Research Paper

Anti-inflammatory and antimicrobial activity of V. aconitifolia

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ABSTRACT

Natural products are still one of the major sources of new drug molecules today. There are several reports in literature regarding the antimicrobial and anti-inflammatory activity of plant extracts. We used crude ethanolic extract of Vigna aconitifolia dissolved in distilled water to measure its antibacterial, anti-inflammatory, and the antipyretic effects. Relative percentage inhibition (RPI) for V. aconitifolia as compared with Amikacin when tested against Escherichia coli was 64.1%, while it was not effective against Staphylococcus aureus and Staphylococcus epidermidis. The anti-inflammatory effects of V. aconitifolia were comparable to standard drug Aspirin when it was used in 300 mg concentration. Similarly, V. aconitifolia showed pronounced antipyretic effect. Percentage reduction in pyrexia was comparable between V. aconitifolia and Aspirin during 1st, 2nd, 3rd and 4th h of administration. Phytochemical screening of V. aconitifolia showed the presence of alkaloids, flavonoids and tannin and these possess anti-inflammatory and antipyretic activity which has been reported in previous studies. Our results suggest further investigation to determine the detail immune-modulatory role of V. aconitifolia in reducing the inflammatory response of the body.

Key words: Natural products, Vigna aconitifolia, antimicrobial activity, anti-inflammatory activity.

INTRODUCTION

Bacterial and fungal pathogens have evolved numerous defense mechanisms against antimicrobial agents, and resistance to old and newly produced drugs is on the increase. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo and Bosio, 1996; Scaccocchio et al., 2001). There are several reports in literature regarding the antimicrobial activity of crude extracts prepared from plants (El-Seedi et al., 2002; Rojas et al., 2003; Duraipandiyan et al., 2006; Nair et al., 2007).

The indiscriminate use of antibiotics has led to rapid spread of multidrug-resistance against conventional antibiotics globally, making it difficult to select an antimicrobial therapy against simple diseases. Antimicrobial susceptibility testing can be used for drug discovery, epidemiology and prediction of therapeutic outcome. At present, natural products are still one of the major sources of new drug molecules. Microbial and plant products occupy the major part of the antimicrobial compounds discovered until now.

Inflammation is manifested by pain, heat swelling and redness. Many a time, the cause of pain is some kind of underlying inflammatory process, regardless of the etiology. With better understanding of the role of inflammatory cytokines, the pathways by which many anti-inflammatory drugs can alleviate inflammation and relieve pain have become more evident. Classically, anti-inflammatory drugs have been used to alleviate inflammation, but the significant adverse effect profiles have always made researchers interested in looking for more safe and effective alternatives. Moreover, the dietary supplement and herbal remedies have been used for
centuries to reduce pain and inflammation (Maroon et al., 2010).

Medicinal plants are valuable for the production of various drugs. These plants are traditionally used to treat various diseases. At present, more than 70% of the populations in developing countries rely on medicinal systems (which used traditionally) known as medicine complementary or unconventional systems (Azaizeh et al., 2010). Absolutely, traditional medicinal plants used by people through the system are source of many essential medicines (Balick, 1996). People consume food plants frequently as a part of their diet; these plant parts may provide a continuous supply of antimicrobial agents against various pathogens, furnishing an added benefit against these microbes at times of need. Many of these plants have been used as anti-inflammatory agents by herbalists.

The plant Vigna aconitifolia, mostly cultivated in India, Pakistan and Myanmar (Marechal and Stainier, 1978) is used as forage, cover crop and green manure. Whole or split moth bean seeds are cooked or fried. In India, particularly in the state of Maharashtra, sprouted moth beans are used for making a spicy stew. Fried splits make up a ready-to-eat traditional spicy dry snack, called dalmoth. It is believed that consumption of the seeds can help treat a fever. Some researchers have advocated that cultivated plants are more than just simple foods and over the period, the use of these foods has changed as the beneficial properties of these foods have been recognized. For example, Lima beans in our country, has been used in curing fever (Phillips, 1993). Scientific evidences relevant to folkloric uses reported are lacking and efforts are needed to validate its claimed pharmacological activities. The present study aims at investigating the antimicrobial as well as anti-inflammatory activity of the Vigna aconitifolia.

