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ANTI-ULCER EFFICACY AND SAFETY OF ANDA LEGHYAM A POLYHERBAL SIDDHA FORMULATION

G.J. Christian*¹, H. Mubarak², K. Yamini³, T. Onesimus⁴, S. Elansekaran¹, K. Vennila¹ and L. Janani¹

National Institute of Siddha¹, Tambaram Sanatorium, Chennai – 47, Tamil Nadu, India

Siddha Clinical Research Unit², Palayamkottai, Tamil Nadu, India

Department of Pharmacognosy, DCRM Pharmacy College³, Inkollu, Andhra Pradesh, India

Department of Pharmacology, DCRM Pharmacy College⁴, Inkollu, Andhra Pradesh, India

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Correspondence to Author:

G. J. Christian

Associate Professor,
National Institute of Siddha,
Autonomous Institution under
Dept. of AYUSH
Ministry of Health & Family
Welfare, Govt. of India, Tambaram
Sanatorium,
Chennai - 47, Tamil Nadu, India.

E-mail: christianvijila@gmail.com

ABSTRACT: *Anda leghyam* (AL) a herbo-animal Siddha formulation is administered for *Gunmam* (Acid peptic diseases) by many Siddha physicians popularly. Anti ulcerogenic activity of the AL has been studied using Aspirin-Pylorus ligation induced gastric ulcer methods (four groups/method, n=6, Aspirin 400mg/kg, Omeprazole 10mg/kg, AL 250,500mg/kg) in wistar albino rat models. Animals were sacrificed and their stomachs were subjected to macroscopic and microscopic ulcer index findings. Statistical data were analyzed by one way ANOVA followed by student's t-test. This study concluded that AL had significant anti-ulcer effects in experimental animals with ulcer induced by aspirin and pylorus ligation; it showed a dose dependent protection against aspirin (400 mg/kg body weight) induced ulcers in rats and it produced a significant reduction of ulcer index in the dose of 500mg/kg bodyweight. AL showed a statistically significant P value < 0.05 and <0.01 at dose level 250 mg/kg and 500 mg/kg respectively as compared to control. Acute toxicity study was carried out as per OECD guidelines and the LD50 was found to be greater than 2000mg/kg body weight with no histopathological or behavioral changes in the experimental animals.

INTRODUCTION: Siddha system of medicine is an ancient medical system being practiced in the southern part of Indian peninsula since ages. This system was conceived and crafted by the super intellectual Sagely Mystics called Siddhars owing to their divine wisdom bestowed on them.

Literature of Siddha system is mainly propagated in Tamil language. Siddha *Materia Medica* comprises of Herbs, metallo-mineral salts and animal and animal products. There are 32 types of dosage forms in Internal medicine of Siddha system according to literature, of which 'Leghyam' (a pliable and palatable medicinal confectionary stuff-electuary) is a household name in India¹. Sage Yugi the founding Father of Siddha pathology, has classified the conditions of acute and chronic abdomen into eight broad types under the title '*Gunmam*' which means the patient doubles up in abdominal pain and depressed².

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It is said to be a result of disequilibrium of three humors viz. Vatham (Gas energy), Pitham (Heat energy) and Kabam (Water energy) due to dietary reasons. Though AL is broadly indicated for a spectrum of abdominal disorders (*Gunmam*), it is presently the mainstay of treatment instituted by the Siddha physicians for Acid peptic disorders with excellent clinical efficacy observed³. *Anda leghyam* ('Anda' means egg) is formulated with Hens' egg white, *Cuminum cyminum* L. seeds, *Trachyspermum ammi* L.⁴ seeds, lemon juice, ghee and honey. This present study is aimed to validate the above traditional Siddha treatment by evaluating the anti-ulcer activity of AL using peptic ulcer rat models with proton pump inhibitor (PPI) Omeprazole as a standard drug⁵.

MATERIALS AND METHODS:

Anda leghyam and Chemicals: AL was purchased from a registered pharmacy. All chemicals used in the present study were of analytical grade and purchased from Micro Labs, Pondicherry. Aspirin and Omeprazole (Standard drugs) were obtained from Hetero Labs, Hyderabad.

