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EVALUATION OF *IN VITRO* ANTI-INFLAMMATORY ACTIVITY OF *HARPAGOPHYTUM PROCUMBENS* AND *URTICA DIOICA* AGAINST THE DENATURATION OF PROTEIN

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Abstract

Anti-denaturation effects of plant extracts in heat treated Bovine Serum Albumin (BSA), are potential therapeutic parameters for finding anti-inflammatory compounds without the use of animal for preliminary pharmacological screening. *Harpagophytum procumbens* and *Urtica Dioica* can bring natural compounds with significant anti-inflammatory effects. The anti-denaturation effects of *H. procumbens* and *U. Dioica* ethanol extracts and their combinations were evaluated using albumin denaturation assay at different concentrations. Aspirin was used as standard drug. The present finding exhibited a concentration dependent inhibition of protein (albumin) denaturation by *H. procumbens* and *U. Dioica* extracts. The effect of Aspirin was found to be less than extracts. In conclusion it can be concluded that *H. procumbens* and *U. Dioica* extracts possessed in vitro anti-inflammatory effect against the denaturation of protein and *H. procumbens* was stronger than *U. Dioica.* Also combination of *H. procumbens* and *U. Dioica* extracts hadn't good effect on inhibition of protein (albumin) denaturation. Further investigations are required to isolate the active component responsible for their anti inflammatory effects.

Key words: Anti-denaturation, anti-inflammatory, *H. procumbens, U. Dioica.*

Introduction

Inflammation is a biological reaction of body tissues against injury, irritant and harmful motivation, such as pathogens and damaged cells. Some factors such as viruses and bacteria, chemical compounds and tissue death are the factors that can motivate inflammation (Ferrero-Miliani *et al.*, 2007). The commonly drugs for treatment of inflammatory have several side effects especially gastric irritation leading to the formation of gastric ulcers (Tripathi, 2008). Natural products can demonstrate a novel source of newer compounds with significant anti-inflammatory activities. The major benefit of herbal medicine seems to be low serious adverse effects and low cost (Bennett and Brown, 2005). Many medicinal plants have shown to exhibit potent anti-inflammatory effect in the treatment of inflammation by using various models. Anti-inflammatory compounds in *Erythrina indica bark* (Ajay Kumar *et al.*, 2010), *Mikania scandens* (Chandra *et al.*, 2012), *Barleria prionitis* (Khobragade and Bhande, 2012), *Zizyphus oenoplia* (Ramalingam *et al.*, 2010) and *Piper betle* (Williams *et al.*, 2002) have been identified. These natural anti-inflammatory products are safer than steroidal allopathic drugs (Ajay Kumar *et al.*, 2010). Free radicals which increase vascular permeability, protein denaturation and membrane alteration are produced in inflammatory disorders; so the use of antioxidant and anti-inflammatory agents which can prevent oxidative and inflammation reactions are needed (Umapathy *et al.*, 2010). Protein denaturation is a marker for inflammatory and arthritic diseases because denaturation of proteins causes the production of auto-antigens in certain arthritic diseases (Dey *et al.*, 2011). Anti-denaturation effects of plant extracts in heat treated Bovine Serum Albumin (BSA), are potential therapeutic parameters for finding anti-inflammatory compounds without the use of animal for preliminary pharmacological screening (When BSA is heated, it undergoes denaturation) (Williams *et al.*, 2008).

