Inhibition on Urease and Thermal Induced Protein Denaturation of commonly used Antiulcer Herbal Products. Study based on *in-vitro* assays

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ABSTRACT

Background: *In-vitro* urease inhibitory and thermal induced protein denaturation inhibitory activities was performed for two commonly used herbal products Endemali and Akseer ULCER in the treatment of ulcers. **Objectives:** To evaluate the antiulcer potential of two commonly used herbal products, Endemali, Akseer ULCER. **Material and Method:** In urease inhibitory assay, enzyme solution, extract, diferent regaents added and absorbance was measured at 630 nm (50 min, pH 8.2) and thiourea used as standard. In protein denaturation assay, the egg albumin was mixed with different concentration of test compounds, buffer absorbance was measured. Aspirin was used as standard. **Results:** The Endemali had a profound effect on the urease activity in a concentration dependent manner with EC_{50}value of 0.468 mg/ml. The Akseer ULCER antagonized the urease activity markedly with EC_{50}value of 0.374 mg/ml.These tested herbal products caused marked inhibition of thermal induced protein denaturation in a concentration dependent manner. The potency in the form of $EC_{50}$for Endemali, Akseer ULCER was measured as 323, 337 μg/ml respectively. **Conclusion:** In short, the tested herbal drug showed strong inhibition on urease activity and inhibition on thermal induced protein denaturation thus our study validated their uses in the treatment of ulcers.

Key words: Akseer ULCER, Antiurease activity, Endemali, *in-vitro* assay, Thermal induced protein denaturation.

INTRODUCTION

The traditional Unani System of Treatment is the integral part of Pakistani community.[1-2] The physician in Unani system of treatment is called Hakim/Tabib and the system as Hikmat/Tibb. The federal government through Unani, Ayurvedic and homeopathic (UAH) Practitioners Act, 1965, regulates the Unani System of Medicine.[3-4] National Council of Tibb and National council for Homeopathy are established as corporate bodies under section 3 of the said Act to promote and popularize the traditional system of education. The registered Hakims and vaids throughout the country are estimated at 39, 584 and 455 respectively. These healthcare professionals are produced by 125 recognized homeopathic medical colleges in the country.[5-7] However, reasonable number of unregistered Hakims are too involved in the practice; based on the empiric knowledge came from ancestors. Like other countries of the world, the ulcer is very common in Pakistani community and badly affecting the lifestyle of people. Along with allopathic drugs, Unani medicines are benefiting large population of the country. However, mostly these formulations are used without a scientific background. Therefore, the current was designed to evaluate the antiulcer potential of two commonly used herbal products, Endemali, Akseer ULCER. The antiulcer potential was rated in terms of urease inhibitory effects of

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these drugs in an *in-vitro* assay.

MATERIALS AND METHODS

Drugs Material

The Endemali strips of Hamdard Laboratories (WAQF) Pakistan, Akseer ULCER tablet of Dawakhana Hakim Ajmal Khan (Pvt) Ltd was purchased from local market.

Chemicals

Urea (Sigma-Aldrich), Sodium Nitroprusside, Phenol Red(BDH Chemicals Ltd, England), Thiourea, Sodium Dihydrogen Phosphate, (Merck, Germany), UreaseJack beans (Avonchem Ltd, UK), Sodium Hypochlorite (HC Haq Chemicals, Pakistan), Dimethyl Sulfoxide(UNI-Chem).

Urease inhibitory assay

Exact 25 ml of enzyme (Jack Bean Urease) solution and test compounds (5 ml, 0.5 mM concentration) were incubated for 15 min at 30°C (Tariq *et al*., 2011). The aliquotwas taken after 15 min and again incubated with 55 µL of buffers containing urea (100 mM) for 15 min at 30 °C. Ammonia production was measured as an urease activity by indophenol method as described earlier (Khan *et al*., 2013c). Final volumes were maintained as 200 ml by adding 45 ml phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprussside), and of alkali reagent (70 ml, 0.5% w/v NaOH and 0.1% active chloride NaOCl). The increase in absorbance was measured at 630 nm after 50 min at pH 8.2. The results (change in absorbance per min) were calculated spectrometrically on different concentrations of drugs. Thiourea was used as the standard inhibitor and percentage inhibitions were calculated as follows

$$\% \text{ Inhibition} = 100 - (OD_{testwell}/OD_{control}) \times 100.$$

The IC_{50} values were calculated using statistical software, Graph Pad version 6.

