Antioxidant and Antiinflammatory Activities of the Flowers of *Tabernaemontana coronaria* (L) R.Br

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The ethanol and aqueous extracts of *Tabernaemontana coronaria* flowers possessed significant *in vitro* superoxide, hydroxyl radicals, nitric oxide scavenging, and lipid peroxidation inhibiting activities. The antiinflammatory activity of the ethanol extract was evaluated by carrageenan-induced acute and formalin-induced chronic antiinflammatory models in mice. The extract showed remarkable antiinflammatory activity in both models, comparable to the standard reference drug diclofenac. The results suggest that the antiinflammatory activity of the ethanol extract of *T. coronaria* is possibly attributed to its free radical scavenging properties.

Tabernaemontana coronaria R.Br (syn. *Ervatamia coronaria*) is a glabrous, evergreen, dichotomously branched shrub, belonging to the family Apocynaceae. It is distributed in upper Gangetic plain, Garhwal, East Bengal, Assam, Karnataka, Kerala, and in Burma[1]. In Ayurveda, the root is acrid; bitter with a flavour; digestible; useful in *kapha*, biliousness, and diseases of the blood. The root has a bitter bad taste. It is aphrodisiac; tonic, especially to the brain, liver, and spleen; and purgative. The milky juice mixed with oil is rubbed onto the head to cure pain in the eye; it kills intestinal worms, and its root, chewed, relieves toothache when rubbed with water; it is applied to wounds to prevent inflammation[1]. The allied species *Tabernaemontana divaricata*, when administered p.o. or i.p. to rats 1 h before subplantar injection of carrageenan, had a significant antiinflammatory activity[2]. A number of chemical constituents including indole alkaloids, phenols, and sterols from the leaves, stems, and roots of the plant have previously been reported[3-6]. Inflammation is elicited by numerous stimuli such as infectious agents, environmental factors, ischemia, antigen-antibody reaction, physiological and pathological factors, and also free radicals. Free radicals have been implicated in the causation of several diseases such as cancer and diabetes, and compounds that can scavenge free radicals have great potential in ameliorating these disease processes[7]. Antioxidants thus play an important role to protect human body against damage by reactive oxygen species[8]. The present study was undertaken to screen the relationship between antiinflammatory and antioxidant activity of the flowers of *T. coronaria.*

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MATERIALS AND METHODS

The flowers of *T. coronaria* were collected during the month of March 2004 from Amala Ayurvedic Hospital premises, Thrissur. The flowers were authenticated at the Department of Botany, St. Mary's College, Thrissur, and a voucher specimen (No.13) was kept at Fr. Gabriel Herbarium, Thrissur.

Preparation of the plant extract:
The flowers of the plant were collected and dried at 40-45° in the oven, and it was powdered and extracted successively using petroleum ether (for defatting), alcohol, and water in a Soxhlet at a temp range of 40-50°, and the extractive values were found to be 12 and 10.5% w/w, respectively.

Male Swiss mice (25-30 g) were supplied by small animals breeding station, College of Veterinary and Animal Science, Mannuthy, Thrissur. The animals were maintained under environmental conditions (22-28°, 60-70% relative humidity, 12 h dark/light cycle) and fed with standard rat feed (Lipton India Ltd.) and water *ad libitum.* All animal experiments conducted during the present study got prior permission from the Institutional Animal Ethics Committee (IAEC) and followed the guidelines of IAEC.

Determination of *in vitro* antioxidant activity:
The superoxide scavenging activity of alcohol and water extracts was determined by the method of McCord and Fridovich[9]. Hydroxyl radical scavenging activity was determined by measuring inhibition of hydroxyl radical generated by Fenton reaction[10]. Lipid peroxidation was induced in rat liver homogenate by incubating with Fe^{2+}-ascorbate for 1h, and lipid peroxide formation was determined by the estimation of thiobarbituric acid reacting substances (TBARS) formation[11].

Nitric oxide scavenging activity of both extracts was determined using Griess reagent. Reaction mixture (3 ml) containing sodium nitropruside (10 m mol), phosphate buffered saline (PBS), and the extracts (50-300 mg/ml) was incubated at 25° for 150 min. After incubation, 0.5 ml of reaction mixture was removed, and 0.5 ml of Griess reagent (1% sulphanilamide, 2% H_3PO_4, and 0.1% naphthyl ethylene diamine dihydrochloride) was added and measured at 546 nm[12].

Determination of antiinflammatory activity:
Male Swiss mice were divided into four groups of six animals each for both inflammation models. Group I was kept as control group (vehicle). Group II and III were given ethanol extract of *T. coronaria* 100 mg/kg body weight and 250 mg/kg body weight, respectively. Group IV was given diclofenac 25 mg/kg body weight.

Carrageenan-induced paw oedema:
In all groups, the inflammation was induced by single subplantar injection of 0.02 ml of freshly prepared 1% carrageenan in normal saline[13]. The groups received extracts of *T. coronaria* at concentrations of 100 and 250 mg/kg body weight, orally, 1 h before the carrageenan injection. The paw thickness was measured using vernier callipers at 1, 2, and 3 h after carrageenan injection. Increase in paw thickness as a measure of inflammatory oedema was calculated using the formula Pt–Po, where Po is the initial paw thickness and Pt is the thickness at time t (3 h). Percentage inhibition of inflammation was calculated by the formula (1–Pt/Pc)×100, where Pt is the increase in paw thickness of the treated and Pc is that of control. Diclofenac was used as the reference drug[14].