Stock solution preparation: The AL was dried and powdered. It was mixed uniformly in 2% CMC solution to achieve 250 and 500 mg/ml as main stock solution and used in this study.

Treatment of animals: Healthy male and female rats weighing 150-200g (Wistar albino) of 4-8 weeks old were selected after physical and behavioral veterinary examination from Institutional Animal House of TRR College of Pharmacy, Hyderabad. The weight range fell within $\pm 20\%$ of the mean body weight for each sex at the time of initiation of treatment. All experiments involving animals complied with the ethical standards of animal handling and were approved by Institutional Animal Ethics Committee (1447/PO/a/11/CPCSEA).

All the selected animals were kept under acclimatization on the same day. The animals were acclimatized for a minimum of 5 days before initiation of dosing. The rats were housed in standard polypropylene cages with stainless steel top grill in groups of 6 rats per cage. Clean autoclaved paddy husk was used as bedding. The paddy husk was changed at least thrice in a week.

The animals were kept in a clean environment with 12-hour light and 12-hour dark cycles. The air was conditioned at $22 \pm 3^\circ\text{C}$ and the relative humidity was maintained between 30-70% with 100% exhaust. Standard rat pellet feed was provided *ad libitum* throughout the study, except overnight fasting prior to blood collection and was offered the feed immediately after completion of blood collection of all the animals.

R.O drinking water was provided *ad libitum* in polypropylene bottles with a stainless steel Sipper tubes throughout study period.

Acute toxicity study: Acute oral toxicity test was carried out as per the guidelines of Organization of Economic Co-operation and Development (OECD), TG 425⁶. Six female albino rats (nulliparous and non-pregnant; 140-160g body weight) were randomized into two groups (3 per group) viz. control and test groups. Animals were kept on overnight fasting and provided water *ad libitum*. Control group received 0.5% carboxy methyl cellulose (prepared in double distilled water) as vehicle at a dose volume of 10ml/kg b.w.t while the test group received single oral dose of 2000mg/kg b.w.t of AL by gavage using a stomach tube.

The dose of the trial drug was determined by the fasted body weight. After the drug had been administered, food was withheld for a further period of two hours. The dose administered was assigned as toxic, if mortality were to be observed in two out of three animals. The animals were observed carefully for mortality and toxic symptoms (general behavior, respiratory pattern, cardiovascular signs, motor activities, reflexes and changes in skin and fur texture) at 30 min, 1, 2 and 4 hours and thereafter once a day for the next 72 hours following AL and vehicle administration⁸.

Body weight was recorded. There was no change in the animal behavior after administration of the trial drug at the dosage level of 2000mg/kg b.w orally (Table 1). LD 50 cut-off value of AL was determined as per the globally harmonized system of classification and labeling of Chemicals⁷.

Aspirin-induced gastric ulcer: Animals were divided into four groups, with each group containing six animals (n = 6).

First group served as control, and were administered Aspirin 400mg/kg, second group served as a positive control and were treated with standard drug Omeprazole (10 mg/kg), third and fourth group served as test groups and were administered AL at the dose level 250 and 500 mg/kg respectively. All the above drugs and vehicle were administered 30 minutes before the administration of aspirin (400mg/kg) orally^{9, 10}. After six hours, animals were sacrificed; their stomachs were removed and 2% formalin was injected into them.

All the stomachs were opened along the greater curvature and immersed in 2% formalin solution. The length of each lesion was measured under the dissecting microscope. The sum of the lengths (mm) of all lesions for each rat was used in the calculation of lesion index. Lesions in the stomach were graded according to the following scale: 0 = normal gray colored stomach, 0.5 = pink to red coloration of stomach, 1 = spot ulcer, 1.5 = hemorrhagic streaks, 2 = number of ulcers <5, 3 = number of ulcers >5, 4 = ulcers with bleeding.