In-vitro protein denaturation assay

The protein denaturation assay was used for the estimation of antiinflammtory potential of herbal product, Endemali, Akseer ULCER. The egg albumin (0.2 ml) was mixed with varying concentrations of decoctions (2 ml, 50-500 µg/ml) and phosphate buffered saline (2.8 ml, pH 6.4) to get a reaction mixture of 5 ml. The mixtures were incubated at 37ºC ± 2 for 15 min and then heated at 70ºC for 5 min. The resulting solutions were cooled and absorbance was taken on a spectrophotometer at 660 nm by using vehicle as blank (Chandra *et al*., 2012, Chatterjee *et al*., 2012, Khan *et al*., 2014). The distilled water served as control while aspirin as a standard. Finally, the % inhibition of protein denaturation was calculated.

$$\text{Protein denaturation (\%)} = \frac{\text{Control Absorbance} - \text{Test Absorbance}}{\text{Control Absorbance}}$$

The IC_{50} values were calculated using statistical software, GraphPad PRISM 6.

Statistical analysis

The resulting data were expressed as the mean ± SEM (n=3) in each group. To determine the differences between groups, one-way analysis of variance(ANOVA) was performed (Graph Pad version 6) usingtheleast significant difference (LSD) test at P<0.5. EC_{50} values were calculated with the help of curve-fitting program.

RESULTS

Effect of tested herbal products in urease inhibitory assay

The results of tested herbal products in urease inhibitory assayare displayed in Figure 1. Endemali had a profound effect in a concentration dependent manner on the urease activity (Figure 1a). The maximum attenuation (78%) was observed at 3 mg/ml. The EC_{50} was calculated as 0.468 mg/ml (table 1). The results of Akseer ULCER against Jack Bean urease is illustrated in (Figure 1b). It antagonized the urease activity which was dependent on drug concentration. In terms of percentage, maximum inhibition was 87% at 3 mg/ml. The calculated EC_{50} was 0.374 mg/ml (table 1). As shown in (Figure 1c), the powder drug also showed concentration dependent antagonism of the urease. The maximum inhibition was 59% inhibition of urease at 3 mg/ml. The calculated EC_{50} was 1.80 mg/ml (table 1). However, Thiourea showed most potent effect (Figure 1d) with EC_{50} value of 0.026 mg/ml (table 1).

Effect of tested herbal products in thermal induced protein denaturation assay

The effect of tested herbal products in thermal induced protein denaturation assay at various concentrations is illustrated in Figure 2. The endemali caused concentration dependent inhibition of heat-induced protein denaturation

