains 1-4% volatile oils that include therapeutically active components. Shankha Bhasma added to this formulation has a good effect on acid neutralising properties as an antacid [17,18]. Kapardik Bhasma has published evidence in the management of Acidity [19]. Furthermore, it has been demonstrated that Shuddha Suuvarnagirik effectively treats Amlapitta (Acidity) [20].

In accordance with OECD guidelines No. 423 [21], this study's objective was to investigate the acute oral toxicity profile of Acideem Plus Tablet in wistar female rats. This study may provide a scientific perspective on the safety of Acideem Plus Tablets.

2. Material and methods

2.1. Polyherbal formulation

The Polyherbal formulation Acideem Plus™ Tablets (Batch No.: ADP 2202) (https://pilospray.com/product/acideem-plus-30-tablets/) were obtained from Healing Hands & Herbs Pvt. Ltd. Pune, India., (www.myhealinghands.in) and it was manufactured under the GMP certified manufacturing unit at Eisen Pharmaceutical Co. (Pvt.) Ltd. Each Tablet contains Amala (*Emblica officinalis*), Mulethi (*Glycyrrhiza glabra*), Guduchi (*Tinospora cordifolia*), Sunthi (*Zingiber officinale*), Bael Ext (*Aegle marmelos*), Shankha Bhasma, Kapardik Bhasma, Shuddha Suvarngairik. In order to create 200 mg/ml and 500 mg/ml solutions, the test suspensions of 2000 mg and 5000 mg were prepared in distilled water. The oral mode of administration was chosen since it was the intended clinical route, based on human clinical dose (1 Tablet 12 hourly) [10], OECD/ WHO recommendations, and the indication of Acideem Plus Tablet. The rat was gavaged orally with just fresh suspension. A dose of 10 ml/kg of suspension was administered.

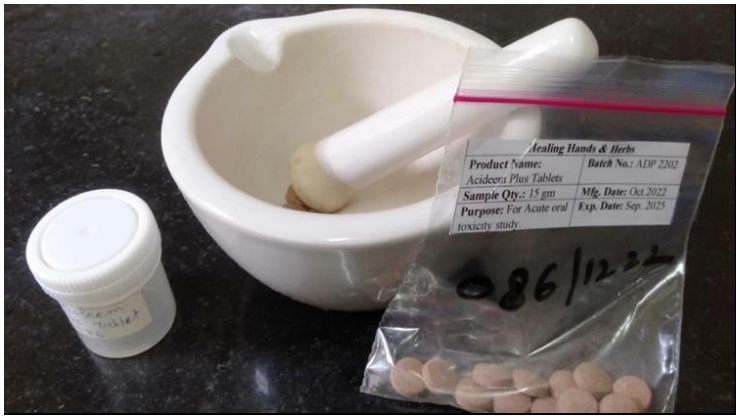

Figure 1 Acideem Plus Tablet dose preparation

2.2. Experimental Animals

The wistar rats were obtained from LACSMI Biofarms Pvt Ltd. Pune, Maharashtra, India. They were kept in cages made of stainless steel and maintained in a room with a 12-hour light/dark cycle, 30–70% humidity, 19–25^{0}C temperature range, and enough ventilation. All animals had complete access to food and water. Throughout the research period, animals were recognised by tail marking, and information from the cage cards was used to identify the group of animals housed in each cage. The IDRAL's standard operating procedures and the regulations established by the Committee for Control and Supervision of Experiments on Animals (CCSEA) were followed and authorised for publication in The Gazette of India on December 15, 1998. Institutional Animal Ethics Committee (IAEC) protocol 086/1222 (IDRAL/IAEC-3-2022) was approved on December 23, 2022.

2.3. Acute oral toxicity study

An acute oral toxicity test was performed on female wistar rats in accordance with the Organization for Economic Co-operation and Development's (OECD's) Test Guidelines 423 [21] and utilising a fixed dose procedure. This method provides information on the substance's hazardous properties and allows it to be rated and classified in accordance with the GHS. The Globally Harmonized System (GHS) is a classification system for chemicals that produce acute toxicity. The OECD guidelines indicate that testing in one sex (usually females) is sufficient [21].

