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Evaluation Of In Vitro Anti-Inflammatory Potential of Andrographis Megamalayana and A.Lawsonii

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ABSTRACT
The present investigation focused on the anti-inflammatory activity of the ethanolic leaf extract of A.megamalayana and A. lawsonii. The anti-inflammatory potential of ethanolic leaf extract of both the plants were assessed using albumin denaturation and HRBC membrane stabilization methods. The outcome of the study indicates that the percent inhibition of albumin denaturation was within the range of 16.31 to 75% and A.megamalayana leaf extract exhibited significantly higher level of inhibition. In HRBC membrane stabilization the inhibition was within the range of 14% to 67%. In conclusion, our findings highlight the promising anti-inflammatory potential of A.megamalayana and A. lawsoniiwarranting further investigation to elucidate its therapeutic implication for the management of inflammatory disorders.

Keywords: Anti-inflammatory, denaturation, membrane stabilization, therapeutics.

I. INTRODUCTION

Nature has been a remedial asset over a period of millennia before the onset of synthetic era. Aboriginal man perceived and treasured the inordinate diversity of plants around him. About 2, 50, 000 higher plants are registered worldwide among which 80, 000 plants exert therapeutic potential and designated as medicinal plants. India, with its opulent tradition of indigenous remedies from the Vedic period is one of the tropical countries harbours 45000 varied plant species, it is being one among the 17 mega biodiversity centres (Krishnamoorthy et al., 2018). It has about 30% of the plants being estimated to be used for its medicinal value in many developed countries. Whereas in fast developing countries such as India and China about 80% of the medicinal plants being contributed for therapeutic utilities.

In recent years due to the exploitation of environment by pollution, noxious lifestyle and environmental toxins extended the chance of ailments. The modern synthetic drug despite so many achievements and progress, with side effects is the major concern for every disorder. To overcome such disputes World Health Organisation focused to harmonize traditional and complementary medicine to elevate global healthcare and to secure the quality, safety and efficacy of medicine (WHO, 1999).

Inflammation is a complex physiological response mediated by the immune system, serving as a protective mechanism against various harmful stimuli. However, dysregulated inflammation can lead to various ailments includes cardiovascular disorders, cancer and autoimmune condition. The vital objectives of this study are to developpotential novel anti-inflammatory therapeutics from the medicinal plants Andrographis megamalayana and A. lawsonii.

II. MATERIALS AND METHODS

Determination of in vitroantiinflammatory activity

Inhibitoin of albumin denaturation assay

Inhibition of albumin denaturation for the ethanolic leaf extract of Andrographislawsoniiwas determined according to the method of Mizushima and Kobayashi (1968) with some modifications. The reaction mixture contained the test extract at different concentrations (50,100,200,400 and 800) and 1% BSA (aqueous solution). 1 N HCl was used to adjust the pH of the reaction mixture. The samples were heated at 37 °C for 20 min and then 57 °C for 20 min, and allowed to cool. The turbidity of the samples was measured at 660 nm. The experiment was performed in triplicate. Percent inhibition of albumin denaturation was calculated as follows:

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$$\text{Percent inhibition} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs control}} \times 100$$

Where, Abs control and Ads Sample are the absorbance (at 600 nm) of the control and sample, respectively.

HRBC membrane stabilization method

Fresh human blood (10 mL) was collected in heparinized centrifuge tubes and centrifuged at 3000 rpm for 10 min and washed 3× with an equal volume of normal saline solution. The volume of the blood was measured and reconstituted as a 10% v/v suspension with normal saline (Sakat et al., 2010). The reaction mixture (2 mL) consisted of 1 mL ethanolic leaf extracts and 1 mL of 10% red blood cell suspension. For the control, saline was added instead of plant extract. Aspirin was used as a standard drug (positive control). The samples were incubated at 56 °C for 30 min, centrifuged at 2500 rpm for 5 min and the absorbance of the supernatant measured at 560 nm. The experiment was performed in triplicate. Percent membrane stabilization activity was calculated by the formula:

$$\text{Protection (\%)} = \frac{100 - (\text{OD sample}/\ \text{OD control}) \times 100}{\text{RE}}$$

