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In Vitro Anti- Inflammatory And Thrombolytic Potential Of Leaf Extract Of *Andrographispaniculata*

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ABSTRACT

Andrographispaniculata(*Acanthacae*) is a widely used medicinal plant in China, India and South Asia. Ethanolic extract of *Andrographis paniculata* was assessed for its thrombolytic, anti-inflammatory activity and phytochemical screening. *In vitro* anti-inflammatory activity was evaluated using HRBC membrane stabilization. Aspirin was used as a standard drug for the study of anti-inflammatory activity. The ethanol extract of *Andrographis paniculata* showed significant activity. In thrombolytic activity using *in vitro* clot lysis assay method, the crude ethanol extract was found to have significant, thrombolytic test showed a maximum effect of 99.12%.while the standard streptokinase showed 90%. The plant has shown the presence of phytoconstituents such as tannins,saponin, carbohydrate which have been reported to show good protective cardio activity However, further detailed studies are required to determine the active components responsible for these effects and mechanism pathway.

Keywords:*Andrographispaniculata*, phytochemical analysis, thrombolytic, anti-inflammatory activity

INTRODUCTION

Medicinal plants have always been considered a healthy source of life for all people. Therapeutically properties of medical plants are very useful in healing various diseases and the advantage of these medicinal plants is being 100% natural

Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic, chemicals, allergens or other noxious stimulian uncontrolled and persistent inflammation may act as an etiological factor for many of these

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chronic illness. Although it is a defense mechanism,The complex events and mediators involved in the inflammatory reaction which can induce, maintain or aggravate many disease.currently used synthetic anti-inflammatory drugs produce fewer side effects hence there is a need for anti- inflammatory drugs with medicinal plants origin.A blood clot (thrombus) developed in the circulating system due to failure of hemostasis causes vascular blockage and while recovering leads to serious consequences in atherothrombotic diseases such as myocardial or cerebral infarction, at times leading to death. Thrombolytic agents that include tissue plasminogen activator (tPA), Urokinase (UK), Streptokinase (SK) etc. are used all over the World for the treatment of these diseases. In India, though SK and UK are widely used due to lower cost, (Mucklow*et al.,* 1995) (Collen*et al.,* 1990). As compared to other thrombolytic drugs, their use is associated with hyper risk of hemorrhage (Rouf*et al*., 1996) severe anaphylactic reaction and lacks specificity. Moreover, as a result of immunogenicity multiple treatments with SK in a given patient are restricted (Jennings, 1996). Because of the shortcomings of the available thrombolytic drugs, attempts are underway to develop improved recombinant variants of these drugs (Nicolini*et al*., 1992; Wu *et al*., 1992). Herbal products are often perceived as safe because they are "natural" (Gesler, 1992). In India, in recent years, there is an increase in the research of traditional ayurvedic herbal medicines on the basis of their known effectiveness in the treatment of ailments for which they have been traditionally applied. Considerable efforts have been directed towards the discovery and development of natural products from various plant and animal sources which have antiplatelet (Demrow*et al.,* 2000), anticoagulant (Leta*et al.,* 2002; Zhiguang*et al.,* 2000), antithrombolytic activity (Rajapakse, 2005).

The activities have been selected because of their great medicinal relevance. Within the recent years, heart diseases have increased to a great extent and side effects of synthetic drug becomes an ever-increasing therapeutic problem (Persoone, 1980). Because natural products of higher plants may give a new source of thrombolytic agents, many research groups are now engaged in medicinal plants research.

*Andrographispaniculata*commonly known as king of bitters, is a perennial herb widely cultivated in China, South Asia, South Africa, India, Pakistan and Srilankathe pharmacological activities such asanti-human immunodeficiency virus (HIV) activity(Change and Yeung 1988),Immunostimulatory activity (Puri*et al.,* 1993), antipyretic activity(Vedavathy and Rao, 1991),antidiarrhoeal activity (Gupta *et al*., 1990; Gupta *et al.,* 1993),anti-inflammatory activity (Shen*et al.,* 2002), antimalarial activity(Mishra *et al.,*

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1992), antimicrobial activity (Wanchaitanawong*et al.,* 2005),antivenom activity (Chang and But, 1987),hepatoprotectiveactivity (Jarukamjorn and Nemoto, 2008; Kapil, 1993; Visen*et al.,* 1993), anti-oxidant activity (Ojha*et al.,* 2009),anthelmintic activity(Padma *et al.*, 2011), dysentery or Gastroenteritis (Akbar, 2011),analgesic and anti-pyretic effects (Rammohan, 2009), immunomodulatory activity (Puri*et al.* (1993), antidiabetic activity (Reyes *et al.,* 2006; Rammohan, 2009), antibacterial activity (Tomoko *et al*., 2002), antiviral and antifungal effects (Aniel Kumaret *al.,* 2010),antifilarial, antiprotozoal and antiplasmodial activity (Arsenault *et al.,* 2008) andnematicidal, larvicidal/adulticial activity (Goel*et al*., 2011).

