Acidon Tablet (an herbal formulation) for the Treatment of Non-ulcer Dyspepsia: Acute toxicity, Preclinical and Clinical Trial

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Abstract

Background: Herbal medicines are becoming increasingly popular particularly for benign, chronic conditions that are self-managed, such as non-ulcer dyspepsia and provide relief from dyspeptic symptoms. Aim: This study aims to critically assess the scientific evidence for using herbal medicinal products for the treatment of non-ulcer dyspepsia. Material and method: Acidon, an herbal formulation of Shree Dhanwantri Herbals, was tested for acute toxicity, preclinical study and clinical study. Result: In acute toxicity study, Acidon treatment did not show any toxic symptoms as revealed by histopathological study of brain, lung, heart, kidney, liver, and spleen in rats. The preclinical studies conducted for the inhibitory effect of Acidon on Helicobacter pylori exhibited significant protection. A clinical study for exploring the effect of Acidon on non-ulcer dyspepsia was carried on patients who were given an oral dose daily for four weeks. Further, the biochemical parameters of the patients used in the clinical study were also found normal. Conclusion: A critical analysis of results revealed that Acidon tablets are safe and can be used for the treatment of non-ulcer dyspepsia.

Keywords- Helicobater pylori, Acidon Tablet, Acute toxicity, Non-ulcer dyspepsia, Herbal medicine

Introduction

Ancient Indian literature comprises a remarkably broad definition of medicinal plants and considers all plant parts to be potential sources of medicinal substances.[1] Herbal medicines have a lengthy therapeutic history and are currently used to treat a huge portion of the world's population.[2] Recently, it has gained extreme research importance for its nutraceutical values. It has been confirmed by WHO that the herbal medicines serve the health needs of about 80% of world's population especially for millions of people in the vast rural areas of developing countries. However, due to the great diversity of chemical components involved, quality control and quality assurance remain a concern. Herbal medications comprise multiple chemicals in complex matrices in which no single active constituent is responsible for overall efficacy. As a result, establishing quality control standards and standardization of final herbal medications is challenging.[3] Each year, up to 40% of adults are affected by dyspepsia which is frequently characterized as functional (non-ulcer) dyspepsia. The defining symptoms are postprandial fullness, early satiation, or epigastric pain or burning in the absence of causative structural disease. [4] It is a lifestyle disorder caused due to erratic lifestyle, rapid socio-economic development and westernization of Asian lifestyles likely to be key factors in changing epidemiology. [5] Its prevalence range is observed in about 11-38.8% of the world population. In western countries, its prevalence is estimated to be around 25% while in India it is 30% around.[6] It is reported that 7.6% of Indians have significant Gastroesophageal reflux disease (GERD) symptoms. About 20-40% of patients, whose dyspepsia is investigated and have GERD, up to 10% have gastric or duodenal ulcers and a small percentage of patients have other diagnoses and up to 60% are diagnosed with Non-Ulcer Dyspepsia.[7] Herbal medicine is becoming increasingly popular, particularly for benign, chronic conditions that are self-managed, such as non-ulcer dyspepsia. Standard reference texts suggest that several herbal medicinal products are promoted for non-ulcer dyspepsia.[8] Although non-ulcer dyspepsia is predominantly a self-managed condition but still it accounts for a significant number of general practitioner consultations and hospital referrals. Herbal medicinal products are often used for the relief of dyspeptic symptoms aims to critically assess the evidence for and against herbal medicinal products for the treatment of non-ulcer dyspepsia.[9] *Helicobacter* is a gram-negative bacterium that colonizes the gastric or intestinal mucosa of many mammalian and avian hosts and induces histologic inflammation. *H. pylori* are significant human pathogens that cause gastritis, peptic ulcer disease and gastric cancers. In the present study, the Acidon tablet provided by Shree Dhanwantri Herbals was assessed for acute toxicity along with preclinical and clinical studies for non-ulcer dyspepsia.

Material and method

Collection of material

The herbal formulation Acidon Tablet was provided by Shree Dhanwantri Herbals, Amritsar, Punjab. Acidon tablet contains *Avipattikar Churna* (whole formulation), *Kamdudha Ras* (whole

formulation), *Suthashekhara Ras* (whole formulation), *Lauha Bhasma* (whole formulation), *Emblica officinalis* (pericarp), *and Glycirrhiza glabra* (root).

