Evaluation of *in vitro* urease and lipoxygenase inhibition activity of weight reducing tablets

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Abstract: Enzyme inhibition is a significant part of research in pharmaceutical field in view of the fact that these studies have directed to the innovations of drugs having remarkable performance in diverse physiological conditions. The present study was aimed to assess urease and lipoxygenase inhibitory activity of weight reducing tablets. For evaluating the urease activity indophenol method was employed using Thiourea as the model urease inhibitor. The lipoxygenase inhibition was evaluated by measuring the hydroperoxides produced in lipoxygenation reaction using a purified lipoxygenase with lionoleic acid as substrate. When formulation of the weight reducing tablets was compared at various concentrations (50, 100 and 500µg/ml). The antiurease activity and lipoxygenase inhibition activity increased in a dose dependent manner. The formulations under test have an excellent antiurease and lipoxygenase inhibition potential and prospective to be used in the cure of a variety of complications associated with the production of urease and lipoxygenase enzymes.

Keywords: Enzyme inhibition; lipoxygenase inhibition; urease activity; weight reducing tablets.

INTRODUCTION

The challenge of pathogenic opposition has inquired the advances of novel structurally varied inhibitors. Enzyme inhibition is a significant part of research in pharmaceutical field in view of the fact that these studies have directed to the innovations of drugs having remarkable performance in diverse physiological conditions. This emergent field has become a dynamic part of pharmaceutical research (Upadhyay, 2012).

As potential innovative anti-ulcer drugs, urease inhibitors have attracted an enormous attention in recent times. It is imperative in the pathological process of different diseases in human beings (Kaleem *et al*., 2013). It provides an apt microenvironment for the subsistence of *Helicobacter pylori* (Hu & Sim, 2000). An enzyme urease takes part in production of a high concentration of the ammonia and carbon dioxide that consequently lead to peptic ulcers by disturbing the mucosal permeability for hydrogen ions (Shirataki *et al*., 2005). Different infections due to urease-producing bacteria can be treated by its inhibition through compelling and explicit compounds that could direct the management of such infections (Zareen *et al*., 2004).

Lipoxygenases (LOXs) consist of a group of non-heme iron-containing dioxygenases, symbolizing the key enzymes concerned with the biosynthesis of leukotrienes (LTs) and catalyses the primary steps in converting arachidonic acid to biologically active LTs (Rackova *et al*., 2007). LTs are deemed as compelling mediators of hypersensitivity and inflammatory reactions (Schneider & Bucar, 2005). Several studies have shown that LTs contributes significantly in the development of pathological conditions including urinary tract infection, kidney stones, peptic ulceration and other inflammatory diseases of digestive tract (Woszczek *et al*., 2002). Concerning their pro-inflammatory possessions the inhibition of 5-lipoxygenase pathway is believed to be remarkable in the management of inflammatory diseases (Prasad *et al*., 2004). The distinctive function of the enzyme in producing LTs makes it a probable goal for biochemical research. Owing to the increase production of LTs concerned in several inflammatory diseases, there has been substantial interest in the generation of 5-LO inhibitors intended for therapeutic purpose. The compounds recognized as 5-LO inhibitors can be divided into antioxidants, substrate-analogous, and miscellaneous grouping of inhibitors (Rask-Madsen *et al*., 1992).

Phytomedicine has demonstrated to be an intact treasure for the innovation of model compounds to treat diseases of various etiologies (Kusters *et al*., 2006; Ramakrishnan & Salinas, 2007; Thukral & Wolf, 2006). Many phenolic/flavonoid compounds originated from vegetables source are revealed to have enzyme inhibition activity. Several natural and synthetic compounds with redox and non-redox potential are identified as inhibitors of 5-LO. In current study we report the anti urease and

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lipoxygenase inhibition activity of polyherbal weight reducing tablets.

MATERIALS AND METHODS

Composition of tablet

Each 500mg tablet contains

Foeniculum vulgare: 10mg; Trigonella foenum-graecum-seed: 10mg; Thea sinensis-leaf: 10mg; Ephedera vulgaris: 10mg; Althaea officinalis: 10mg; Zingiber officinale: 10mg; Apium graveolens: 10mg; Moringa oleifera: 10mg, Glycyrrhiza glabra 10 mg, Ruta greveolens 10 mg and Mallotus philippensis: 10mg.

