

Immunostimulant activities of *Aristolochia indica* L. and *Tinospora cordifolia* L. in Azathioprine induced immuno suppressed rats

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Abstract

The present study gives details of the qualitative phytochemical analysis and the respective immuno stimulating activities of the herbs *Aristolochia indica* L. and *Tinospora cordifolia* L. in Azathioprine induced immune suppressed rats. Alterations in the haematological and biochemical parameters due to azathioprine treatment revealed immuno suppression which were ameliorated by the ethanolic extracts of the herbs.

Keywords: azathioprine, *Aristolochia indica*, immunomodulation, phytochemistry, *Tinospora cordifolia*

INTRODUCTION

Humans have been fighting against a variety of diseases since prehistoric periods. The immune system of vertebrates consist of many types of proteins, cells, organs and tissues which interact in an elaborate and dynamic network. As part of this more complex immune response, the vertebrate immune system adapts over time to recognize particular pathogens more efficiently. This adaptation process creates immunological memories and allows even more effective protection during future encounters with these pathogens. This process of acquired immunity is the basis of vaccination (Beck and Gail, 1996). Thus the immune system is a remarkably effective structure that incorporates specificity, inducibility and adaptation. However, failures of host defense do occur which fall into three broad categories *viz.*, Immunodeficiencies, autoimmunity and hypersensitivities.

Malnutrition is the most common cause of immunodeficiency in developing countries. Diets lacking sufficient protein are associated with impaired cell mediated immunity. Deficiency of single nutrients such as Zinc, Selenium, Iron, Copper, Vitamins A, C, E, B₆ and B₉ also reduces immune responses (Chandra, 1997). Fetal undernourishment can cause a lifelong impairment of the immune system (Langley – Evans and Carrington, 2006).

Environmental factors such as changes in weather, dry and overheated rooms, pollutants appear to reduce one's resistance as well. The obvious effect of a weakened immune system is an increased susceptibility to illness.

Herbal drugs are known to possess immunomodulatory properties and generally act by stimulating both specific and non-specific immunity (Wagner and Proksh, 1985).

The genus *Aristolochia* consists of 350 species of which 15 are native to India. *Aristolochia indica* is a climbing plant commonly found in eastern and southern India (Bhattacharjee, 2001).

Roots, leaves, fruits, rhizome, stem and seeds are therapeutic in nature. Leaves are used to treat bowel complaints. Seeds are good for inflammation. The plant is good for treating snake bite (Chopra *et al.*, 1982). Decoction of stem is stimulant, tonic and febrifuge.

Tinospora cordifolia is a glabrous, succulent, climbing shrub native to India, particularly fond of climbing up the trunks of large neem trees. The viscous sap of leaves has a nauseating bitter taste (Stanley *et al.*, 2000; Prince *et al.*, 1999). The plant possesses adaptogenic effect (Rege *et al.*, 1989), antidiabetic, antioxidant, hypolipidemic, anti inflammatory activities (Gupta *et al.*, 1967; Wadood *et al.*, 1992; Rege *et al.*, 1994; Sharma, 1995; Mathew and Kuttan, 2001).

This paper gives details about a preliminary phytochemical analysis and immuno stimulant activities of ethanolic extracts of *Aristolochia indica* L. and *Tinospora cordifolia* L. in azathioprine induced immuno suppressed rats.

MATERIALS AND METHODS

Collection of plant materials and preparation of extracts:

The aerial parts of the plant *A. indica* and *T. cordifolia* were collected from Thanjavur, Tamil Nadu, India, dried in shadow separately, pulverized and then subjected to soxhlet extraction. The ethanolic extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature. The

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concentrated materials were stored separately in airtight containers for future use. Qualitative phytochemical analysis were carried out using the procedures of Kokate (1996).

Animals

Male albino rats (Wistar strain, weighing 150-200g) were purchased from Animal house, Mayiladuthurai housed under standard husbandry conditions (30°C±2°, 60-70% relative humidity and 12h:12h day-night cycle), and were allowed standard pellet rat feed and water *ad libitum*.

Induction of immuno suppression

Azasan (Glaxo smithkline) chemically known as Azathioprine was used as the immuno suppressive drug. Azasan 200mg/kg body weight was administered orally.

Experimental design

Animals were divided in to 8 groups of 6 animals each.