Ethical statement

The rats were kept in regular animal husbandry settings at the Central Animal Facility. The Animal Ethical Committee of Guru Nanak Dev University (GNDU), Amritsar, authorized the experimental protocol Approval no. 226/CPCSEA/2018/27. The rules and regulations of control and supervision of experimental animals, (CPCSEA) were followed according to the Ministry of Environment and Forests, Government of India. All experiments were performed by following the Animal Research: Reporting of In-Vivo Experiments (ARRIVE) guidelines. A clinical study of Acidon Tablet in patients with mild to moderate Non-Ulcer Dyspepsia was approved by the Institutional Ethics Committee of Rajiv Gandhi Govt. Post Graduate Ayurvedic College and Hospital, Paprola. The clearance for the proposed clinical research work was obtained before the commencement of trial (AYU/IEC/2019/1210). Written informed consent was obtained from the study subjects before the registration of patients in the trial. Patients were explained about various aspects of the clinical study including trial drug and their probable side effects if any. Detailed case record performa was prepared including details of the patients, disease, and demographic profile, detailed history followed by general physical examination, systemic examination and criteria for assessment.

Acute toxicity studies

Acute toxicity was conducted as per OECD-423 guidelines to determine the median lethal oral dose (LD_{50}) of Acidon tablet. In the present study, 10 female Wistar rats (200-220g) were divided into two groups with each having five animals. The first group was a control group with no treatment. The second group was administered with Acidon tablet (2000 mg/kg body weight) according to the OECD guidelines, Annexure 2d(1).[10] Rats were observed for 24 h followed by 14 days for body weight changes, signs of behavioral changes and (or) mortality.

Animals were weighed at the beginning and end of the study to assess the net effect of the test drug on the body weight, behavioral changes such as skin and fur coat as well as color, eye irritation, piloerection, handling, tremors, gait, respiration rate and mortality at specified intervals (OECD-423 guidelines).

At the end of the study, all animals were starved overnight sacrificed and their organs (Brain, Heart, Lungs, Liver, Kidney and Spleen) were collected and preserved in 10% neutral buffered formalin for studying anatomical changes. The fixed, collected organs were cut into 5 mm thick slices, dehydrated with graded concentrations of ethanol (70, 95, and 99 percent of absolute ethanol), cleaned in xylene, and embedded in paraffin wax. The embedded tissues (Brain, Lung, Heart, Liver, Kidney and Spleen) were sectioned at a thickness of 6 mm, stained with hematoxylin and eosin (H & E), and viewed under a light microscope, following Gurr's 1959 methodology.[11] With the microscope, the sections were imaged at a magnification of x100 (Nikon ECLIPSE Ti2).

Pre-clinical study

In this experiment, a 3 % percent solution of Tryptone soy broth was prepared. Culture flasks were inoculated with 100 µl of the log phase culture of *H. pylori* and were placed in CO_2 incubator at 37^{o}C with 10 % CO_2. The Acidon tablet extract was prepared at three different concentrations. The cultures were monitored each day till they reached OD_{550} value of 0.5. Out of this, 5 ml of culture broth was taken in vials to which 200 µl of Acidon tablet solution (5000, 10000 and 20,000 ppm) was added. Vials were placed in an incubator at 37^{o}C. OD_{550} was determined after 24 hours and percentage inhibition was calculated using the formula in equation 1

$$Equation\ 1 \quad = \frac{X - Y}{X} * 100$$

Where X is the absorbance of the culture

Y is the absorbance of different concentrations of the Acidon tablet.

Clinical study

A study was conducted on 10 selected patients. They were managed with Acidon tablet with a dose of 2 tablets (500 mg each) TID with water. The trial was for 4 weeks and patients were followed up weekly till the completion of therapy. Various parameters like Upper Abdominal Pain, Heartburn /Retrosternal burning sensation, Bloating, Bitter and Acidic belching, Fullness after meal/Flatulence, Nausea and Vomiting (occasional sour and bitter) were considered for scoring.[12] The scoring system was adopted for the assessment of these subjective parameters as given below: Grade 0- Absent; Grade 1: Occasional sensation; Grade 2: Occur sometimes and relieved by water and food; Grade 3: Frequent feeling relieved by antacids; Grade 4: Frequent feeling relieved by antacids.

