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Research Article

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Acute toxicity study and Antiulcer Activity of Siddha Polyherbal Formulation Narasinga Rasayanam against Aspirin plus Pylorus Ligation Induced Ulcer in Rats

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Abstract

Narasinga Rasayanam (NR) a poly herbal Siddha formulation is administered for Gunmam (Acid peptic disease) by many Siddha physicians popularly. Anti ulcerogenic activity of NR has been studied using Aspirin- Pylorus ligation induced gastric ulcer methods (four groups/method, n=6, Aspirin 500mg/kg, Ranitidine 20mg/kg, NR 200,400 mg/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 NR had significant anti ulcer effects in experimental animals with ulcer induced by aspirin and pylorus ligation methods; it showed a dose dependent protection against aspirin (500 mg/kg body weight) induced ulcers in rats and it produced a significant reduction of ulcer index in the dose of 400 mg/kg body weight. NR showed a statistically significant P value < 0.05 and <0.01 at dose level 200 mg/kg and 400 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.

Keywords: Narasinga Rasayanam, Gunmam, Anti ulcerogenic activity, OECD guidelines.

Introduction

Siddha system of medicine is a holistic medical science which cares body and mind. Life style selections, adoption of functional foods and person oriented treatment regimen are the uniqueness of Siddha system of medicine. Health is an indispensable part of human beings. Siddha system of medicine defines health as a comprehensive composition of physical, mental, spiritual, emotional, and social wellness [1].The basic concept of Siddha is "*Food itself is a Medicine and Medicine itself is a Food*". Siddha system of medicine educates on how our food choices affect our health and well being. Since the beginning of mankind, people have used foods to not only fill their stomachs, but also to heal their bodies [2].

According to *Thiruvalluvar*, after digestion, eat in right measure is the way for possessor of the body to prolong its being. However with the development of chemical drugs and pills, they have forgotten that the food they eat are directly linked to their health. To save more time, many chose to grab a quick breakfast on their way to the office and eat while reading and watching the computer screen. These are indeed bad eating habits [3].The digestive process in the stomach

can be really stressful on the body. It requires a certain amount of blood supply in the stomach in order to digest and this is hard to meet if the body or brain is in motion. In long term such an unhealthy diet habit can undermine the digestion capacity of the human stomach and leads to many diseases such as Peptic ulcer (*Gunmam)* [4], gastritis, etc..

Peptic ulcer is produced by an imbalance between gastro duodenal mucosal defence mechanisms and the aggressive factors, particularly gastric acid and pepsin. It is one of the major diseases affecting the human population. About 10% of the population may develop peptic ulcer in their life time. It affects 9.5% among women and 10.5% among men[5]. Ulceration is reported for high chances of recurrence and mortality. Thus there is a need for more effective and safe anti-ulcer agents aiming to relieve pain, heal the ulcer and delay ulcer recurrence. Therefore herbal medicines are considered safer alternatives because of natural ingredients with no side effects [6].

The present study involves *Narasinga Rasayanam* one of the poly herbal Siddha preparation which is mentioned in *Sarabenthira Vaithiya Muraigal -Gunma Roga Sigitchai* text, indicated for *Gunmam* (Peptic ulcer), *Mahodharam* (Ascites), *Paandu* (Anaemia) and *Kuttarogankal* (different skin diseases). So far no scientific studies were carried out to evaluate its medicinal values. Therefore, an attempt had been made to validate its traditional claim for its anti ulcer property by using the models of acute gastric lesions induced by pyloric ligation and aspirin in rats.

Materials and Methods

Preparation of *Narasinga Rasayanam* (NR):

The preparation of NR was based on the Siddha classical literature '*Sarabenthirar Vaidhyamuraigal – Gunmaroga Sigichai'* [7].

Collection and authentication of the test drug:

The ingredients of NR are *Kodiveli Ver* (*Plumbago zeylanica*), *Serankottai* (*Semecarpus anacardium*), *Thannervittan Kizhangu* (*Asparagus racemosus*), *Nerunjil* (*Tribulus terrestris*), *Nilapanai Kizhangu* (*Curculigo orchioides*), Palm jiggery, Honey, Cow's Ghee. All these raw materials were procured from a well reputed country shop in Parrys corner, Chennai and authenticated by Botanist, National Institute of Siddha. All the herbal ingredients were purified (detoxification) by the suitable method specified in the Siddha literature.