- Group 1 : Rats served as control, received routine feed and water.
- Group 2 : Immunesuppression induced rats (Azasan 200mg/kg b.wt., orally)
- Group 3 : Rats administered with Azasan (200mg/kg b.wt., orally) along with *Aristolochia indica* (50mg/kg b.wt., orally)
- Group 4 : Rats administered with Azasan (200mg/kg b.wt., orally) along with *Aristolochia indica* (100mg/kg b.wt., orally).
- Group 5 : Rats administered with Azasan (200mg/kg b.wt., orally) along with *Aristolochia indica* (200mg/kg b.wt., orally)
- Group 6 : Rats administered with Azasan (200mg/kg b.wt., orally) along with *Tinospora cordifolia* (50mg/kg b.wt., orally)
- Group 7 : Rats administered with Azasan (200mg/kg b.wt., orally) along with *Tinospora cordifolia* (100mg/kg b.wt., orally)
- Group 8 : Rats administered with Azasan (200mg/kg b.wt., orally) along with *Tinospora cordifolia* (200mg/kg b.wt., orally).

After stipulated time (21 days), the effectiveness of the herbal drug was evaluated through the blood and serum samples. The hematological parameters such as

Haemoglobin content, RBC, WBC, and Mean Corpuscles Volume (MCV) were evaluated from the blood samples. Serum was used for the estimation of AST, ALT, and Vitamin C using standard procedures.

RESULTS AND DISCUSSION

Immunomodulation by medicinal plants is comparatively a recent concept in pharmacognosy. Although human immune system is quite able to fight against various infections, certain infectious agents, unhealthy living conditions, chronic illness, environmental pollution and stress are harmful to immune system. In an effort to win the battle over such diseases, earlier researches have examined a variety of natural substances that demonstrate immuno modulatory potentials. Immuno modulation is described as the ability of a nutrient, herb or other substances to promote healthy immune function.

Certain plant compounds have been shown in experimental studies to have immuno stimulating properties by activating immune cells such as macrophages, lymphocytes and the cytokines (Roesler *et al.*, 1991; Puri *et al.*, 1993; Suresh and Vasudevan, 1994).

Medicinal plants have curative properties due to the presence of various complex chemical substances of different composition, which are found as secondary plant metabolites. In the present study, qualitative phytochemical analysis of herbal extracts revealed the presence of carbohydrates, alkaloids, tannins and phenols, saponins, fixed oils and fats, gums and mucilage, proteins and flavonoids (Table,1).

Corinna *et al.*, (2008) reported that azathoprine triggered suicidal erythrocyte death, an effect presumably contributing to azathioprine induced anaemia, Similarly in the present study, haematological profile was highly altered by azathioprine. Administration of the ethanolic extracts in increasing concentrations (50mg, 100mg, 200mg/kg b.wt.,) brought back the altered parameters with regard to RBC, WBC, Hb, MCV to near normal levels (Table,2).

Azathioprine acts upon rat hepatocyte mitochondria and leads to hepatic necrosis which inturn leads to the leakage of liver marker enzymes in to the blood (Mitra *et al.*, 1998). Azathioprine produced double fold increases in the levels of AST and ALT in the present study (Table,3). Highly elevated levels of liver marker enzymes have been reduced and restored to near normal in the treatment of 200mg/kg herbal extract.

One of the most studied nutrients with regard to immune functions is Vitamin C. It is believed to have an effect on the infectious diseases primarily because of its role in the phagocytic function of leucocytes. Research suggests that supplemental Vitamin C may

Table 1. Qualitative phytochemical properties of ethanolic extracts of *Aristolochia indica* and *Tinospora cordifolia*

S. No.	Name of the Compounds	Name of the test	<i>Aristolochia indica</i>	<i>Tinospora cordifolia</i>
1	Carbohydrates	1. Fehling's 2. Benedict's	++ ++	+++ +++
2	Alkaloids	1. Mayer's 2. Hager's 3. Wagner's 4. Dragen Dorff's	+++ ++ +++ ++	+ + - -
3	Steroids	Chloroform+acetic acid +H ₂ SO ₄	-	-
4	Tannins & Phenols	1. 10% Lead acetate 2. 5% Ferric chloride 3. 1% gelatine	+ + +	++ + +
5	Saponins	Foam test	+	+++
6	Fixed oils and fats	Spot test	+	++
7	Gums and Mucilage	Alcoholic Precipitation	+	++
8	Proteins	Biuret test	++	+++
9	Flavonoids	NaOH/HCl	+	++
10	Volatile oils	Hydro distillation method	-	-