III. RESULTS

Antiinflammatory analysis of ethanolic leaf extracts of A. megamalayanaand A. lawsonii

The inflammation is a cascade of biochemical events propagates the coordinate activation of signalling pathways that normalize inflammatory mediator levels in injured tissue cells and inflammatory cells recruited from the blood. The current study is focused on the inhibition of albumin denaturation and HRBC membrane stabilization assay of various concentration of A. megamalayanaand A. lawsoniiethanolic leaf extracts and were illustrated in Table 1 and 2.

Inhibitory effect of ethanolic leaf extracts of A. megamalayanaand A. lawsoniion albumin denaturation.

Albumin denaturation is generally concerned with the process by which albumin denature their formal structure by the application of external stress factors. As part of the present investigation on the mechanism of the anti-inflammatory activity, the potential inhibitory ability of the ethanolic leaf extracts of both Andrographis species against albumin denaturation were assessed and depicted in Table 1. The anti-albumin denaturation potential of both the plant samples at the concentration ranges from 50 to 800 µg/mL exhibited 50% effectiveness in inhibiting heat induced albumin denaturation. Among the test sample assessed A. megamalayanaethanolic leaf extract registered the highest degree of inhibition at 800 µg/mL concentration with 75.5 % protection. But the lowest inhibitory effect was marked to be 16.3% at the concentration of 50µg/mL by A. lawsoniiethanolic leaf extract. Meanwhile, the non-steroidal anti-inflammatory drug (NSAIDs) Asprin used as standard exhibited relatively higher inhibition capacity of 76.2 and 88.2% at 400 and 800 µg/mL concentration, respectively.

Effect of ethanolic leaf extracts of A. megamalayanaand A. lawsoniion membrane stabilization assay

Stabilization of lysosomal enzymes is considered to be significant in regulating the inflammatory response by precluding the release of lysosomal constituents of activated neutrophil. In the present context, ethanolic leaf extracts of both the test samples has been manifested a marked percentage of inhibition against hypotonicity induced membrane destabilization. However, the concentrations (50 – 800 µg/mL) of both the leaf extract effects the leading outcome. Among the assessed samples A. megamalayanaleaf extracts registered evidently high protection. As shown in Table 2. Maximum percentage of inhibition was registered by 800 µg/mL of A. megamalayanaleaf extract with 67.1% protection. While the lowest percent of inhibition was observed in ethanolic extract of A. lawsonii leaf at 50 µg/mL with 14.1% protection. Surprisingly, the outcome of the extracts analysed were nearly comparable with the percentage of hypotonicity induced membrane

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destabilization efficacy of the standard Asprin (72.3%) at 800µg/mL concentration.

Table 1. Effect of ethanolic leaf extract of A. megamalayana and A. lawsoniion albumin denaturation assay

Concentration (µg/ml)	Ethanolic Leaf extract of A. megamalayana	Ethanolic Leaf extract of A. lawsonii	Aspirin (Standard)
50	18.20 ± 0.10[a]	16.31 ± 0.60[a]	35.45 ± 0.05
100	38.45 ± 0.89[b]	27.53 ± 0.72[a]	53.25 ± 0.02
200	50.22 ± 0.20	41.16 ± 0.10[c]	65.11 ± 0.32
400	64.60 ± 0.20	59.10 ± 0.50 [c]	76.28 ± 0.26
800	75.50 ± 0.10	67.80 ± 0.20	88.23 ± 0.37

Values are expressed as mean ± SD (n=3)

Table 2. Effect of ethanolic leaf extract of A. megamalayana and A. lawsonii on membrane stabilization assay