Figure 2 Female wistar rats before dose administration

Figure 3 Injecting Acideem Plus Tablet Suspension

This experiment was conducted with healthy female wistar rats weighing 180–189 g and aged 8–9 weeks. They were randomly assigned into 4 subgroups G1, G2, G3, and G4. Each group contained three rats, with Group 1 (G1) serving as the control group. Before the experiment began, the animals were acclimated in the experimental room for a minimum of five days. Prior to the dose, all rats were starved for the whole night. Three to four hours following the dose, food was given to the rats. The 2000 mg and 5000 mg test suspensions were made in distilled water to create 200 mg/ml and 500 mg/ml solutions, respectively, and all the rats were dosed orally in a constant dosage volume of 10 ml/kg body weight. The 14-day acute oral toxicity observation period was conducted on all animals. All the animals were observed

for 14 days for signs of acute oral toxicity. In accordance with the Globally Harmonized System (GHS) for chemical classification that causes acute toxicity, the Acideem Plus Tablet will subsequently be ranked and categorised.

Figure 4 Female wistar rats after dose administration

2.4. Observational Parameters

All animals were closely monitored for treatment-related clinical symptoms, morbidity, and mortality following oral administration of Acideem Plus Tablet at various time intervals of 30 min, 1 hr, 2 hr, 4 hr, and 6 hr post-dosing on the first day and once daily thereafter for 14 days.

2.5. Body weight

Body weight measurements were taken on the day before the dose was given (day 0), the day of the dose (fasting body weight), then every week after that until the day of the death. Group mean body weights and weight gain was calculated on day 7 and 14 of post-dosing.

2.6. Necropsy and Gross Pathology

At the termination of the study, all the surviving animals were humanely killed by carbon dioxide asphyxiation. All of the study animals underwent significant pathological alterations that were documented.

3. Results

3.1. Acute Oral Toxicity Assessment

Table 1 Overall Incidence of Mortality after receiving polyherbal formulation (Acideem Plus Tablet) for 14-day observations in the acute oral toxicity study

Group	Dose (mg/kg)	Mortality	
		Females	
		Absolute	%
G1	0	0/3	0
G2	2000	0/3	0
G3	2000	0/3	0
G4	5000	0/3	0

The Acideem Plus Tablet was administered orally to female wistar rats at doses of 2000 mg/kg and 5000 mg/kg, and it had no adverse effects on the rat's health. All of the animals behaved normally during the course of the investigation and persisted through the entire 14-day testing period. In the vehicle control group (G1), all animals were healthy, and no deaths were observed throughout the post-dosing observation period of 14 days (Tables 2 & 3). Additionally, none

of the animals in the control group (G1) (Table 4) exhibit any unusual characteristics in terms of their individual fates or necropsy results.

Table 2 Individual Animal Clinical Signs & Mortality (G1) (Distilled water treatment)

Animal ID	**Observation at: hrs.**					**Days**												
	½	**1**	**2**	**4**	**6**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
	Female wistar Rats																	
1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Total mortality	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mortality %	0																	

N-Normal

Table 3 The rat body weight (G1) (Distilled water treatment)

Group	**Animal ID**	**Sex**	**Dose (mg/kg)**	**Body weight(g) before and after receiving distilled water**			**Weight gain**	
				Day 0	**Day 7**	**Day 15**	**% Gain (Day 7)**	**% Gain (Day 15)**
G1	1	Female	0	181	196	211	8.29	16.57
	2			182	195	218	7.14	19.78
	3			182	192	211	5.49	15.93
Mean ± SD				181.66 ± 0.57	194.33 ± 2.08	213.33 ± 4.04	6.97 ± 1.40	17.42 ± 2.06

The formula for the % weight gain is as follows:

$$\% \text{ Weight Gain (Day 7 or 15)} = \frac{\text{Day 7 or 15 (Weight after 7 or 15 days)} \times 100}{\text{Day 0 (Initial weight)}}$$

% Weight Gain (Day 7 or 15) = Answer-100

Table 4 Individual animal fate and necropsy finding (G1) (Distilled water treatment)

Animal No.	**Fate**	**Necropsy Findings**	
		External Observations	**Internal Observations**
1	TS	NAD	NAD
2	TS	NAD	NAD
3	TS	NAD	NAD

NAD: No Abnormalities Detected; TS: Terminal Sacrifice

Acideem Plus Tablet was administered to the animals in groups 2 and 3 (G2 & G3) at a dose of about 2000 mg/kg. No deaths were reported during the 14-day post-dosing monitoring period, and all the animals were in good health. (Table 5, 6 & 7). Additionally, neither the necropsy results nor the individual animal fate for any of the animals in groups 2 or 3 revealed any discrepancies (Table 8).