Ulcer index was calculated by adding the total number of ulcers plus the severity of ulcer. The ulcer score was determined on inspection using a 10 × magnifying hand lens. The scoring of severity of ulceration was as follows: 1 mm (pin point) = 1; 1-2 mm = 2; > 2 mm = 3; > 3 mm = 4. The mean ulcer score was determined by dividing the total ulcer indices in a group by the total number of animals in that group^{11, 12}.

Ulcer Score = Total ulcer index (UI) in a group / Total number of animals in that group.

Ulcer index¹³:

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

U_I = Ulcer Index

U_N = Average number of ulcers per animal

U_S = Average number of severity score

U_P = percentage of animals with ulcers

Determination of Percentage protection:

% Protection =

Control mean ulcer index - Test mean ulcer Index

Control mean ulcer index

Pylorus- Ligation induced gastric ulcer: Male albino rats weighing 150-200g were selected for pyloric ligation ulcer model¹⁴. Rats were divided into four groups, each group consisting of six animals. Animals were fasted for 24 hours. One group received normal saline 2 ml/kg (negative control), the second group received Omeprazole 10 mg/kg by oral route (positive control) and the third and fourth groups received AL at dose level of 250 and 500 mg/kg by oral route respectively, 30 min prior to pyloric ligation. 4 hours later all animals were sacrificed by decapitation and the stomach was opened to collect the gastric contents.

The total volume of gastric content was measured. The gastric contents were centrifuged at 1000 rpm for 10 min. One ml of the supernatant liquid was pipetted out and diluted to 10 ml with distilled water. The solution was titrated against 0.01N NaOH using Topfer's reagent as indicator, to the endpoint when the solution turned to orange color. The volume of NaOH needed was taken as corresponding to the free acidity. Titration was further continued till the solution regained pink color. The volume of NaOH required was noted and was taken as corresponding to the total acidity. Acidity was expressed as;

$$\text{Acidity} = \text{Volume of NaOH} \times \text{Normality} \times 100 \text{ mEq}/0.1$$

RESULTS AND DISCUSSION: Acid peptic disease conditions like peptic ulcer, gastritis, gastric esophageal reflux disease have long been recognized as some of the most debilitating and common gastrointestinal problems. Peptic ulcer denotes the condition in which there is a discontinuity in the entire thickness of the gastric or duodenal mucosa that presents as a result of acid and pepsin in gastric juice and, gastritis, a diffuse inflammation with pin pointed gastro-duodenal mucosal lesions. Acid peptic disorders (APD) are common and potentially serious conditions which require a well-targeted therapeutic strategy. It includes treatment with number of drugs such as proton pump inhibitors, H2 receptor antagonists and antacids¹⁵. Acid peptic diseases occur due to an imbalance between aggressive (acid, pepsin) and defensive (gastric mucosal barrier) factors of gastric mucosa¹⁶.

The ulcer index parameter was used for the evaluation of ulcer severity. Moreover the disturbance of defensive factors like mucus secretion, bicarbonate secretion and mucosal blood flow has been reported to cause ulcer. With the ever growing interest in herbal and traditional medicine, this formulation has been screened and reported here to be useful in treating and managing acid peptic disease in the natural dietary way, than managing with synthetic drugs just as the dictum of Siddha medicine rightly says, '*food is administered*

as medicine (curative) and medicine is served (preventive) as food'. Though this formulation is in clinical practice for years, it has not been validated so far by screening for the anti-ulcer activity. So this is a maiden attempt of reverse pharmacological analysis validating this age old gastro intestinal remedy here.

The study elucidates that AL has significant anti-ulcer effect by virtue of its anti-secretagogue and antacid/acid neutralizing activities on experimental animals with ulcer induced by aspirin (**Fig. 1-6**).



FIG. 1: CONTROL (ASPIRIN 400MG/KG)



FIG. 2: STANDARD (OMEPRAZOLE 10 MG/KG)



FIG. 3: ANDA LEGHYAM (250MG/KG)



FIG. 4: ANDA LEGHYAM (500MG/KG)

It showed a dose dependent protection against aspirin (400mg/kg body weight) induced ulcers in rats and it produced a significant reduction of ulcer index in the dose of 500mg/kg body weight. AL showed statistically significant P values < 0.05 and < 0.01 at the dose levels of 250mg/kg and

500mg/kg respectively as compared to control. The resultant anti ulcerogenic activity of AL by virtue of its anti secretagogue property in pylorus ligation model is evident (**Table 1, Fig. 6**) from its significant reduction in gastric volume, total and free acidity.