Concentration (µg/ml)	Ethanolic Leaf extract of A. megamalayana	Ethanolic Leaf extract of A. lawsonii	Aspirin (Standard)
50	16.50 ± 0.50[a]	14.11 ± 0.20[a]	25.88 ± 0.33
100	28.40 ± 0.10[b]	24.30 ± 0.15[b]	34.40 ± 0.20
200	40.72 ± 0.10[c]	36.40 ± 0.30[b]	52.22 ± 0.35
400	53.67 ± 0.45[c]	49.50 ± 0.1[c]	64.98 ± 0.20
800	67.10 ± 0.40	64.80 ± 0.20	72.34 ± 0.50

Values are expressed as mean ± SD (n=3)

IV. DISCUSSION

Anti-inflammatory analysis of ethanolic leaf extracts of A. megamalayanaand A. lawsonii

Inflammation is an intricate mechanism of the immune system that can be triggered by the harmful stimuli associated with several events like cellular damage, pathogens and toxic compounds (Medzhitov, 2010). Denaturation of tissue proteins is a significant impact of inflammation related illness by which the protein loses their biological function. They act as a significant marker to check the root of inflammation (Banerjee et al., 2014; Anitha et al., 2014). In the present study, inhibition of heat induced protein denaturation was taken as a measure of anti-inflammatory activity for the ethanolic extracts of A. megamalayanaand A. lawsoniileaf. As it was evidenced (Table 1) that the examined plant extracts manifested substantial inhibitory effect at the concentration ranged between 50 and 800 µg/ml and were considerably comparable to the standard drug reference Aspirin. It was evinced that the ethanolic leaf extract of A. megamalayanashowed highest inhibitory effect in dose dependent manner than the other examined plant species. The increased absorbance by the examined plant samples with respect to control attributed the protein stabilization activity. It was reported that the anti-inflammatory potential of the extract relies on the phytochemical constituents present in it, as increase in concentration may possess higher the amount of active principles like flavonoids, terpenoids and related polyphenols may accountable for maximum inhibitory effect (Dey et al., 2011; Sangeetha and Vidhya, 2016). The bioentity responsible for the alteration of electrostatic force, hydrogen, hydrophobic and disulphide linkage can readily prevent the thermal induced denaturation of protein and therefore would be a possible candidature for the anti-inflammatory drug discovery (Kar et al., 2012).

Biogenesis of lysosomal enzymes in response with inflammation may impact the pathogenesis of number of disorders. The extra cellular activities of these enzymes were attributed to acute or chronic inflammation (Anosike et al., 2012; Amin et al., 2014). In this study, the anti-inflammatory effect of ethanolic extract of A. megamalayanaand A. lawsoniileaf was determined by HRBC membrane stabilization method and the outcome was depicted in Table 2. It was noticed that the ethanolic extracts showed membrane stabilization effect through hypotonic induced lyses RBC membrane. The haemoglobin was liberated as response to lyses of erythrocyte membrane, due to reduced stabilization of membrane and this erythrocyte membranes are the analogues of lysosomal membrane (Chou, 1997). Among the

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plant assessed ethanolic extract of A. megamalayanadepicted maximum percentage of stabilization effects on lysosomal membrane in dose dependent manner. In similar trend it was observed from earlier reference that the plant extracts might likely to stabilize the lysosomal membrane by inhibiting the release of lysosomal constituents at the site of inflammation. Stabilization of lysosomal contents is significant in restricting the release of lysosomal components of stimulated neutrophil from microbial enzymes and proteases (Sakat, et al., 2010; Veena and Manu, 2013). The anti-inflammatory mechanism of the extracts and the reference drug could be correlated with the linking to the RBC membrane with successive modification of the surface charge of the cells (Seema et al., 2011). Suprisingly, it was noted that the phytochemical constituents like flavonoids, tannins and Saponins exerted efficiency to bind cations, thus stabilizing RBC membranes and other biomacromolecules (Leela and Dass, 2011). Hence the effect could be extended in restraining the inflammatory stimuli and signifies A. megamalayana would serve as an alternative source for the novel anti-inflammatory drug development.