Table 5 Individual Animal Clinical Signs & Mortality (G2) (2000 mg/kg)

Animal ID	**Observation at: hrs.**					**Days**												
	½	**1**	**2**	**4**	**6**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
	Female wistar Rats																	
4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Total mortality	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mortality %	0																	

N-Normal

Table 6 Individual Animal Clinical Signs & Mortality (G3) (2000 mg/kg)

Animal ID	**Observation at: hrs.**					**Days**												
	½	**1**	**2**	**4**	**6**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
	Female wistar Rats																	
7	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
8	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
9	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Total mortality	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mortality %	0																	

N-Normal

Table 7 The rat body weight (G2 & G3) (2000 mg/kg)

Group	**Animal ID**	**Sex**	**Dose (mg/kg)**	**Body weight(g) before and after receiving Acideem Plus Tablet**			**Weight gain**	
				Day 0	**Day 7**	**Day 15**	**% Gain (Day 7)**	**% Gain (Day 15)**
G2	4	Female	2000	184	193	214	4.89	16.30
	5			189	196	216	3.70	14.29
	6			190	199	218	4.74	14.74
Mean ± SD				187.66 ± 3.21	196 ± 3.0	216 ± 2.0	4.44 ± 0.64	15.11 ± 1.05
G3	7	Female	2000	187	192	216	2.67	15.51
	8			185	196	218	5.95	17.84
	9			186	197	216	5.91	16.13
Mean ± SD				186 ± 1.0	195 ± 2.64	216.66 ± 1.15	4.84 ± 1.88	16.49 ± 1.20

Table 8 Individual animal fate and necropsy finding (G2 & G3) (2000 mg/kg)

Animal No.	Fate	Necropsy Findings	
		External Observations	Internal Observations
4	TS	NAD	NAD
5	TS	NAD	NAD
6	TS	NAD	NAD
7	TS	NAD	NAD
8	TS	NAD	NAD
9	TS	NAD	NAD

NAD: No Abnormalities Detected; TS: Terminal Sacrifice

Animals in Group 4 (G4) received a dose of roughly 5000 mg/kg of Acideem Plus Tablet. No deaths were noted throughout the 14-day post-dosing monitoring period, and all the animals remained healthy. Furthermore, none of the animals in Group 4 showed any anomalies based on the individual animal fate or the necropsy results.

Table 9 Individual Animal Clinical Signs & Mortality (G4) (5000 mg/kg)

Animal ID	Observation at: hrs.					Days												
	½	1	2	4	6	2	3	4	5	6	7	8	9	10	11	12	13	14
	Female wistar Rats																	
10	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
11	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
12	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Total mortality	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mortality %	0																	

N-Normal

Table 10 The rat body weight (G4) (5000 mg/kg)

Group	Animal ID	Sex	Dose (mg/kg)	Body weight(g) before and after receiving Acideem Plus Tablet			Weight gain	
				Day 0	Day 7	Day 15	% Gain (Day 7)	% Gain (Day 15)
G4	10	Female	5000	188	198	214	5.32	13.83
	11			189	196	216	3.70	14.29
	12			184	197	218	7.07	18.48
Mean ± SD				187 ± 2.64	197 ± 1	216 ±2	5.36 ± 1.68	15.53 ± 2.56

Kruskal Wallis test was used at a 5% level of significance to check the percentage weight gain in wistar rats varies with the dose.

H0: The median percent weight gain across the three-dose level is equal. Vs. **H1:** At least one of the median percent weight gains is different from others

Where,

H0 = Null Hypothesis and **H1**= Alternative Hypothesis

P- value for the above test is 0.3377 (> 0.05) so we accept the null hypothesis at a 5% level of significance. i.e., group population medians are equal. It shows that the weight gain percentage in wistar rats does not differ significantly across the dose.

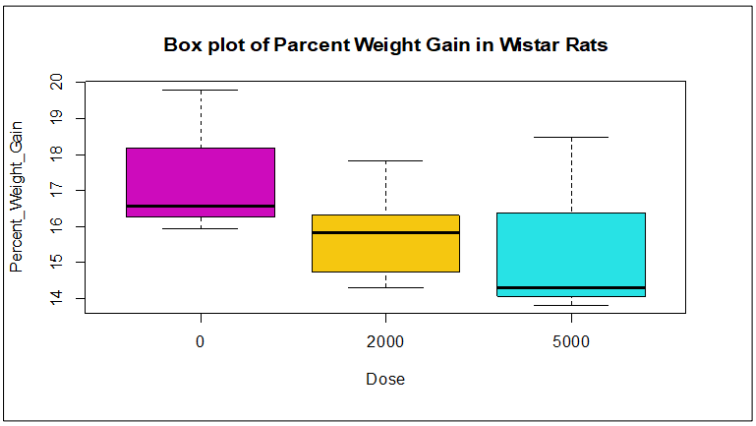

Figure 5 Box plot of Percent weight gain in wistar rats

Table 11 Individual animal fate and necropsy finding (G4) (5000 mg/kg)