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The plant sources can bring natural compounds with significant anti-inflammatory effects and the major suitability of them is their low incidence of side effects, and low cost. *Harpagophytum procumbens* DC is from Pedaliaceae family and it is native to southern Africa, also the anti-inflammation activity has been attributed to this plant (Ernst and Chrubasik, 2000; Baghdikian *et al.*, 1997; Lanhers *et al.*, 1992; Mahomed and Ojewole , 2004). The roots of *H. procumbens* are used to treat fever, indigestion, allergic reactions and rheumatism (Van Haelen, 1986; Leung and Foster, 1996; Schulz *et al.*, 1998; Der Marderosian, 1999). Scientific studies revealed that *H. procumbens* exhibits anti-inflammatory, anti-oxidant, analgesic, anti-epileptic, anti-diabetic, antimalarial and antimicrobial activities (Mncwangi *et al.*, 2012). Iridoid glycosides and phenylpropanoid glycosides are common compounds in *H. procumbens* and they are known to possess anti-inflammatory activity (Mncwangi *et al.*, 2012). *Urtica dioica* L. is from Urticaceae family and grows in many regions of the world (Rechinger, 1963). In Iran, it grows widely in Northern provinces (Rechinger, 1963). In Iran, *U. dioica* has been used as anti-turgid treatment in ancient Persian medicine (Riehemann *et al.*, 1999; Zargari, 1994). Also it has antioxidant (Kanter *et al.*, 2005) and blood fat decreasing effects (Aveci *et al.*, 2006). Many studies have reported anti-inflammatory effects of this herb, especially knee and femoral bone arthritis (Bondarenko *et al.*, 2003; Khalili *et al.*, 2012; Hajhashemi and Klooshani 2013). Caffeic malic acid and polysaccharides are extent in all parts of plant and demonstrated anti-inflammatory activity (Obertreis *et al.*, 1996). The objective of this study was to investigate the anti-inflammatory effects of *U. dioica* and *H. procumbens* extracts and the potential interactions of their combinations against the denaturation of protein *in vitro*.

Material and methods

Plant material

The whole parts of *H. procumbens* and aerial parts of *U. Dioica* were prepared from greenhouse of Research Center of Plant Sciences in Ferdowsi University of Mashhad; Khorassan Razavi of Iran. The plants were dried under shade and powdered.

Preparation of extracts

50 grams of the *H. procumbens* and *U. Dioica* powders were mixed with ethanol in a clean conical flask separately. The mixtures were kept for 48hrs in room temperature. Extracts were filtered by using Whatmann filter paper and solvent was evaporated under vacuum at 40°C to afford 2.47 g extract of *H. procumbens* and 2.27 g extract of *U. Dioica* (yield 3.29% and 4.54% respectively).

Assessment of in vitro anti-inflammatory activity (Inhibition of albumin denaturation)

The anti-inflammatory activity of ethanol extracts of *U. Dioica* and *H. procumbens* was studied using inhibition of albumin reaction denaturation technique. Test solution containing different concentrations (50, 100, 200, 400, 600, 800, 1000 µg/ml) of samples (1 mL) was mixed with 1 mL of 1% albumin solution. PH of the reaction mixture was controlled by using HCl (PH<7). The samples were incubated at 37^0C for 20 min. Denaturation was induced by keeping the reaction mixture at 51^0C in water bath for 20 min. After cooling, the absorbance was measured at 660 nm by spectrophotometer. The experiment was performed in triplicate. The Percent inhibition of protein denaturation was calculated as Equation.

$$Percentage\ Inhibition = \frac{Acontrol - Asample}{Acontrol} x100 \quad (1)$$

Statistical Analysis

All the experiments were performed in triplicate and the data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Duncan's multiple range tests were carried out to determine significant differences ($p < 0.05$) between the means by SPSS.

Results and discussion

The denaturation of proteins is one of the causes of inflammation. In denaturation of protein process tertiary and secondary structure of protein will missed by application of heat, acid or base, an inorganic salt and organic solvent. The mechanism of denaturation involves alteration in electrostatic hydrogen, hydrophobic and disulphide bonding (Grant *et al.*, 1970). The activation of phagocytes, production of O^{2-} and OH^- radicals can damage tissues (Gilham *et al.*, 2000) and then damage tissues stimulate inflammatory response by production of chemotactic factors (Gilham *et al.*, 2000). Plant extracts that showing inhibition of denaturation are often tested for anti-inflammatory activity. So in present study, the protein denaturation bioassay was selected for in vitro assessment of anti-inflammatory property of *H. procumbens* and *U. Dioica* ethanol extracts. The findings of present study exhibited a concentration dependent inhibition of protein denaturation by *H. procumbens* and *U. Dioica* extracts throughout the concentration range of 50-1000 µg/mL. A number of anti-inflammatory drugs are known to inhibit the denaturation of proteins such as

Table 1: % Anti-denaturation of ethanol extracts of *U. Dioica* and *H. procumbens* and their combinations in various concentrations