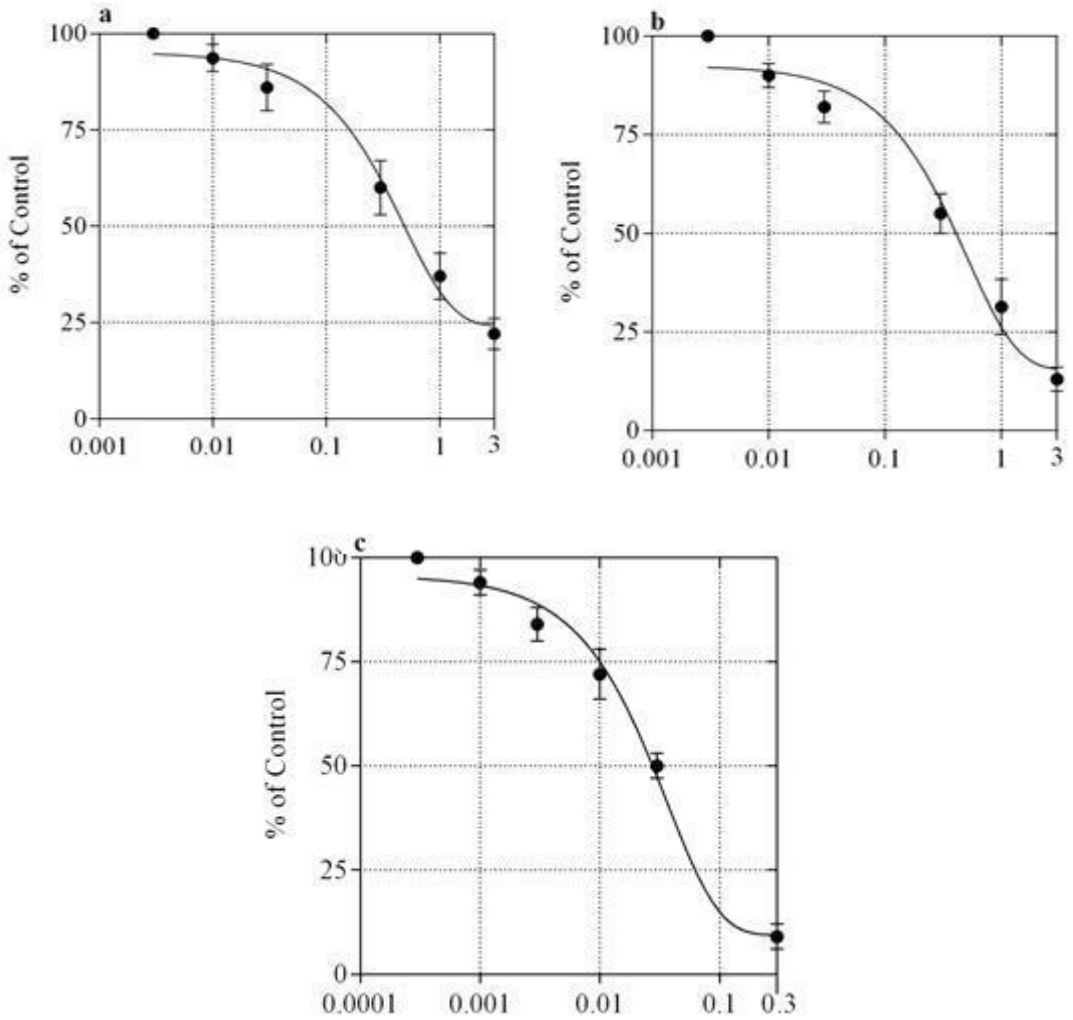

Figure 1: The percent effect of herbal products on urease inhibition. (a) Endemali, (b) Akseer ULCER, and (c) Thiourea. The resulting data were expressed as the mean ± SEM (n=3) in each group.

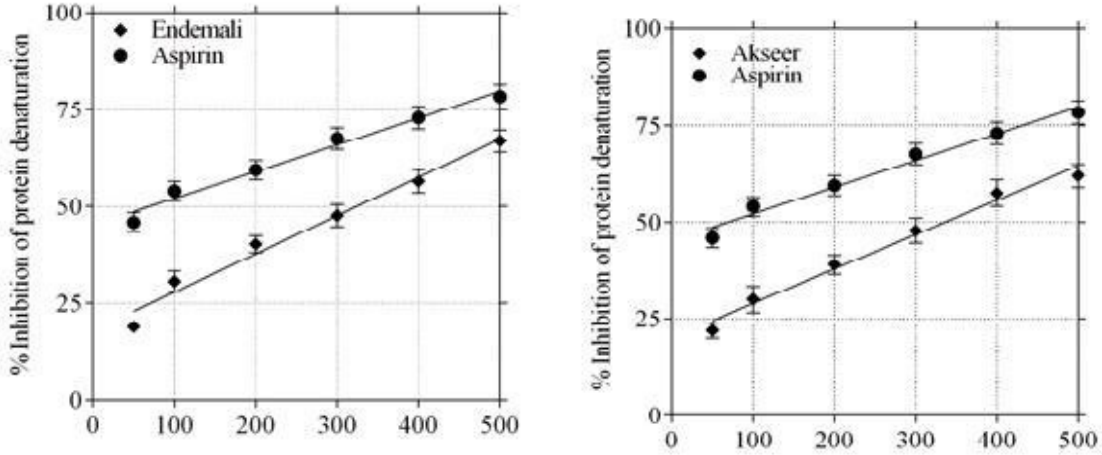

Figure 2: The percent inhibition of effect of herbal products on thermal induced protein denaturation assay. The resulting data were expressed as the mean ± SEM (n=3) in each group.

Table 1: Half maximual concentration (IC_{50}:mg/ml) of herbal drug in urease inhibitory assay. The resulting data were expressed as the mean ± SEM (n=3) in each group.

Drugs	EC_{50} (mg ml)
Endemali	0.468
Akseer ULCER	0.374
Thiourea	0.026

Table 2. Half maximual concentration (IC_{50}:mg/ml) of herbal drug in thermal induced protein denaturation assay. The resulting data were expressed as the mean ± SEM (n=3) in each group.