Animal No.	Fate	Necropsy Findings	
		External Observations	Internal Observations
10	TS	NAD	NAD
11	TS	NAD	NAD
12	TS	NAD	NAD

NAD: No Abnormalities Detected; TS: Terminal Sacrifice

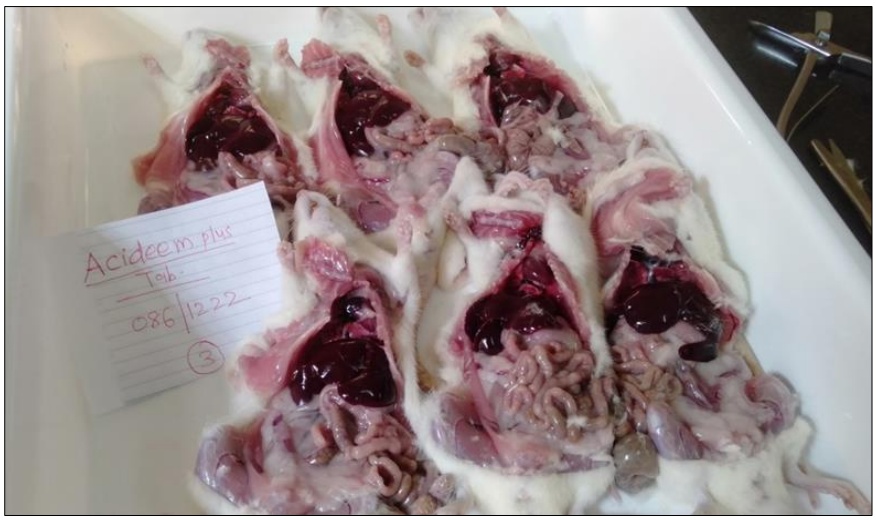

Figure 6 Gross Pathology Image of Vital organs – Liver, kidney, Heart, Spleen, Adrenals, Sex organs & G.I

4. Discussion

The use of herbal treatments has seen a significant rise in interest globally, along with corresponding improvements in phytomedicinal therapy. Herbal treatments have established themselves as dietary supplements, mono- or polyherbal medications, food additives, etc. They have also developed into recognizable and reliable economic commodities [22]. The widespread use of herbal preparations/remedies has been influenced by the belief that they are both safe and effective, especially in rural areas where they can be used for extended periods of time without worrying about the dose or concentration that will result in toxic side effects [23]. Therefore, a scientific assessment of oral toxicity is required and will aid in identifying the safe dose ranges. Acideem Plus Tablet is an ayurvedic polyherbal formulation used in Acid peptic diseases [10]. This current study evaluated the Acute oral toxicity study of Acideem Plus Tablet in female wistar rats. After receiving the study product in a single dosage of 2000 and 5000 mg/kg, acute tests did not cause mortality in rats. According to OECD guidelines [21], it indicates that the LD_{50} is more than 5000 mg/kg. As a result, the Acideem Plus Tablet is classified in category 5 of the Global Harmonization System of Chemical Substances. Throughout the administration of study drug suspension at all dosages, there were no statistically significant changes in the percentage weight gain in the animals seen when compared to the control animals. Also, throughout the study period, no significant differences were observed in the food and water consumption of any of the animals when compared with the control. Additionally, after 15 days of the study, when the internal organs were evaluated, there was no sign of any change in any organ compared to the control group.

5. Conclusion

The acute oral toxicity study of Acideem Plus Tablet in wistar rats demonstrated no adverse effects at doses up to 5000 mg/kg body weight. Based on the parameters of the current study and the data gathered, the acute oral LD50 of Acideem Plus Tablet in wistar rats is greater than 5000 mg/kg body weight, and the GHS classification category is 5 or Unclassified (>5000). These results proved the oral administration of the Acideem Plus Tablet was safe.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Authors declared no conflict of interest.

Statement of ethical approval

All the animals were handled in accordance with regulations established by the Committee for Control and Supervision of Experiments on Animals (CCSEA).

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