Chemicals and reagents

Linoleic acid and lipoxygenase were procured from Sigma (St. Louis, MO, USA). All the analytical grade solvents were used obtained from Merck. Sodium nitroprusside and urease (EC 3.5.1.5) from Jack beans were obtained from Sigma (St. Louis, MO, USA).

Extract preparation

The herbs used in the preparation were sieved through mesh #60. Each grinded herb was taken into extractor and water was added as solvent in the proportion of 1:10 (herb: solvent). The decoction was obtained by heating the extractors with steam for 2 - 3 hours. Filtration was done and the filtered decoction was shifted to evaporators to eradicate the additional solvent.

Antiulcer/anti urease activity

For determining Urease activity ammonia production was measured using the indophenol method (Arfan *et al*., 2010). 25µL of enzyme (Jack bean Urease) solution and 55µL of buffers containing 100 mM urea were mixed and incubated at 30^{o}C for 15 min in 96-well plates. Phenol reagent comprising of 1% w/v phenol and 0.005% w/v sodium nitroprusside in quantity of 45µL and alkali reagent comprising of 0.5% w/v NaOH and 0.1% active chloride NaOCl in amount of 70µL were added to every well. An absorbance at 630 nm was measured after 50 min, by means of a microplate reader (Molecular Device, USA). Percentage inhibitions were calculated from the formula 100–($OD_{testwell}$/$OD_{control}$) x100.

Lipoxygenase inhibition activity

Lipoxygenase enzyme solution was prepared in sodium phosphate buffer with such concentration to give 130 U per well (Rackova, 2007). Sodium phosphate buffer having pH 8.0 (160µl:100mM) was taken in each one well of plate. The plates were labeled as Blank (B *substrate* and (B *enzyme*), Control and Test. In each well labeled as test the test compound solution in methanol (10-1000µM: 10µl) was added. Lipoxygenase solution (LOX: 20µl) was poured in every well including B *enzyme*, Control and Test except B *substrate* and the mixture was incubated at 25°C for ten minutes. Substrate solution was prepared by adding linoleic acid (155µl: 0.5 mM) into 0.12% w/v tween 20 (257µl). The mixture was mixed and 0.6ml NaOH (1N) was added to remove turbidity and volume was making up to 20ml with deionized water. This mixture was flushed with nitrogen gas to avoid autoxidation before adding to each well. The reaction was started by the adding 10µl substrate in every well except B (*enzyme)* and the absorbance was noted after five minutes at 234 nm.

RESULTS

When formulation of the weight reducing tablets were compared at concentrations of 50, 100 and 500µg/ml, antiurease activity improved in a dose dependent way just like standard Thiourea. At 50µg/ml tablets possess 21.8%, at 100µg/ml tablets exhibit 35.9% and at 500 µg/ml tablets possess 44.9% inhibition activity. The standard Thiourea revealed 64.5%, 76.5% and 89.9% antiurease activity at concentrations of 50, 100 and 500µg/ml respectively. Results showed that formulation of weight reducing tablets have excellent antiurease potential. (fig. 1).

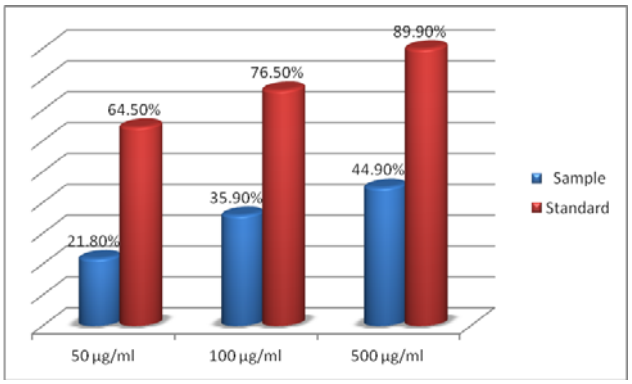

Fig. 1: Comparison of Antiurease activity of weight reducing tablets and Standard

When formulation of weight reducing tablets was compared at various concentrations (50, 100 and 500 µg/ml), lipoxygenase inhibition activity increased in a dose dependent manner just like standard. At 50µg/ml tablets possess 14.8%, at 100µg/ml tablets exhibit 53.9% and at 500µg/ml tablets possess 64.9% inhibition activity. The standard bacilein revealed 61.3%, 73.4% and 85.3% lipoxygenase inhibition activity at concentrations of 50, 100 and 500µg/ml respectively. All these results showed that lipoxygenase inhibition activity increased in a dose dependent manner and formulation has good lipoxygenase inhibition potential when compared to bacilein. (fig. 2)