Data was collected and recorded in detail in clinical Performa. The obtained data were analyzed statistically and expressed in the terms of the mean score before treatment (BT), after treatment (AT), the difference of means (BT-AT), Standard deviation (SD) and standard error (SE). Overall percentage improvement of each patient was calculated. The overall assessment of the effect of therapy on the patients was categorized according to the following grades. The symptoms were evaluated and the response of the drug was recorded in terms of improvement of symptoms as given: Total symptoms free-100%; Marked improvement in symptoms-76-99%; Moderate improvement-51-75%; Slight improvement-26-50%; No improvements: ≤ 25%.

Biochemical Analysis

Biochemical parameters like Total Serum Bilirubin, Direct Serum Bilirubin, Serum Glutamate Oxaloacetic Transaminases, Serum Glutamate Pyruvic Transaminases, Blood Urea and Serum Creatinine were carried out using Erba kits.

HPLC fingerprinting

HPLC instrument system (Shimadzu, Kyoto, Japan) consisted of auto-sampler, stable thermostatic, column compartment, a quaternary pump with a vacuum degasser, and a UV detector. The column used in analysis was a reverse phase C18G column (250 × 4.6 mm, 5 µm) and column temperature was maintained at 30˚C. The mobile phase consisted of a binary solvent system, Solvent A: 900 mL of HPLC grade water was taken, to which 1.36 g KH2PO4 and 5 mL ortho-phosphoric acid were added. Volume was made up to 1000 mL and filtered through a 0.22 µm membrane filter. Solvent B: Acetonitrile (HPLC grade). Gradient elution program was used to run the mobile phase as 0 min, 5% solvent B; 8 min, 5% solvent B; 15 min, 40% solvent B; 30 min, solvent B 45%; 35 min, 5% solvent B; 40 min, 5% solvent B. Flow rate was set at 1.0 mL/min, injection volume was20 µL and peaks were detected at the wavelength of 280 nm. [13]

Results

Acute toxicity study

The results of changes in body weight and behavioral changes are presented in Figure 1 and Table 1. It was observed that the bodyweight of animals increased in both the control and the treated group that indicating that this herbal formulation did not affect the appetite of animals (Fig 1). As reported in the literature, to determine the effect on target organs, mechanism of action and the variation in organ weight is important.[14] An increase in body weight is not regarded as a sign of toxicity instead decrease in the body weight can be an indication of the harmful effect.[15]

Furthermore, all animals were subjected to various observations such as skin and fur coat as well as color, eye irritation, piloerection, handling, tremors, gait, respiration rate and mortality. Animals were lethargic for about 2 minutes after dose administration. There was no sign of skin and/or eye irritation, tremors, convulsion, salivation, sleep, or coma as well any mortality as in Table 1.

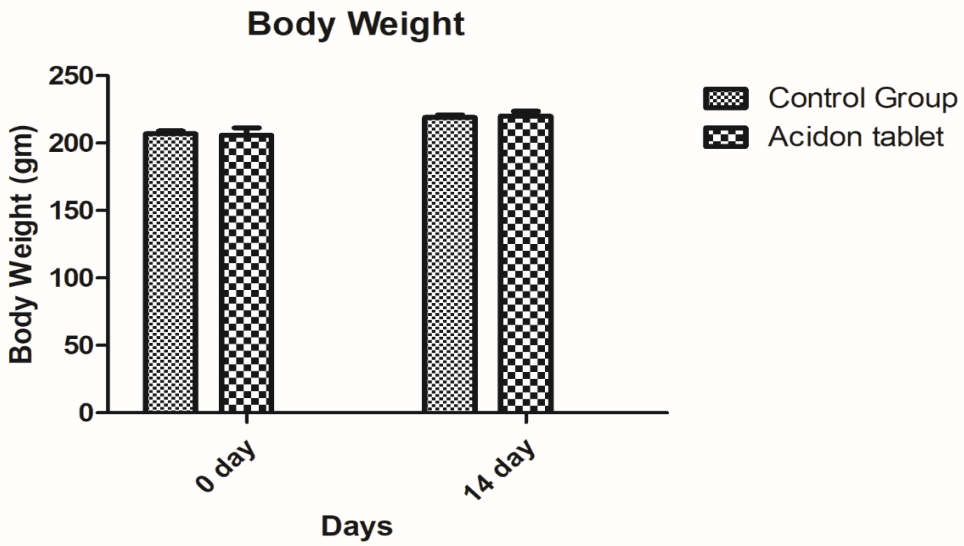

Fig1: Body weight changes in animals administered with Acidon tablet. Data includes Mean±S

Table 1: Behavioral changes observed in animals treated with Acidon tablet.