Andrographispaniculata recommended for prominent 26 Ayurvedic formulations treatment of various ailments include immunostimulant (Puri*et al*.,1993), asthma, gonorrhea, piles (Rao, 1914), dysentery and dyspepsis (Bhalla*et al*.,1982), influenza (Dey, 1986), gastric complaints, diarrohea (Gupta *et al*., 1990), pharyngitaonsillitis (Thamlikitkal*et al.*, 1991), fever (Ahmad *et al*., 1992), loss of scalp hair (Home *et al.*,1992), snake bite (Gupta *et al*.,1994), myocardialischemia (Guo*et al.*, 1995), common cold (Melchior*et al.*,1996), diabetes (Zhang*et al*., 2000), respiratory tract infections (Coon *et al.*, 2004), Jaundice (Tomar *et al.*,1983), antiulcerogenic (Viswanathan *et al*., 1981), antityphoid (Anonymous, 1985), antisnake venom (Selvanayagam*et al*., 1994), antifertility (Akbarsha*et al*., 2000), anti-inflammatory (Shen*et al.*, 2002), and antihyperglycemic (Rao *et al.*, 2006) activitythe present study was to screen the extract of *Andrographis paniculata* for its clot lysis property (thrombolytic activity) and anti-inflammatory activity by using an *in vitro* procedure.

Materials and Methods

Collection, Identification and Authentication of plant materials

The plant species namely *Andrographis paniculata* was collected by in and around Koothanallur, Thiruvarur District, Tamil Nadu, India. The plant was identified with the help of the Flora of Presidency of Madras and authenticated by Dr.S. John Britto, RAPINAT Herbarium and Center for Molecular Systematics, St. Joseph's College, Tiruchirappalli (Voucher number of the specimen, ME 004) (Gamble, 1997).

Preparation of plant powder

The plant was air dried under shade for 10-15 days. Then the dried material was grinded to fine powder using an electric grinder and stored in air tight bottles. The powder matter was used for further analysis.

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Preparation of the Ethanolic extract

Ethanolic extract was prepared according to the methodology of Indian pharmacopoeia (Anonymous, 1996). The coarse powder material was subjected to Soxhlet extraction separately and successively with 210 ml ethanol and 90 ml distilled water. These extract were concentrated to dryness in flash evaporator under reduced pressure controlled at a temperature (40℃-50℃). The paste form of the extracts was put in an air tight containeran astored in refrigerator.

Preliminary Phytochemical analysis

The preliminary phytochemical investigation of the whole plant of *Andrographis paniculata* was carried out with the standard protocol. The extracts are subjected to preliminary phytochemical analysis (Kokate*et al.*, 1995).

***In vitro* anti- inflammatory activity**

The human red blood cell membrane stabilization method (HRBC) has been used as a method to study the *in vitro* anti- inflammatory activity (Gandhisan*et al.*, 1991).

Human red blood cell (hrbc) membrane stabilization

Blood was collected from healthy human volunteer who was not taken any NSAIDS for 2 weeks prior to the experiment. The collected blood was mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% NaCl in water) and centrifuged at 3000 rpm. The packed cells were washed with isosaline (0.85%, pH 7.2) and a 10% (v/v) suspension was made with isosaline.

Various concentrations of extracts were prepared (100,200, and 300µg/ml) using distilled water and to each concentration 1ml of phosphate buffer (0.15M, pH 7.4), 2ml of hyposaline (0.36%) and 0.5 ml of HRBC suspension were added. It is incubated at 370°c for 30 min and centrifuged at 3,000 rpm for 20 min. The hemoglobin content in the supernatant solution was estimated spectrophotometrically at 560nm. Aspirin (100µg/ml) was used as reference standard and a control (distilled water) was prepared omitting the extracts. The percentage haemolysis was calculated by assuming the hemolysis produced in presence of distilled water of as 100% the percentage of HRBC membrane stabilization or hemolysis was calculated using the formula

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% of Inhibition of Hemolysis=100×OD1-OD2/OD1

Where,

OD1and OD2 are optical density of aspirin and test extracts respectively.