MATERIALS AND METHODS

Preparation of Vigna extracts

Dried beans of V. aconitifolia were identified, collected and a specimen was submitted to herbarium of the Taxonomy Department of the Institute of Pure and Applied Biology, Multan. Coarsely milled powder material (1-1/2 kg) was soaked in 30:70 aqueous ethanol for 10-12 day by intermittent shaking. Then, filtration was done by the muslin cloth and Whatman filter paper simultaneously. The filtrate was evaporated on a rotary evaporator (Buchi R-200 Switzerland) under reduce pressure (760 mmHg) to a thick, semi-solid paste of light yellow color (Khan et al., 2013). The crude ethanolic extract of V. aconitifolia (0.3) was dissolved in 1 ml of distill water to prepare 0.3 g/ml w/v (300 mg/ml) of stock solution. The serial dilutions (30 and 3 mg/ml) concentration of plant extract was made from this stock solution.

Test culture

Escherichia coli, staphylococcus aureus, and Staphylococcus epidermidis bacterial cultures were used to screen antimicrobial activity. These cultures were isolated from the patients in the local hospitals and collected for experimental use. The cultures were grown in nutrient agar slant, streaked on agar plates, and stored at 4°C. Single colony of pure culture was picked up from the stock culture, incubated in nutrient broth at 37±1°C for 24 h.

Animals

24 local strain rabbits (1.25-1.8 kg) and 32 Wistar rats were purchased and housed in the animal house of the Faculty of Pharmacy, Bahauddin Zakaryaa University, Multan, under controlled environmental condition (23-25°C) after IACUC approval. The animals were watered and fed with fresh green fodder (rabbits) and certified Purina Rat Chow(R) ad libitum. We used two sets of rabbits, an experimental group and two control groups (positive control and a negative control) with six animals in each set of animals to assess the antipyretic effect of Vigna aconitifolia. The control groups were utilized in the successive repeats of experiment to minimize the animal use. The Wistar rats were divided in to four groups with four animals in each group. The experiment was repeated in triplicates reusing the two control groups.

Measuring the antibacterial activity of Vigna aconitifolia

Paper disc diffusion method was used to evaluate the antibacterial activity (Aida et al., 2001). 20 ml of sterile nutrient agar was spread in a Petri dish, over agar media and 1 ml of pure inoculum was spread using a sterile glass rod. Filter paper disc was immersed in 100 mg/ml plant extract and it was placed in the spread inoculum aseptically. These plates were placed in incubator for 24 h at 37°C. Zone of inhibition around disc showed the antimicrobial activity. Ciprofloxacin, Lincomycin and Amikacin were used as standard drugs for the various bacteria. The relative percentage inhibition of test material with respect to standard drug was calculated. The test was repeated in triplicate.

Measuring the Anti-inflammatory activity of Vigna aconitifolia

Edema was induced in 24 rats by sub-plantar injection of 0.1 ml of 1% aqueous gel of carrageenan-λ into the right hind paw of the animals (Vinegar et al., 1969). The size of paw was assessed by displacement method using
plathysmometer (II-520MR, World Precision Instruments). Calculating the percentage of increase in paw volume assessed the intensity of the edema which was maximum after 3 h of the carrageenan injection (Lee and Crosbym, 1999). These rats were divided into 4 groups with 6 rats in each group. An intraperitoneal injection of test and control doses were given to four groups and volume of paw was assessed after 1, 2, 3, and 4 h (Ferreira, 1979). The positive control group was injected 10 mg/kg aspirin, negative control was injected with 10 mg/kg saline and two study groups were injected with 100 and 300 mg/ml of Vigna extract. The percentage of edema inhibition in treated animal in comparison with negative control group was measured by percent inhibition = (1 - VT/V0) × 100, where VT is the volume of the paw in the test sample and V0 is the volume of the paw in the negative control sample.