Formulation	Observation Parameter	Change at Specific time interval (hr)									
		0 mir	0-30 mi	1 hr	2 hr	3 hr	4 hr	4-12 hr	12-24 h	After 24 hr	After 14 da
Acidon Tablet	Skin	N	N	N	N	N	N	N	N	N	N
	Fur	N	N	N	N	N	N	N	N	N	N
	Eye	N	N	N	N	N	N	N	N	N	N
	Piloerection	N	N	N	N	N	N	N	N	N	N
	Respiratory Pattern	N	N	N	N	N	N	N	N	N	N
	Handling	N	N	N	N	N	N	N	N	N	N
	Lethargy	P	P	N	N	N	N	N	N	N	N
	Tremor	N	N	N	N	N	N	N	N	N	N
	Gait	N	N	N	N	N	N	N	N	N	N
	Mortality	N	N	N	N	N	N	N	N	N	N

*N- Normal

Histopathological details of organs were normal. No sign of toxicity was found in the tissues of the organs (brain, lungs, heart, kidney, liver and spleen). The architecture of the brain shown in Figure 2 (Control group) and Figure 3 (Treated group) was normal with glial cells and fibrillary material. As there was no difference in the architecture of the brain between the control and treated groups, therefore, it can be concluded that herbal Acidon tablet had no toxic effect on brain cells. Similarly, in other organs also normal architecture was found in control as well as treated groups in the histological reports. This data points towards the notion that the Acidon tablet didn't cause any harm in the organs at the tested dose for 14 days

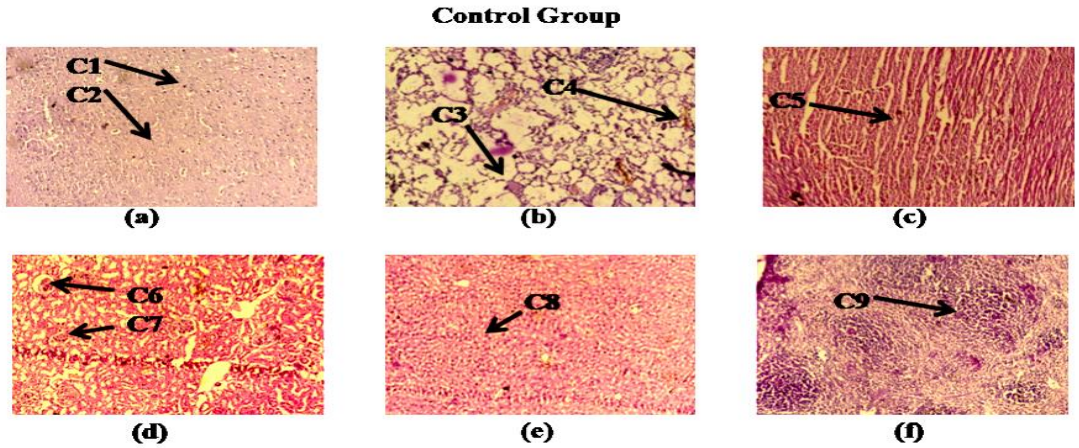

Fig 2: Histopathological study of different organs of control animals. (a) Brain, (b) Lung, (c) Heart, (d) Kidney, (e) Liver and (f) Spleen.