***In vitro* thrombolytic activity**

2 ml venous blood drawn from healthy volunteers was distributed in three different pre weighed sterile micro centrifuge tube (0.5 ml/tube) and incubated at 37°c for 45 minutes. After clot formation, serum was completely removed (aspirated out without disturbing the clot formed) and each tube having clot was again weighed to determine the clot weight (clot weight=weight of clot containing tube-weight of tube alone) to each micro centrifuge tube containing pre weighed clot, 100µl of ethanol extract (10mg/ml) of was added. As a positive control, 100µl of streptokinase and as a negative non-thrombolytic control, 100µl of distilled water were separately added to the control tubes numbered. All the tubes were then incubated at 37° c for 90 minutes and observed for clot lysis. After incubation,fluid released was removed and tubes were again weighed to observe the difference weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as constant oxygen supply for 48 hours.The experiment was repeated twice for better statistical results.

%clot lysis= (Weight after lysis clot/ Weight of clot before lysis) ×100

RESULTS AND DISCUSSION

Phytochemical Analysis

The preliminary phytochemical screening of leaves of*Andrographispaniculata r*evealed that ethanolic extract of *Andrographis paniculata* recorded the presence of alkaloid, flavonoid, tannins, fixed oils and fats, proteins and amino acids, diterpenes, terpenoids, gums and mucilage, phytosterol and glycosides (Table 1). Phytochemical constituents such as tannins, flavonoids and several aromatic compounds or secondary metabolic of plants serves as defense mechanism against predation by many microorganisms.

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Table 1: Qualitative phytochemical analysis ethanolic extract of *Andrographis paniculata*

S. No.	Name of the test	Ethanolic Extract
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Glycosides	+
4.	Saponins	+
5.	Phytosterols	+
6.	Fixed oils and fats	_
7.	Resins	_
8.	Phenols	+
9.	Tannins	+
10.	Flavonoids	+
11.	Proteins and amino acids	+
12.	Diterpenes	_
13.	Terpenoids	_
14.	Gums and Mucilage	_
15.	Coumarins	_
16.	Chlorogenic acid	_
17.	Steroids	_

(+) Indicates presence

(-) Indicates absennce

Anti-inflammatory activity

The lysosomal enzyme released during inflammation produces a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The nonsteroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. Since HRBC membrane is similar to lysosomal membrane, the study was undertaken to check the stability of HRBC membrane by ethanol extracts to predict the anti-inflammatory activity. The extracts at the concentration of 100,

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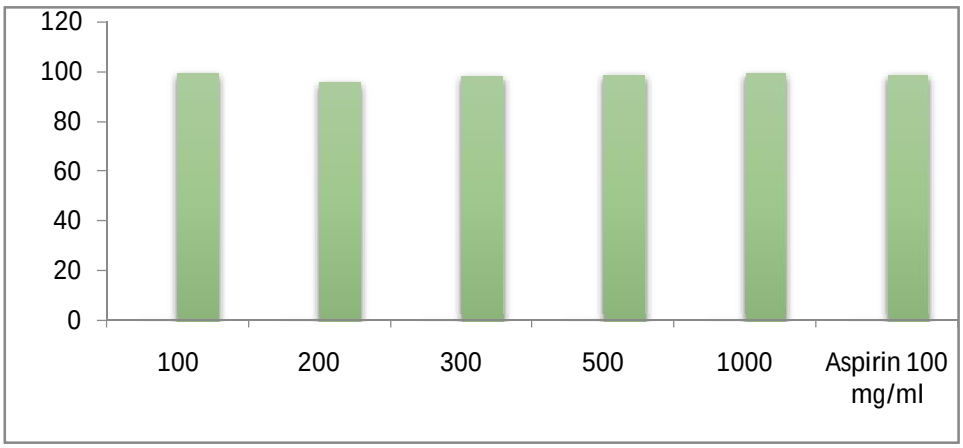
200, 300, 500, 1000 µg/ml were incubated separately with HRBC solution and 99.12 %haemolysis was compared with standard drug aspirin at the same concentration. The ethanol extracts at a concentration of 1000 µg/ml exhibited significant result (Figure2).