Method of Preparation:

Kodiveli, Serankottai, Thanneervittan, Nerunjil, Nilappanai Kizhangu were purified and dried in sun shade, and then it was made into fine powder separately and finally mixed together. After that in the mixture of all herbal powder, the ghee, honey and palm jiggery were added little by little and ground in a *Kalvam* (stone mortar) until it attained waxy consistency. Then it was stored in an air tight container.

Selection of experimental animals:

Healthy Wistar albino rats of either sex weighing (150-250 gms) were used for this study. The animals were obtained from animal house, TANUVAS, Madhavaram, Chennai. Animals were housed at a temperature of 24±2ºC and relative humidity of 30-70%. At 12:12 light, day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pellet. All the experimental procedures and protocols used in this study were reviewed by (IAEC) Institutional Animal Ethics Committee of C.L. Baid Metha, College of Pharmacy and were in accordance with the guidelines of the IAEC.

Acute toxicity studies

Acute toxicity study was conducted to determine the safe dose by staircase method. The overnight fasted rats were orally administered with NR suspended in 0.5% lukewarm water at limit test dose of 2000 mg/kg body weight. They were later on observed closely for 1 hr, frequently for the next 4 hrs, periodically once in 4 hrs and then on a daily basis, i.e. once 24 hrs. Animals surviving the first 24 hrs were observed for the next 14 days [8,9].

Anti- ulcer activity: Experimental design

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 ± 3°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.

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 500mg/kg, second group served as a positive control and were treated with standard drug Ranitidine 20mg/kg third and fourth group served as test groups and were administered NR at the dose level 200 and 400 mg/kg respectively. All the above drugs and vehicle were administered 30 minutes before the administration of aspirin (500mg/kg) orally [10, 11]. After six hours, animals were sacrificed; their stomachs were removed and 2% formalin was injected into them.

Stomach of animal was 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 [12, 13].

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

Ulcer index [14]:

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

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:

$$\% \text{ Protection} = \frac{\text{Control mean ulcer index - Test mean ulcer Index}}{\text{Control mean ulcer index}}$$

Pylorus- Ligation induced gastric ulcer:

Male albino rats weighing 150-200g were selected for pyloric ligation ulcer model [15]. 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 Ranitidine 20 mg/kg by oral route (positive control) and the third and fourth groups received NR at dose level of 200 and 400 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;

Acidity = Volume of NaOH x Normality x 100 mEq/0.1

Histopathological analysis:

The tissues were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and were embedded in paraffin wax. Serial sections were obtained using a microtome. This was followed by staining with hematoxylin and eosin. Parameters including mucosal congestion, edema, desquamation and necrosis were observed and recorded.

Results and Discussion

The study elucidates that NR 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. 1-4).**