REFERENCES

[1]. Krishnamoorthy Y, Vijayageetha M, Kumar SG, Rajaa S, Rehman T. Prevalence of malnutrition and its associated factors among elderly population in rural Puducherry using mini-nutritional assessment questionnaire. J Family Med Prim Care. 2018 Nov-Dec;7(6):1429-1433.

[2]. Mizushima Y and Kobayashi M. 1968. Interaction of anti-inflammatory drugs with serum preoteins, especially with some biologically active proteins. J of Pharma Pharmacol. **20,** Pp:169- 173.

[3]. Sakat S, Juvekar AR, Gambhire MN. 2010. In vitro antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata Linn. I. J Pharm Sci. **2**(1). Pp: 146- 155.

[4]. Medzhitov R. Inflammation 2010: New adventures of an old flame. Cell. **140** Pp:771–776.

[5]. Banerjee et al., 2014Michael Peake, Malcolm Whiting. 2006. Measurement of Serum Creatinine – Current Status and Future Goals. Clin BiochemRev**27**Pp: 173.

[6]. Anitha Rani A., S. Mary Josephine Punitha, M. Rema. 2014. antiinflammatory activity of flower extract of Cassia auriculata – An in vitro study. Int. Res J Pharm. App Sci., **4**(1), Pp: 57-60.

[7]. Dey P, Chatterjee P, Chandra S, Bhattacharya S. 2011. Comparative in vitro evaluation of antiinflammatory effects of aerial parts and roots from Mikania scandens. J Adv Pharm Educ Res. **1** Pp:271–277.

[8]. Godhandaraman Sangeetha, Ramalingam Vidhya. 2016. In vitro anti-inflammatory activity of different parts of Pedalium murex (L.). International Journal of Herbal Medicine**4**(3) Pp: 31-36.

[9]. Amin Chowdhury, Shofiul Azam, Mohammed Abdullah Jainul, Kazi Omar Faruq, and Atiqul Islam. 2014. Antibacterial activities and in vitroantiinflammatory (Membrane Stability) properties of methanolic extracts of Gardenia coronaria leaves. International Journal Of Microbiology. Article Id 410935, Pp: 5 .

[10]. Biswakanth Kar RB, Suresh Kumar, Indrajit Karmakar, Narayan Dola, Asis Bala, Upal K Mazumder, Pallab K Hadar. 2012. Antioxidant and in vitro anti-inflammatory activities of Mimusopselengi leaves. Asian Pacific Journal of Tropical Biomedicine. **2**(2), Pp: 976-980.

[11]. Chioma A Anosike, OnyechiObidoa and Lawrence US Ezeanyika. 2012. Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (Solanum aethiopicum). Daru. **20**(1), Pp: 76.

[12]. Chou CT. 1997. The antiinflammatory effect of Tripterygium wilfordii F on adjuvant induced paw edema in rats and inflammatory mediator's release. Phytother. Res., **11**. Pp: 152-154.

[13]. Veena S and Manu Singh. 2013. In vitro antiarthritic and hemolysis preventive: Membrane stabilizing efficacy of ethanolic root extract of Operculinaturpethum.World. J. Pharm and Pharmac. Sci. **2**(1). Pp: 302-312.

[14]. Seema Chaitanya Chippada, Sharan Suresh luri, Srinivasa Rao Bammidi and Meena Vangalapati. 2011. In

vitroantiinflammatory activity of methanolic extract of Centella asiatica by HRBC membrane stabilisation. Rasayan J. Chem. .**4**, No.2, Pp :457-460.

[15]. Leelaprakash G, S.Mohan Dass. 2011. In vitro anti-inflammatory activity of methanol extract of Enicostemmaaxillare. International Journal Of Drug Development and Research. .**3** (3): 189-196.