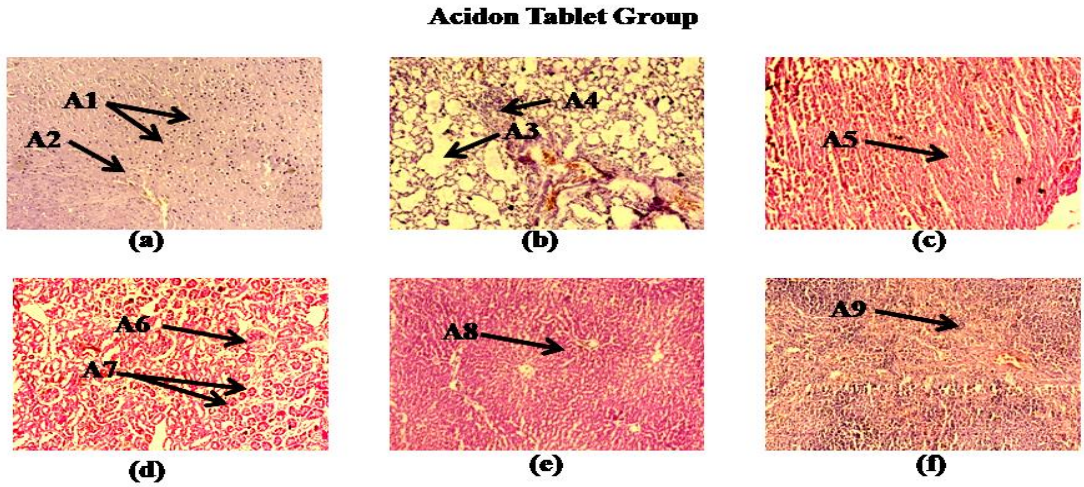

Fig 3: Histopathological study of different organs of test animals treated with Acidon tablet. (a) Brain, (b) Lung, (c) Heart, (d) Kidney, (e) Liver and (f) Spleen

Preclinical Study

The inhibitory effect of Acidon tablet on *H. pylori* was calculated using percentage inhibition after 24 hours of incubation (Fig 4). Acidon tablet showed the highest percentage inhibition of 65.04% at 10 mg/ml concentration. *H. pylori* are thought to be responsible for non-ulcer dyspepsia.[16] Acidon tablet was successful in inhibiting the growth of *H. pylori* so it can be concluded that it can potentially cure non-ulcer dyspepsia. The dose of 10 mg/ml gave good

results so can be used to treat the non-ulcer dyspepsia or gastric at this concentration as it produced no toxicity at this amount of dose.

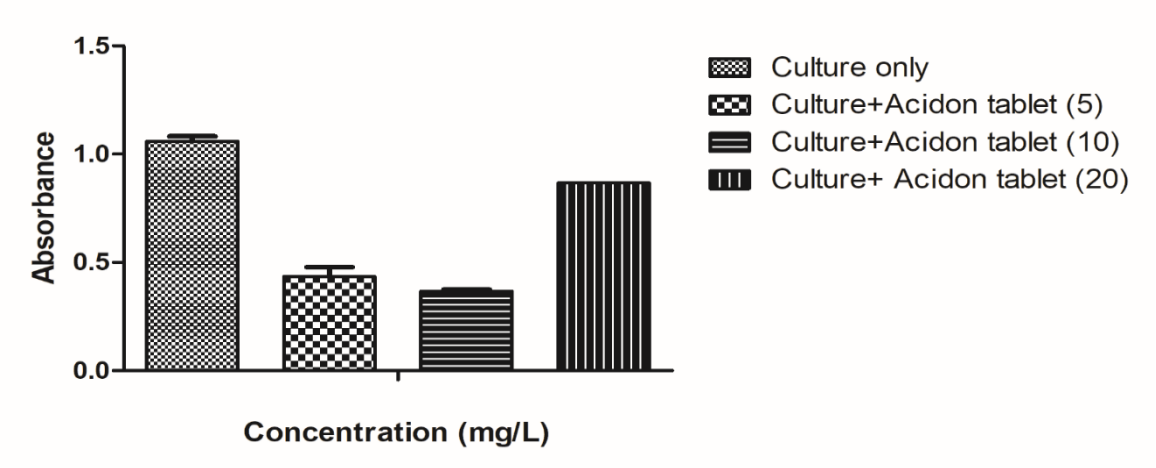

Fig 4: *In vitro* inhibitory activity of Acidon tablet against *H. pylori* expressed as the absorbance of the culture observed after 24 hours of incubation.