TABLE 1: EFFECT OF ANDA LEGHYAM AGAINST ASPIRIN AND PYLORUS LIGATION INDUCED GASTRIC ULCER IN RATS

Treatment	Dose (mg/Kg b.wt)	Ulcer index	% Inhibition	pH	Free acidity	Total acidity	Vol. of gastric juice
Control	400	29.6±15	-	2.0±0.08	31.3±2.8	82.3±4.63	4.48±0.117
Standard	10	11.6±0.8	60%	3.68±0.20	17.3±1.8	43.5±2.88	2.68±0.18**
Test dose	250	18.80±1.5	40%	2.85±0.10	25.1±2.3	59.7±2.95	4.28±0.09*
Test dose	500	10.44±2.1	54%	2.97±0.50	18.3±1.6	47.5±4.96	3.15±0.16**

Results are shown in Mean ±S.E, one way ANOVA followed by Dunnet t test *P<0.05, **P<0.01 when compared with control

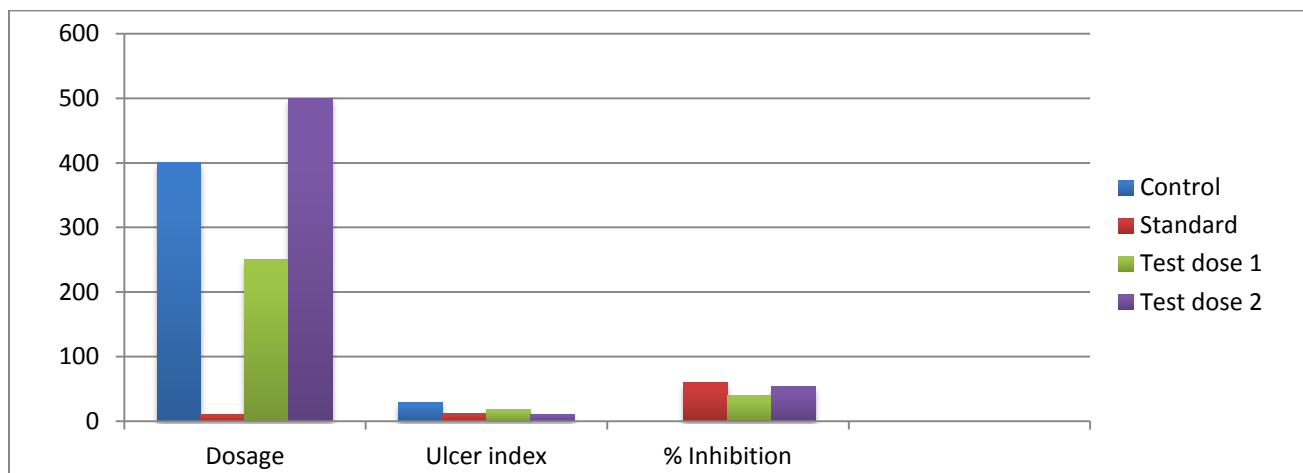


FIG. 5: EFFECT OF AL ON PYLORUS LIGATED RAT MODEL INDICATING DOSAGE, ULCER INDEX AND PERCENTAGE OF INHIBITION

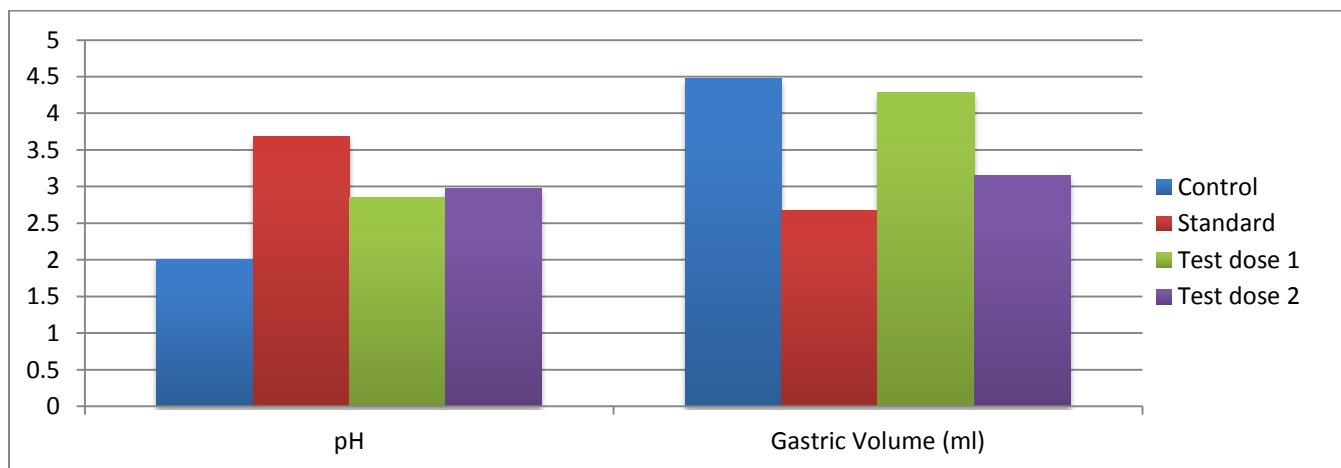


FIG. 6: EFFECT OF AL ON PYLORUS LIGATED RAT MODEL INDICATING PH AND GASTRIC VOLUME OF GASTRIC JUICE

There were no treatment related toxicity signs or death observed in both the control and test groups throughout the study. No significant change in body weight was observed (Table 2).

TABLE 2: ACUTE TOXICITY STUDIES OF AL- CHANGES IN THE ANIMAL BEHAVIOR AFTER ADMINISTRATION OF 2000 MG/KG

Animals' behavior/activities	Dosage	Mean weight of animals		Signs of toxicity	Onset of toxicity	Duration of study
		Before test	After test			
Activities*	2000 mg/kg	174 kg	180 kg	Nil	Nil	72 hrs

*Respiration, Writhing, Tremor, Convulsion, Salivation, Diarrhoea, Mortality, Hind limb paralysis, Sedation, Skin irritation, Eye irritation and CNS depression