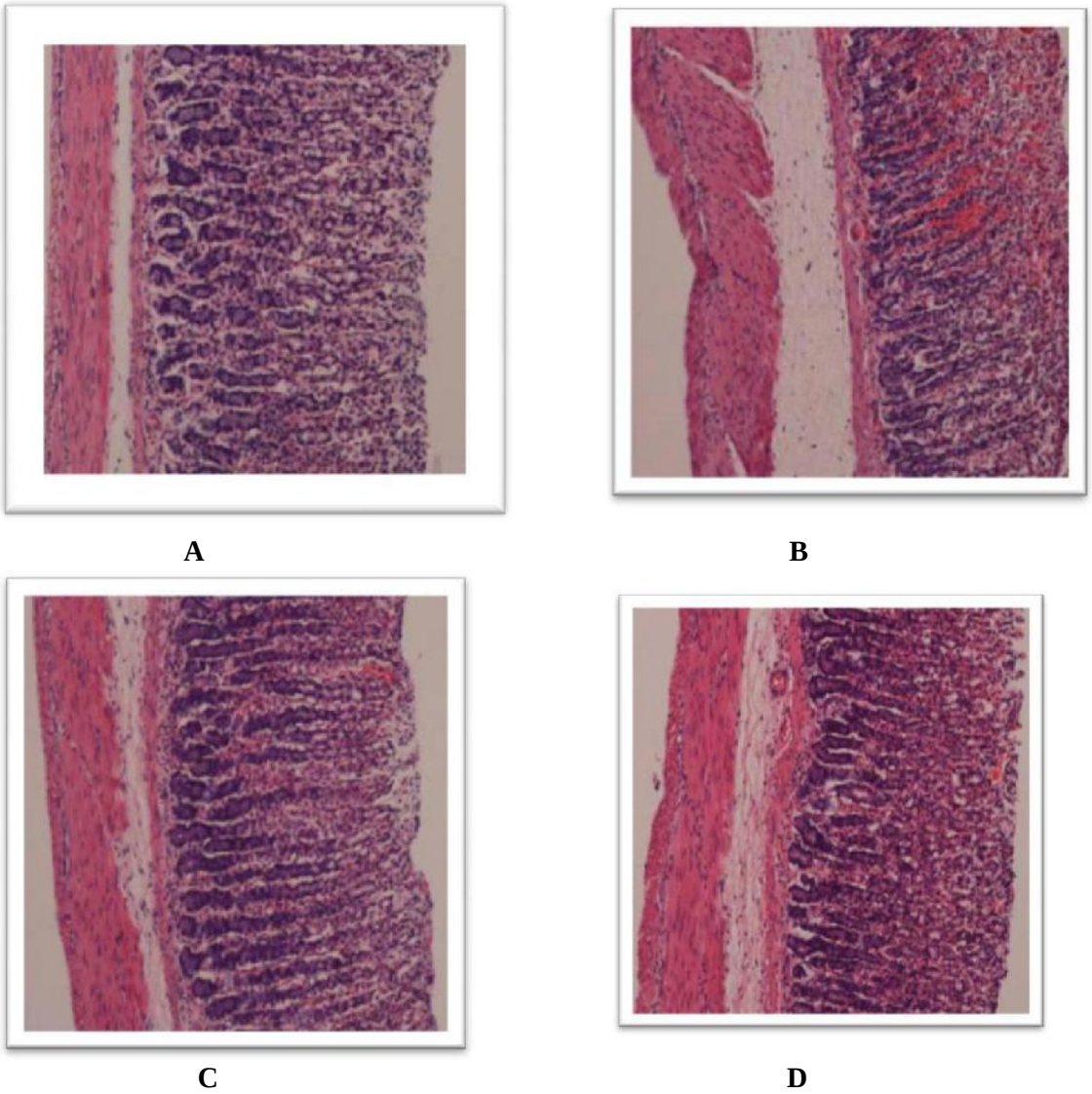

Figure Histopathological Examination of Open Excised Stomach in Aspirin Induced Ulcer Method:

A. Inhibition in gastric lesions at control
B. Gastric lesions induced by Aspirin (500mg/kg)
C. Absence of gastric lesions in Ranitidine (20mg/kg)
D. Fraction inhibition of gastric lesions at *Narasinga Rasayanam* (400mg/kg)

It showed a dose dependent protection against aspirin (500mg/kg body weight) induced ulcers in rats and it produced a significant reduction of ulcer index in the dose of 400mg/kg body weight. NR showed statistically significant P values < 0.05 and < 0.01 at the dose levels of 200mg/kg and 400mg/kg respectively as compared to control. The resultant anti ulcerogenic activity of NR by virtue of its anti secretagogue property in pylorus ligation model is evident **(Table 1)** from its significant reduction in gastric volume, total and free acidity.

Table: 1 Effect of *Narasinga Rasayanam* on Volume of gastric juice and pH

Group No.	Body weight (gms)	Treatment	Volume of Gastric juice	pH	Free acidity	Total acidity
I	181.3±1.51	Control(distilled water 2ml/kg)	4.02±0.11	1.84±0.14	26.84±0.08	70.16±0.30
II	181.5±0.84	Standard drug Ranitidine (20mg/kg)	1.94±0.06 **	4.96±0.18**	10.42±0.02**	22.24±0.18**
III	181.8±1.60	*Narasinga Rasayanam (NR)*(200mg/kg)	3.79±0.16 *	3.20±0.14	16.91±0.38*	27.08±1.06**
IV	181.3±1.63	*Narasinga Rasayanam (NR)*(200mg/kg)	3.28±0.21 **	3.88±0.16	13.68±0.02**	35.45±0.33**

Effects are statistically significant. *P<0.05;**P<0.01 (in comparison with standard). Values are expressed in terms of mean ± SEM of 6 rats (ANOVA).

The LD_{50} 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, *Narasinga Rasayanam* 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, NR 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 NR (400 mg/kg) was slightly less than that of Ranitidine 20mg/kg , the ulcer index was slightly lower for NR treated group. Thus the present study explores that the NR has significantly decreased the ulceration in aspirin induced and pylorus ligation induced ulcers in rats.

Conclusion

Hence, based on the results, it can be concluded that the *Narasinga Rasayanam*, significantly p<0.05 and p<0.01, decreased the ulceration in Aspirin induced ulcer model in rats which suggest a direct ulcer protective effect on the gastric mucosa at the dose level of 200mg/kg and 400mg/kg respectively. Furthermore, studies should be done to identify the active principle involved in the antiulcer activity of *Narasinga Rasayanam.*

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