**Measuring the anti-pyretic activity of Vigna aconitifolia**

Rectal temperature of rabbits were recorded by inserting a well-lubricated bulb of a thermometer of about 5 cm in the rectum prior to beginning the experiment. Boiled cow’s milk was injected intraperitoneally into rabbit at the dose of 0.5 ml/kg body weight to induce pyrexia. Induction fever took 1-2 h (Brasseur, 1989). The experimental group received crude extract of Vigna dissolved in saline at dose of 80 mg/kg intraperitoneally, while the positive and negative controls were given 10 mg/kg body aspirin and normal saline, respectively through intraperitoneal injection. Finally, rectal temperatures were noted at one-hour interval for four hours. Percentage reduction = (B - Ca)/B × 100 was the formula used to assess the reduction in rectal temperature, where A is the normal rectal temperature, B is the rectal temperature after 2 h of boiled administration, C is the rectal temperature after treatment and n represents 1st, 2nd, 3rd, and 4th h.

**RESULTS**

**Antibacterial activity of Vigna aconitifolia**

Antimicrobial action of Vigna aconitifolia was tested against three bacterial strains namely E. coli, S. aureus and S. epidermidis. V. aconitifolia showed antibacterial activity against Gram negative E. coli at the dose 100 mg/ml, whereas no significant effect was observed against Gram positive bacteria, that is, S. aureus and S. epidermidis. Table 1 shows the zone of inhibition (mm) and relative percentage inhibition of the V. aconitifolia and standard drugs for the three bacteria used in the experiment. There was a 64.1% relative percentage inhibition (RPI) for V. aconitifolia as compared with the Amikacin when tested against E. coli, while it did not show any activity against other two Gram positive strains of bacteria.

**Anti-inflammatory activity of Vigna aconitifolia**

In this study, the anti-inflammatory activity was measured by inducing edema through Carrageenan on rat paw. Our results showed that the extract of V. aconitifolia reduced the edema induced by carrageenan at dose of 100 and 300 mg/ml significantly as shown in Figure 1. The effects were comparable to standard drug Aspirin when Vigna was used in 300 mg concentration.

**Anti-pyretic activity of Vigna aconitifolia**

Intraperitoneal injection of boiled milk induced pyrexia in rabbits. The ethanolic extract of V. aconitifolia (80 mg/kg) administered intraperitoneally reduced pyrexia corresponding to antipyretic activity of Aspirin. The reduction in rectal temperature of rabbit by the crude extract of V. aconitifolia was significant as compared with the control group at different time intervals. Percentage reduction in pyrexia was compared between V. aconitifolia and Aspirin during 1st, 2nd, 3rd and 4th h of administration (Figure 2).
Figure 1: Average percentage inhibition of inflammation by Aspirin and various doses of *V. aconitifolia* at different intervals.

Figure 2: Inhibition of crude extract *V. aconitifolia* and Aspirin on boiled milk induced pyrexia model at different time intervals.

Ethanol extract of *V. aconitifolia* had significant antipyretic activity induced by boiled milk in rabbit, which is comparable with the Aspirin (standard drug). Our results showed a better response to *V. aconitifolia*. Pyrexia was well-defined by the International Union of Physiological Sciences Commission as a state of prominent increase in core temperature which is part of the defensive responses of hosts to microorganisms or nonliving matter.
known as pathogenic or foreign particle. Sepsis is implicated in hyperthermia in most of the hospitalized cases (Kaul et al., 2006). Fever may be seen as body's response to malignancy, tissue ischemia or drug reactions (Bor et al., 1998). Apart from exogenous pyrogens (e.g. microorganisms), many of the endogenous pyrogens, such as interleukin (IL)-1, IL-6, tumour necrosis factor (TNF)-α and interferon, may interact with cellular structures of anterior hypothalamus. These cellular structures lack blood brain barrier thus allowing the pyrogens to directly stimulate them. This lead to activation of the arachidonic acid pathway that increases the formation of prostaglandin E2 (PGE2) which acts on preoptic nucleus of the hypothalamus, consequently increasing the body temperature (Walter et al., 2016). PGE2 is the final mediator for febrile response. Most of synthetic anti-pyretic drugs inhibit COX-2 enzyme, thus inhibiting PGE2 biosynthesis and reduce body temperature. Natural COX-2 inhibitors have been reported previously and these have been shown to play a significant role as anti-pyretic as well as anti-inflammatory agents (Cerella et al., 2010). Phytochemical screening of *V. aconitifolia* showed the presence of alkaloids, flavonoids and tannin and these possess anti-pyretic activity which has been reported in previous studies (Forestieri et al., 1996; Kim et al., 2000; Sengar et al., 2015).