Drugs	EC_{50} (µg ml)
Endemali	323
Akseer ULCER	337
Aspirin	65

with maximum attuneation of 67% at 100 µg/ml and EC_{50} of 323 µg/ml (table 2). The akseer ULCER also exhibited marked inhibition on thermal induced protein denaturation with maximum effect of 62% at 100 µg/ml and EC_{50} of 337 µg/ml (table 2). The various concentrations of powder drug showed profound inhibition on thermal induced protein denaturation with maximum effect of 68.50% at 100 µg/ml and EC_{50} of 297 µg/ml.

DISCUSSION

The results of our study revealed profound effect on commonly used herbal formulations on the expression of urease in an *in-vitro* assay.

Urease (urea amidohydrolase) is usually found in different bacteria, fungi, algae and plants, an enzyme that catalyzes the hydrolysis of urea to ammonia and carbamate, which is the final step of nitrogen metabolism in living organisms.[8] Carbamate rapidly and spontaneously decomposes, yielding second molecule of ammonia. These reactions may causes significant increase in pH and are responsible for negative effect so urease activity in human health and agriculture.[9-10] From the medical view point, infections induced by these bacteria such as *Helicobacter pylori* and *Proteus mirabilis* usually have a high urease activity. Urease is vital to *H. pylori* metabolism and virulence, as necessary for its colonization of the gastric mucosa, and is a potent immunogen that elicits a vigorous immune response. This enzyme is used for taxonomic identification and for diagnosis and follow-up after treatment, and is a vaccine candidate. Ureas represent an interesting model for metalloenzyme studies. *H. pylori* contributes in urinary tract and gastrointestinal infections, probably augmenting the severity several pathological conditions like peptic ulcers and stomach cancer. Ureases are also involved in the development of urolithiasis, pyelonephritis, hepaticencephalopathy, hepatic coma and urinary catheter encrustation.[11-12]

In this regards, targeting urease for treating pathogenic disorders caused by it may open a new line of treatment for infections caused by urease producing bacteria. The results of our study showed profound inhibition of urease (Jack Bean) by two commonly used herbal products in a concentration dependent manner. Therefore, our study provided the scientific basis for the traditional uses of these herbal agents of plant origin as antiulcer.

The development of an inflammatory response is a complex but well regulated process. Arachidonic acid is a polyunsaturated fatty acid that liberated from cell membrane phospholipids via the hydrolysis by phospholipase A2 enzymes (PLA2). The arachidonic acid is then metabolized by two distinct enzymatic pathways; cyclooxygenase (COX) in to prostaglandins (PGs) and lipoxygenase in to leukotrienes.[13-15] Prostaglandins (PGs) are members of the eicosanoid family produced by almost all cells of the human body; the principal mediator of inflammation in most of the inflammatory diseases.[16-17] The inflammatory mediators approach from plasma proteins or cells including mast cells, platelets, neutrophils and monocytes/macrophages which are activated by bacterial products or host proteins. They bind to specific receptors and elicited vascular permeability, neutrophils chemotaxis, stimulate smooth muscle contraction, excite pain or mediate oxidative damage. Most of the mediators are short - lived but produce harmful effects.[18-19] The non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely prescribed medicine in the management of inflammatory conditions. Clinically, they are useful for the symptomatic relief[20-21] by acting through several mechanisms though causing various side effects.[22-23]

It was already proved that conventional NSAID's like phenyl butazone and indomethazine do not act only by the inhibition of endogenous prostaglandins production by blocking cyclo oxygen as enzyme but also by prevention of denaturation of proteins.[24-25] The protein denaturation is a practice in which proteins are unable to maintain their structural integrity in the presence of external stimuli such as strong acid or base, concentrated inorganic salt, an organic solvent or thermal treatment. It has been observed that proteins lose their biological potency upon denaturation. Denatured proteins are considered

as one of the inflammatory mediator therefore, agents that cause prevention of precipitation of denatured protein aggregates and protein condensation are useful in diseases like rheumatic disorders, cataract and Alzheimers disease.[26] In the current study, the tested herbal products demonistrated marked attenuation of urease activity at various test concentrations. Additionally, the urease inhibition of these herbal products was augmented by their strong inhibition on thermal induced protein denaturation. Thus urease inhibition coupled with anti inflammtory effect could be more effective the rapeutic approach in the treatment of ulcers as inflammation is primerly observed with hyperacidic conditions.

CONCLUSION

It is concluded that the tested two herbal Pakistani drugs showed marked inhibition against urease. The study therefore, provided scientific evidence for their use in the treatment of ulcers. Furthermore, the inhibition on thermal induced protein denaturation could be more effective therapeutic approach in the treatment of ulcers.

CONFLICT OF INTEREST

The author has no conflict of interest.

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