Clinical study

Effects of Acidon treatment on subjective criteria

The results of non-ulcer dyspepsia are presented in Table 2. The parameters undertaken were: upper abdominal pain, heartburn, bloating, bitter and acidic belching, fullness after a meal, nausea, and vomiting. The mean gradation of Upper Abdominal Pain before therapy in Group-I was 2.00 that reduced to 0.60 after intervention with a change of 70% with a p-value <0.001 that shows statistically highly significant results. The result was statistically highly significant (p-value=0.001) for Heartburn as there was a change of 76.8 % and the mean value before and after treatment was 1.30 and 0.30 respectively. The mean gradation of bloating before therapy was 1.20 that reduced to 0.20 after the intervention. This change of 83.33% with a p-value of 0.004 shows statistically significant results. The mean value of Bitter and Acidic belching was 1.10 that reduced to 0.20 with a change of 81.82%. There was a statistically significant change. The mean gradation of Fullness after the meal before therapy was 0.90 that reduced to 0.10 after intervention with a change of 88.89% that shows statistically significant results. The mean of Nausea before therapy was 1.10 that reduced to 0.20 after intervention with a change of 81.82% which shows statistically highly significant results. The mean value of Vomiting was 0.60 that reduced to 0.10 with a change of 83.33%. There was a statistically insignificant change. All these results indicate that Acidon tablet has shown significant improvement in all parameters such as upper abdomen pain, heartburn, bloating, bitter and acidic belching, and fullness after meal, nausea and vomiting. All this data indicates that herbal Acidon tablet possesses antacid properties as it has shown good results in all the parameters under consideration.

Table 2: Effect of therapy on subjective criteria before and after therapy using paired t test

Criteria	N	Mean score		% Change	Mean Diff.	SD	SE
		BT	AT				
Upper Abdominal Pain	10	2.00	0.60	70.00%	1.40	0.516	0.163
Heart burn	10	1.30	0.30	76.87%	1.00	0.667	0.211
Bloating	10	1.20	0.20	83.33%	1.00	0.816	0.258
Bitter and Acidic belching	10	1.10	0.20	81.82%	0.90	0.738	0.233
Fullness after meal	10	0.90	0.10	88.89%	0.80	0.789	0.249
Nausea	10	1.10	0.20	81.82%	0.90	0.316	0.100
Vomiting	10	0.60	0.10	83.33%	0.50	0.707	0.224

Effect of Acidon tablet treatment on hematological profile

The mean value of Hemoglobin (gm) before treatment was 11.94 that increased to 12.48 with a change of 4.52%. This change was statistically insignificant. So, it indicates test substance had no harmful effect on Hb content indirectly suggesting the good effect on oxygen transport within the body. The mean value of TLC before treatment was 8760.0 per cu mm (cubic millimeter) that reduced to 7655.0 per cu mm with a change of 12.6%. This change was statistically insignificant. In the case of Lymphocytes, the mean value before treatment was 26.01% that reduced to 24.0% with a change of 7.73%. The change was statistically insignificant indicating no detrimental effect on the immune system. The mixed cell value was 10.97% before treatment that reduced to 11.02% after treatment with a percentage change of 0.45% and the changes were statistically insignificant. In Neutrophils, 0.06% change was recorded as the mean value changed from 63.02% before treatment to 62.98% after treatment. This change was statistically insignificant. The mean value of ESR before treatment was 23.40 mm fall in the first hour that reduced to 14.10 mm fall in the first hour with a change of 39.7%. This change was statistically significant, which indicates that it might show signs of inflammation.[17] Similarly, all other parameters of the hematological analysis were normal suggesting no harmful effect of the test drug on the blood parameters (Table 3).

Effect of Acidon treatment on other biochemical parameters

There was a decrease in Total Serum Bilirubin (TSB) value by 34.5% and was statistically insignificant. Direct Serum Bilirubin (DSB) value was reduced by 18.2% which was statistically insignificant. It indicates that function of the gall bladder was not affected. Serum Glutamate Oxaloacetic Transaminases (SGOT) level was decreased by 5.6% and the results were statistically insignificant. After the treatment, the Serum Glutamate Pyruvic Transaminases (SGPT) level was decreased by 9.42%. The result was statistically insignificant.