The LD₅₀ value was found to be more than 2000mg/kg b.w.t. As per the reference of Globally Harmonized system of Classification and labeling of chemicals, *Anda leghyam* can be classified as Category-5 and this provides evidence that it is safe for administration in human and animals. And it is also evident that, AL may exert its action as an H₂ receptor antagonist or proton pump inhibitor which may be delineated in further study. It was observed that there was statistically significant decrease in ulcer index, gastric acid volume, total acidity, free acidity, ulcer score and also increase in pH of gastric juice.

Though the anti-secretagogue activity of AL (500mg/kg) was slightly less than that of omeprazole (**Table 1**), the ulcer index was slightly lower for AL group. Hence it may be deduced that AL has got other pharmacological activities also like antacid/acid neutralizing, ulcer healing etc. Thus the present study exhibit that the AL has significantly decreased the ulceration in aspirin induced and pylorus ligation induced ulcers in rats.

CONCLUSION: The safety of *Anda leghyam* is evident that there is no significant behavioral change or death in the animals noted in the acute toxicity study. Also the experimental studies on animal models demonstrated the protective and curative activities of the AL against gastric ulceration compared with the standard drug Omeprazole. AL at both doses (250mg/kg and 500 mg/kg) had shown anti-ulcer activity, but marked response was observed at the dose level of 500 mg/kg body weight and the results were more similar to that of the reference drug Omeprazole (10mg/kg).

Hence, it is concluded that the *Anda leghyam* significantly decreases stomach ulcerations in Aspirin and pylorus ligation rat models of stomach ulcer; and is safe for oral administration. Further studies are needed to determine the degree of

protection and mechanism of action of *Anda leghyam*.

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