Figure 2*: In vitro* anti- inflammatory activity of whole plant extract of *Andrographis paniculata*



Pain is a symptom of many disease requiring treatment with analgesics, it is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Pain can also be elicited by inflammation. Progress has been made in elucidating the role of various endogenous substances such as prostaglandins and peptides in the inflammatory process. Most of the so called non-steroidal anti-inflammatory agents have analgesic activity. Some plant active constituents also possess anti-inflammatory activity (Manohara, 2009). In the present investigation an authenticated leaves of *A. paniculata* was extracted with ethanol. Using the extracts the anti-inflammatory activity has been performed by *in vitro* HRBC membrane stabilization method. Among the concentrations of the plant extracts at a concentration of 1000µg/ml showed better results may be due to the presence of phytochemicals.

***In vitro* Thrombolytic Activity**

In thrombolytic activity using *in vitro* clot lysis assay method, the crude ethanol extract showed average clot lysis of 76 % while the standard streptokinase showed 90 %

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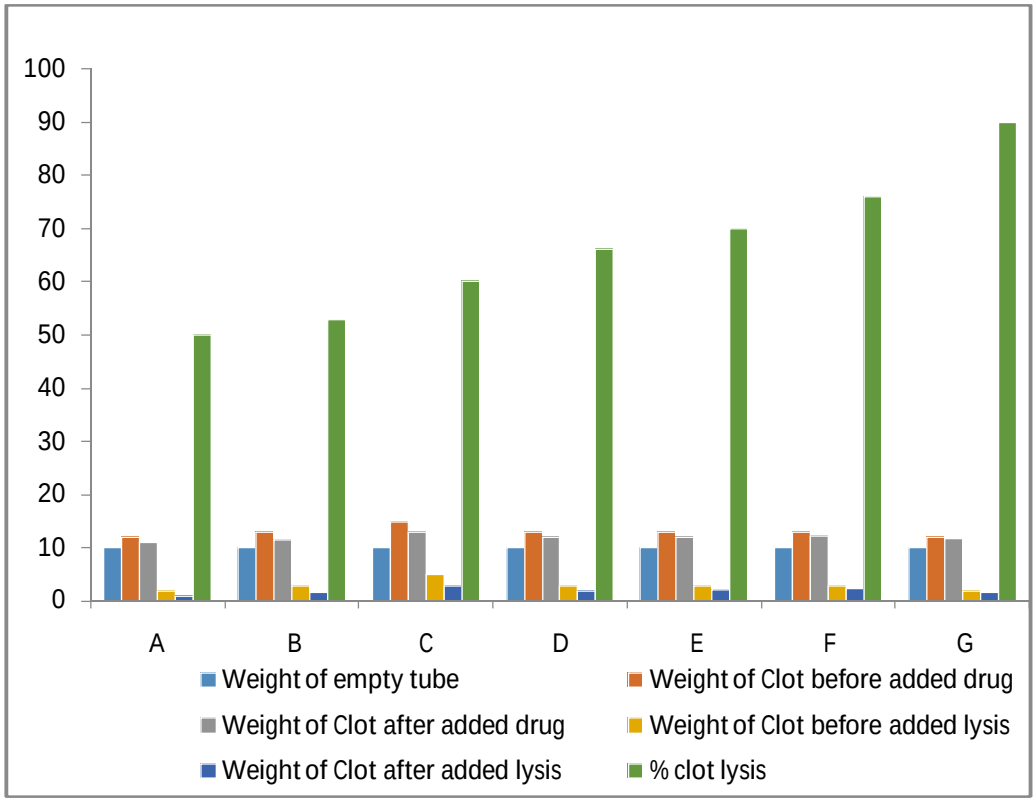
which is shown in Figure 3. The percentage of clot lysis was found to be significant when compared with the vehicle control.Therefore, it is evident that the sample and ethanol extract possess thrombolytic activity as well as biologically active.Several herbal drugs are being evaluated for their thrombolytic activity (Sikandar*et al*., 2013; Sekendar Ali *et al*., 2013; FahadHussain*et al.,* 2014). Since the formation of thrombus leads to fatal diseases such as venous thrombosis, portal vein thrombosis, Paget-Schroetter disease, Budd-Chiari syndrome.

Figure 3: Values of the clot lysis Experiment



On the basis of the results obtained in the present study, it is concluded that the *Andrographis paniculata*possess significant anti- inflammatory activity as well as thrombolytic activity. In addition, positive result in thrombolytic activity test led us to the interference that the plant extract may contain bioactive compounds, which may aid ongoing cardiovascular drug discovery from the floristic resources. Hence, further studies are suggested to be undertaken to pin point the exact compounds and to better, understand its actions scientifically.

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