This data implies that test tablets had no side effects on the liver enzymes. There was a percentage change in Blood urea level by 4.1% but the changes were statistically insignificant. The Serum creatinine level decreased by 5.95% which was statistically insignificant. Both these parameters point out that no side effects of formulations were observed on the kidney (Table 4). In the overall study, Acidon tablets had yielded good results. All the patients have shown improvements.

Table 3: Hematological scores before and after therapy using Paired‘t’ test

Category	Mean score		% Change	Mean Diff.	SD	SE
	BT	AT				
Hb (gm%)	11.94	12.48	4.52%	0.54	0.897	0.284
TLC (per cu mm)	8760	7655	12.6%	1105	2259.8	714.6
LYM (in %)	26.01	24.00	7.73%	2.01	4.930	1.559
MXD (in%)	10.97	11.02	0.45%	0.05	2.396	0.757
NEUT (in %)	63.02	62.98	0.06%	0.04	5.107	1.615
ESR(mm fall in first hour)	23.40	14.10	39.7%	9.30	12.04	3.806

Table 4: Biochemical parameters scores before and after therapy using paired t-test

Category	Mean score		% Change	Mean Diff.	SD	SE
	BT	AT				
TSB (mg/dl)	0.82	0.53	34.5%	0.29	0.477	0.151
DSB (mg/dl)	0.33	0.27	18.2%	0.06	0.158	0.049
SGOT (IU/L)	28.6	27	5.6%	1.60	7.633	2.414
SGPT (IU/L)	24.4	22.1	9.42%	2.30	10.11	3.197
B.UREA (mg/dl)	22.2	21.3	4.1%	0.90	4.653	1.471
S.CREAT (mg/dl)	0.84	0.79	5.95%	0.05	0.217	0.068

HPLC fingerprinting

Acidon tablet was analyzed for the determination of the 11 polyphenolic compounds such as gallic acid, catechin, chlorogenic acid, epicatechin, caffeic acid, umbelliferone, rutin, ellagic acid, quercetin and kaempferol and coumaric acid (Fig 5). Among all, gallic acid, catechin, chlorogenic acid, epicatechin, caffeic acid, umbelliferone, rutin and kaempferol were present.

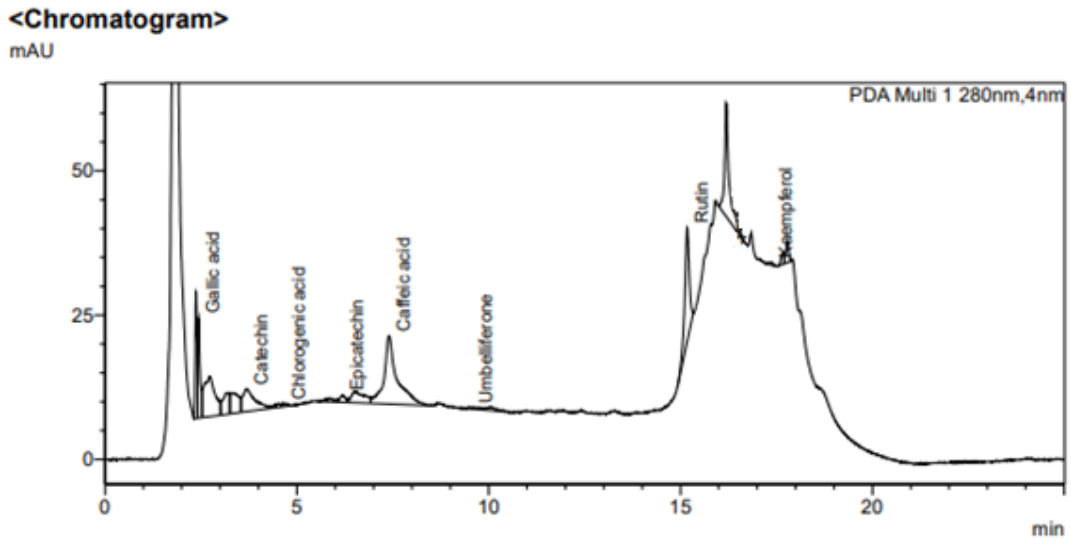

Fig. 5: Detection of different phytoconstituents present in the Acidon tablet by HPLC fingerprinting

Discussion

The present study revealed that there was no acute toxicity observed in animals. In the preclinical study, Acidon tablet has shown a significant antimicrobial activity against *H. pyloris* indicating potential protective effect against non-ulcer dyspepsia. In the clinical study, a significant protective effect was observed against non-ulcer dyspepsia in all patients.