Note:

- ++++ - Highly rich amount
- +++ - Rich amount
- ++ - Moderate amount
- + - Minimum amount
- - Absent

Table 2. Effects of ethanolic extracts of *Aristolochia indica* L. and *Tinospora cordifolia* L. on Haematological parameters of different treatment groups

S. No.	Treatment Groups	RBC (Millions/cu. mm)	WBC (Cells / cu.mm)	Hb g/dL	MCV (µ ³)
1.	Normal	6.2 ± 0.43	10.4±0.63	16.7±1.66	94.6±8.34
2	Disease control	6.1±0.22	7.8±0.32	15.4±1.23	89.2±6.74
3	<i>A. indica</i> 50 mg/Kg	6.2±0.31	7.8±0.27	15.3±1.12	89.1±7.23
4	<i>A. indica</i> 100 mg/Kg	6.2±0.43	8.2±0.38	15.7±1.08	90.3±7.32
5	<i>A. indica</i> 200 mg/Kg	6.2±0.31	8.5±0.34	15.8±1.11	90.8±7.51
6	<i>T. cordifolia</i> 50 mg/Kg	6.2±0.22	8.1±0.24	15.6±1.33	90.3±7.21
7	<i>T. cordifolia</i> 100 mg/Kg	6.2±0.38	8.4±0.26	15.9±1.37	91.5±7.41
8	<i>T. cordifolia</i> 200 mg/Kg	6.2±0.49	9.4±0.25	16.4±1.29	93.1±7.38

Table 3. Effects of ethanolic extracts of *Aristolochia indica* L. and *Tinospora cordifolia* L. on AST and ALT of different treatment groups

S. No.	Treatment Groups	AST U/L	ALT U/L
1.	Normal	40.37±4.31	35.28±3.56
2	Disease control	87.45±7.05	78.34±7.12
3	<i>A. indica</i> 50 mg/Kg	86.87±7.56	76.45±6.88
4	<i>A. indica</i> 100 mg/Kg	78.57±7.12	71.32±6.57
5	<i>A. indica</i> 200 mg/Kg	69.38±5.67	59.51±5.46
6	<i>T. cordifolia</i> 50 mg/Kg	81.34±7.22	72.34±6.36
7	<i>T. cordifolia</i> 100 mg/Kg	72.05±6.54	63.15±5.64
8	<i>T. cordifolia</i> 200 mg/Kg	55.33±5.37	48.23±4.21

Table 4. Effects of ethanolic extracts of *Aristolochia indica* L. and *Tinospora cordifolia* L. on Vitamin C of different treatment groups

S. No.	Treatment Groups	Vitamin C mg/dL
1.	Normal	10.41±0.38
2	Disease control	8.72±0.22
3	<i>A. indica</i> 50 mg/Kg	9.68±0.41
4	<i>A. indica</i> 100 mg/Kg	9.72±0.27
5	<i>A. indica</i> 200 mg/Kg	9.84±0.23
6	<i>T. cordifolia</i> 50 mg/Kg	9.68±0.21
7	<i>T. cordifolia</i> 100 mg/Kg	9.82±0.23
8	<i>T. cordifolia</i> 200 mg/Kg	10.22±0.45

help to enhance leucocyte activity and mobilization (Heuser and Vojdani, 1997).

Leucocytes contain very high concentrations of ascorbic acid that diminish with infection and return to normalcy after recovery. Only very high doses of Vitamin C are able to restore ascorbic acid levels to normalcy during an infection. Vitamin C is reported to reduce blood histamine levels, which may help to alleviate some of the respiratory symptoms associated with the infections. The reduced level of Vitamin C in immuno suppressive rats was remarkably restored by the herbal extracts of the present study.

The scientific evidences collected from the present study supports the traditional claims behind usage of *A. indica* and *T. cordifolia* for medicinal purposes. The effectiveness of extract treated animals in overcoming the effects of drug induced immuno suppression provided evidence for balancing and adaptogenic effectiveness of extracts. The inference drawn from the present study is that *T. cordifolia* is a better immuno stimulant than *A. indica*.

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