DISCUSSION

Community interest in herbal medicine has increased exponentially during the past decades, including both developed and developing countries (Mark Blumenthal,

1999; De Smet, 1997; Foster, 2012). It is predictable that around 25% of all modern medicines are direct or indirect derivatives from plant sources (Shu, 1998). This resurgence of herbal medicines has increased the international trade enormously. Pharmaceutical companies have established renewed concern in exploring plants as a major source for innovative lead structures with promising safety, efficacy and quality. Researchers are coming together established facts with investigational methodology for determining the safety and efficacy of these plant based remedies (M Blumenthal, 1999)-(2000). The present study is a part of these efforts. The weight reducing formulation under test comprised of several useful herbs including *Foeniculum vulgare, Trigonella foenum-graecum-seed, Thea sinensis-leaf, Ephedera vulgari plants, Althaea officinali roots, Zingiber officinale rhizome, Apium graveolens leaf, Moringa oleifera leaves, Glycyrrhiza glabra rhizome, Ruta greveolens leaf* and *Mallotus philippensis seeds.*

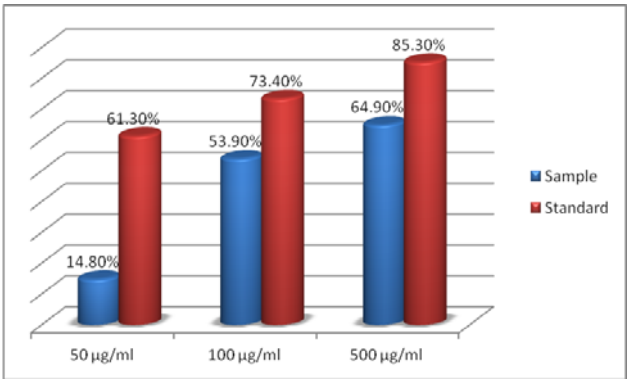

Fig. 2: Comparison of Lipoxygenase inhibition activity of weight reducing tablets and Standard

When formulation of weight reducing tablets were compared at concentrations of 50, 100 and 500µg/ml, antiurease activity improved in a dose dependent way just like standard Thiourea. Urease diffuses along the cytoplasmic membrane, increases the preplasmic space pH and as a result allows the bacteria growth in the present of extra cellular gastric acid. Additionally, urease activity will lead to kidney stones formation and also conduct the development of urolithiasis, pyelonephritis and hepatic encephalopathy. It has been shown that *H. pylori*, a pathogen which is colonized in the digestion system of human beings and considered as one of the important factors leading to gastric disease, is incapable of causing infection in the absence of urease. Natural medicines especially medicinal plants have been considered as one of the options to cure the diseases in some cases for many decades and their basic ingredients are used in medicine industry at present time (Biglar *et al*., 2014). In current study the formulation under test has the probability to be used in the cure of a variety of complications associated with the production of urease enzymes. Urease inhibitors can act by binding in a substrate or active-site directed mode or by binding in a non-substrate like or mechanism-based directed mode. Major examples of the substrate-like urease inhibitors include Thiourea and hydroxyurea. The common anti urease compounds contained strong basic groups for instance mimics of the amide bond of its substrate molecule i.e. urea. Lipoxygenases convert the adding up of molecular oxygen to fatty acid containing a cis-1, 4-pentadiene system. The primary product is a 4-hydroperoxycis trans-1, 3-conjugated pentadienyl moiety within unsaturated fatty acid. This assay analyzes the hydroperoxides produced in the lipoxygenation reaction by means of a purified lipoxygenase with lionoleic acid as substrate. Eun-Mi Choi reported that fruit methanolic extract of *F. vulgare* decreases the possibility of inflammation-related diseases (Choi & Hwang, 2004). The anti-inflammatory characteristics of *Zingiber officinale* have been acknowledged for centuries as evidenced by its inhibitory effects on prostaglandins synthesis (Ali *et al*., 2008). When formulation of weight reducing tablets was compared at various concentrations, lipoxygenase inhibition activity increased in a dose dependent manner just like standard revealing that formulation has good anti lipoxygenase potential.

CONCLUSION

The formulations under test have an excellent potential of urease and lipoxygenase inhibition. It is expected that this formulation could be possibly used in the cure of a variety of complications associated with the production of urease and lipoxygenase enzymes.

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