Acute toxicity studies

Acute toxicity studies are of prime importance in drug development.[18] If drug is to be delivered only once or twice for treatment then acute toxicity studies are sufficient as in this study animals are monitored for 14 days after single high dose exposure to drug. By this method LD_{50} (Lethal Dose) value of drug can be estimated which is essential parameter in drug development. Hence, we conducted the acute toxicity study of Acidon tablet. Our drug had not shown any behavioral signs of toxicity. After the completion of study, animals were sacrificed to ascertain the internal organ damage due to drug. It was revealed that no histological damage was observed in tissue of all organs (Brain, lungs, heart, kidney, liver and spleen).

Preclinical study

Helicobacter pylori are gram-negative bacteria that colonize the gastric or intestinal mucosa of many mammalian and avian hosts and induce histologic inflammation. *H. pylori* cause gastritis, peptic ulcer disease and gastric cancers.[19] All these conditions can lead to dyspepsia. Hence, we evaluated our drug for antimicrobial activity against this microbe. It was revealed that

Acidon tablet possessed significant antimicrobial activity against *H. pylori* indicating potential to treat dyspepsia.

Clinical studies

Clinical studies are the final crucial step to validate the pharmacological potential as well as the safety of drug in humans with their consent. Well-designed observational studies can play a key role in supporting the evidence base for drugs and therapies.[20] Clinical study of Acidon tablet was conducted in 10 patients to whom tablet was administered orally with water. It was observed that Acidon tablet has shown significant improvements in all parameters such as upper abdomen pain, heart burn, bloating, bitter and acidic belching, and fullness after meal, nausea and vomiting. Additionally, hematological and biochemical profile of patients was analyzed. It was revealed that hematological as well as biochemical parameters were normal in all patients indicating that the consumption of Acidon tablet is safe. This study indicates that Acidon tablet can be a potential drug to treat dyspepsia.

HPLC Analysis

HPLC analysis is an instrumental technique to detect the presence of the constituents of the drug. Polyphenol are natural compounds present in plants which contain hydroxyl groups with benzene rings. They are known to possess various medicinal properties and their use to treat dyspepsia is common.[21] As Acidon tablet is polyherbal formulation, it is certain it might contain medicinally important polyphenolic compounds possibly responsible for its activity against non-ulcer dyspepsia. HPLC analysis revealed polyphenols were present in Acidon tablet such as gallic acid, catechin, chlorogenic acid, epicatechin, caffeic acid, umbelliferone, rutin, and kaempferol.

Conclusion

In the present study, Acidon tablet was evaluated for acute toxicity and pharmacological activity (Pre-clinical and Clinical study). Results have shown that there was no acute toxicity observed in the study. In the preclinical study, Acidon tablet has shown a significant reduction in the growth of *H. pyloris* indicating potential protective effect against non-ulcer dyspepsia. In the clinical study, a significant protective effect was found against non-ulcer dyspepsia in all patients under study. In a nutshell, herbal Acidon tablet has shown a potential role in treating non-ulcer dyspepsia.

Declarations

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Authors are thankful to Shree Dhanwantri Herbals for providing funds.

Ethical statement

The Animal Ethical Committee of Guru Nanak Dev University (GNDU), Amritsar, authorized the experimental protocol Approval no. 226/CPCSEA/2018/27. A clinical study of Acidon Tablet in patients with mild to moderate Non-Ulcer Dyspepsia was approved by the Institutional Ethics Committee of Rajiv Gandhi Govt. Post Graduate Ayurvedic College and Hospital, Paprola. The clearance for the proposed clinical research work was obtained before the commencement of trial (AYU/IEC/2019/1210). Written informed consent was obtained from the study subjects before the registration of patients in the trial.

Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contribution

AR, PS and SA conceived the idea; GS provided the material; AR, PS, and TK perform the experimental work; AR and PS wrote the paper; MS helped in histopathological evaluation; BS and SA reviewed the manuscript and made the corrections. All the authors approved the final manuscript.

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