Proportion of *U. Dioica* to *H. procumbens* extracts	% Anti-denaturation						
	(1000µg/ml)	(800µg/ml)	(600µg/ml)	(400µg/ml)	(200µg/ml)	(100µg/ml)	(50µg/ml)
2.9±0.002	4.0±0.002	18.2±0.002	24.7±0.005	60.3±0.004	70.8±0.186	69.5±0.002*	10 to 0
16.9±0.003	9.0±0.002	20.2±0.001	26.0±0.002	35.1±0.004	28.6±0.006	33.6±0.003	9 to 1
5.3±0.002	23.0±0.002	22.7±0.004	23.9±0.001	33.4±0.004	35.3±0.004	35.1±0.002	8 to 2
4.8±0.001	27.4±0.002	22.5±0.002	22.7±0.003	32.9±0.001	36.4±0.003	34.9±0.002	7 to 3
14.8±0.002	4.7±0.003	27.4±0.003	14.2±0.002	34.5±0.003	34.6±0.004	35.7±0.004	6 to 4
6.7±0.003	5.3±0.003	27.4±0.003	10.0±0.001	30.0±0.003	35.3±0.004	33.6±0.002	5 to 5
28.6±0.003	7.3±0.002	26.1±0.002	20.2±0.001	35.8±0.002	35.5±0.004	35.5±0.003	4 to 6
10.0±0.002	18.0±0.003	32.4±0.003	28.5±0.002	35.6±0.002	35.6±0.005	35.7±0.004	3 to 7
17.7±0.002	12.0±0.003	31.1±0.001	26.1±0.001	36.6±0.002	37.0±0.002	36.0±0.002	2 to 8
16.1±0.004	27.2±0.003	28.8±0.001	30.8±0.002	36.6±0.003	36.3±0.005	34.6±0.004	1 to 9
16.7±0.001	25.0±0.004	54.8±0.002	63.3±0.003	69.5±0.001	70.8±0.001	71.9±0.004	0 to 10

*All data are the mean ± SD of three replicates.

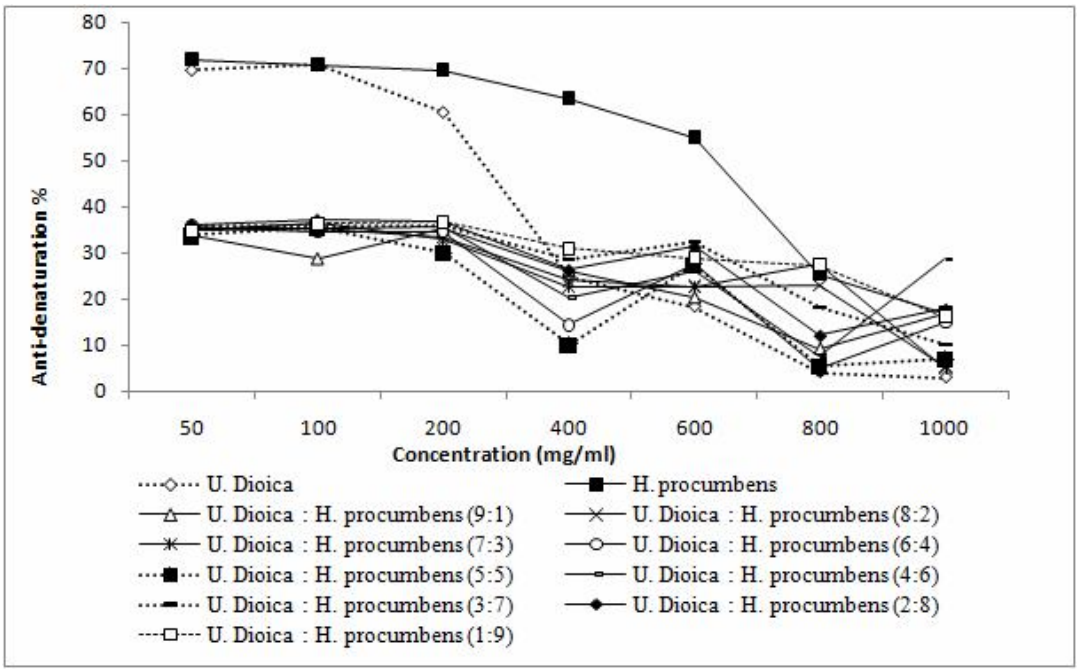

Fig. 1: Anti-denaturation activity of *U. Dioica* and *H. procumbens* extracts and their combinations in various concentrations