The aqueous ethanolic extract of *V. aconitifolia* showed a significant effect against gram negative bacteria *E. coli*. The bacteria, *E. coli*, are most abundant facultative anaerobes. Virulent strains of *E. coli* cause various diseases in humans, such as urinary tract infection, gastroenteritis, meningitis, peritonitis and hemolytic uremic syndrome, along with some rare diseases (Griffin and Tauxe, 1991; Nataro and Kaper, 1998; Darfeuille-Michaud et al., 2004). The comparison of activity was done with amikacin which is a customarily used drug for treating *E.coli* infections. The zone of inhibition was 14.74 mm at dose of 100 mg/ml of *V. aconitifolia* as compared Amikacin which showed 23 mm zone of inhibition, indicating that *V. aconitifolia* is active against Gram negative. However, it did not show any effect on the two gram positive strains. The anti-bacterial activity of various plants has been reported earlier including *Vigna radiata* (Parekh and Chanda, 2007). The antibacterial activity of flavonoids, saponins and alkaloids has been well documented (Barbieri et al., 2017).

The anti-inflammatory activity of *V. aconitifolia* was also evaluated. The crude extract of *V. aconitifolia* inhibited edema in the first hour, but more pronounced effect was seen in the 3rd and the 4th h. Aspirin was used as positive standard control drug. Aspirin caused reduction in inflammation as previous studies showed anti-inflammatory effect of NSAIDs on rat induced by Carrageen (Brune and Hinz, 2004; Ratchford et al., 2017). Inflammatory response is also related to the release of chemical mediator as histamine, bradykinin, serotonin and prostaglandins (Dong et al., 2014; Serhan et al., 2015). Many studies have shown that prostaglandins are involved in pathophysiological procedures, such as inflammation and pain. COX has two isoforms: COX-1 and COX-2. COX-1 is constitutive, while COX-2 is inducible and not detected in most tissues. COX-2 can be prompted by a variety of factors, such as pro-inflammatory cytokines and play a significant role in inflammation (Seibert et al., 1997). Carrageenan-induced rat paw edema is a biphasic event where the early stage is due to the release of serotonin and histamine and the late phase is due to production of arachidonic metabolites, protease, and lysosomal enzymes (Vinegar et al., 1969). The flavonoids and alkaloids showed a significant anti-inflammatory activity against arachidonic acid metabolites, various pro-inflammatory cytokines, serotonin and histamine. Numerous studies have suggested that the chemical constituents of *V. radiata* (Mung) show great potential to improve the clinical symptoms of inflammation-associated diseases, such as allergies and diabetes (Bellik et al., 2012).

Findings of the present study on *V. aconitifolia* are being reported for the first time. As a commonly used food, it has the potential to provide a continuous anti-inflammatory activity during various diseases including diabetes and atherosclerosis, furnishing an additional benefit in such patients. Our results suggest that crude extracts of *V. aconitifolia* play a significant role in reducing inflammation. Therefore, further investigation is required to determine the detail immune-modulatory role of *V. aconitifolia* constituents in reducing the inflammatory response of the body.

**REFERENCES**