Phenylbutazone, salicylic acid, flufenamic acid, etc (Mizushima and Kobayashi, 1968); in present study Aspirin was used as standard drug and all the results were compared with it at 100 µg/ml. The results of anti-denaturation were given in the table 1 and fig. 1. As results of this study, it becomes evident that ethanol extracts of *H. procumbens* and *U. Dioica* were more active than Aspirin (% Anti-denaturation of Aspirin at 100 µg/ml= 68% ± 0.12). Therefore, form the results of the present study it can be concluded that *H. procumbens* and *U. Dioica* extracts have in vitro anti-inflammatory effects against the denaturation of protein. The results showed all of extracts protected the Bovine Serum Albumin (BSA) against heat induced denaturation. The percentage of BSA protection against heat was increased with decreasing concentration. Both of extracts were found to have the good anti-denaturation at lowest concentrations. This result is coinciding with the statement given by William *et al.*, that the anti-denaturation action of extract is more when the concentration is less (Williams, 2009). Ethanolic extract of *H. procumbens* showed greater percentage of protection than *U. Dioica* at 50 µg/ml. As shown in table 1, among the two extracts under the study, ethanol extract of *H. procumbens* at 50 µg/ml has shown better inhibition of BSA denaturation (-71.94±0.0047 %) compared to ethanol extract of *U.*

Dioica (69.54±0.0025 %). The in-vitro anti-inflammatory activities of the extracts were comparable to the Aspirin, a reference drug at 100 µg/ mL (68.0 ±0.12%). The anti-inflammatory activity of plants may due to the presence of active compounds such as flavonoids and triterpenoids (Sakat *et al.*, 2010). Phenolic compounds possess a potentially beneficial lipoxygenase inhibitory and antioxidant properties; so they have been used for the treatment of inflammatory diseases (Sreejayan and Rao, 1996). Therefore the anti-inflammatory activity of *H. procumbens* and *U. Dioica* in this work seems to be due to the high polyphenolic compounds in them. In previous studies *H. procumbens* was found as rich source of polyphenolic compounds (Motlhanka, 2012). Several phytochemical constituents were isolated from *H. procumbens* including iridoids, harpagoquinones, amino acids, flavonoids, phytosterols and carbohydrates (Gruenwald, 2002). In Fiebich et al research, the results demonstrates that *H. procumbens* release pro-inflammatory factors by affecting intracellular signal transduction pathways such as the activation of the transcription factor AP-1 (Bernd, 2011). Kaszkin *et al.* showed both extracts of Harpagophytum (one containing 8.9% harpagoside and the other containing 27% harpagoside) have inhibitory effects on the inducible enzyme nitric oxide synthase (iNOS), which is known for its role, in inflammatory processes (Kaszkin *et al.*, 2004). The major chemical constituents of *H. procumbens* were iridoid glycosides and phenylethonoid acteoside (Clarkson *et al.*, 2006). Acteoside was shown to exert anti-inflammatory effects (Reinke *et al.*, 2015). Inflammation effect of *H. procumbens* is due to its ability to block the production of inflammatory mediators such as PGE2 (Aberham *et al.*, 2007). The previous studies showed that the *U. dioica* extract has anti-inflammatory properties such as in Khalili study, alcoholic extract of *U. dioica* in 400 mg/kg could have diminished inflammation 24.08 ± 2.1 % (Khalili *et al.*, 2012). The commonly phytochemical compounds from *U. dioica* are flavonoids, tanins, volatile compounds and sterols (Krystofova *et al.*, 2010; Gul *et al.*, 2005). As shown in fig. 1, ethanol extract of *H. procumbens* (100%) has higher anti-inflammatory effect than combined extracts in all concentration; but ethanol extract of *U. Dioica* (100%) was weaker than combined extracts in concentrations of 400, 600, 800 and 1000 µg/ml.

Conclusion

In conclusion, the present study revealed that *H. procumbens* and *U. Dioica* extracts produced marked in-vitro anti-inflammatory activity and they protected BSA from denaturation at a concentration 50 µg/ml (69-71%); but the combination of them hadn't positive effect on the denaturation of protein. Further investigations are required to isolate the active component responsible for their anti inflammatory effects.

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