Formalin-induced paw oedema:
The animals were divided into four groups of six animals each. In animals of all groups, inflammation was produced by subplantar injection of 20 µl of freshly prepared 2% formalin in the right hind paw of mice. The paw thickness was measured using vernier callipers 1h before and after formalin injection. The drug treatment was continued for 6 consecutive days. In both models, the degree of oedema formation was assayed as increase in paw thickness. The increase in paw thickness and the percentage inhibition were calculated as above with substitution of P_0 by Pc, as Pc is higher than P_T.

Statistical analysis:
Experimental data were expressed as mean ±SD. Student's *t*-test was applied for expressing the significance, and *P* value less than 0.001 was considered as significant.

RESULTS AND DISCUSSION

The reactive oxygen species (ROS), such as superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^·$), have been implicated in pathophysiology of various clinical disorders, including ischemia, reperfusion injury, atherosclerosis, acute hypertension, diabetes mellitus, and cancer[15]. The ethanol extract of *T. coronaria* showed significant superoxide radical, hydroxyl radical, nitric oxide, and lipid peroxidation scavenging activities when compared with the aqueous extracts. Concentrations required for 50% inhibition (IC_{50}) of superoxide, hydroxyl radical, nitric oxide scavenging activities, and lipid peroxidation inhibiting activity were 235.5, 410.6, 250.7, and 255.6 µg/ml, respectively. The concentrations required for the 50% inhibition of free radicals by water extracts were 280.4, 300.7, 660.1, and 295.3 µg/ml, respectively. Carrageenan-induced acute inflammation and formalin-induced chronic paw oedema in animals are the most suitable test procedures to screen antiinflammatory agents. The ethanol extract showed better antioxidant activity than aqueous extract, and it was selected for the inflammatory studies.

Antiinflammatory activities of the ethanol extract of *T. coronaria* in two models reduced the paw oedema significantly ($P < 0.001$) (Tables 1 and 2). The concentrations required to inhibit the paw oedema in both type of inflammations were comparable to the standard reference drug diclofenac.

Recent studies suggest that the inflammatory tissue damages are due to the liberation of reactive oxygen species from phagocytes invading the inflammation sites[16-18]. In addition to this, nitric oxide is also implicated in

TABLE 1: EFFECT OF DIFFERENT EXTRACTS OF *T. CORONARIA* FLOWERS *IN VITRO* ANTIOXIDANT ACTIVITY

Extracts	Concentration needed for 50% inhibition of oxygen radicals (µg/ml)			
	Superoxide	Hydroxyl radical	Lipid peroxidation	Nitric oxide
Ethanol	235.5±7.2	410.6±15.6	250.7±10.2	255.6±6.5
Water	280.4±10.5	300.7±11.3	660.1±20.6	295.3±9.2

n=3 observations, values are mean ±SD

TABLE 2: ACUTE ANTIINFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACTS OF *T. CORONARIA* IN CARRAGEENAN-INDUCED INFLAMMATION

Groups	Initial paw thickness (cm)	Paw thickness after 3 hrs (cm)	Increase in paw thickness (cm)	Inhibition (%)
Control	0.192±0.023	0.340±0.024	0.148±0.021	-
Diclofenac (25 mg/kg)	0.182±0.032	0.245±0.008	0.063±0.030	57
Dose –I (100 mg/kg)	0.195±0.020	0.242±0.008*	0.047±0.027	68
Dose-II (250 mg/kg)	0.240±0.014	0.240±0.014*	0.030±0.020	79

Values are mean±SD, *P<0.001, values are compared with control group

TABLE 3: CHRONIC ANTIINFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF *T. CORONARIA* IN FORMALIN-INDUCED INFLAMMATION

Groups	Initial paw thickness (cm)	Paw thickness after 6 days (cm)	Increase in paw thickness (cm)	Inhibition (%)
Control	0.192±0.023	0.320±0.007	0.128±0.030	-
Diclofenac (25 mg/kg)	0.182±0.032	0.260±0.007**	0.075±0.039	42
Dose-I (100 mg/kg)	0.195±0.020	0.242±0.027**	0.047±0.042	63
Dose-II (250 mg/kg)	0.210±0.012	0.247±0.031**	0.037±0.035	71

Values are mean±SD **P<0.001, values are compared with control

inflammation, cancer, and other pathological conditions[19-21]. Interactions between superoxide and nitric oxide regulates the vascular tone or inflammation[16]. The significant *in vitro* antioxidant activity of *T. coronaria* is in a concentration dependent manner.

The development of carrageenan-induced oedema is biphasic; the first phase is attributed to the release of histamine, 5-HT, and kinins, while the second phase is related to the release of prostaglandins[22-23]. Carrageenan induces paw oedema by inducing protein-rich exudates containing a large number of neutrophils[24]. Formalin induces paw oedema. The formalin-induced is most suitable, as it closely resembles human arthritis[25]. The nociceptive effect of formalin is also biphasic, an early nuerogenic component followed by a later tissue-mediated response[26]. The results suggest the possible usefulness of *T. coronaria* in the treatment of inflammation-associated diseases like arthritis. The effect of our preparations becomes significant within 3 h, during the phagocytic phase of carrageenan-induced inflammation, when the mast cells release cytoplasmic enzymes and serotonin[27]. Superoxide is known to participate in the formation of chemotactic factors and recruitment of Poly Morpho Nuclear cells (PMNs). Hence our studies indicated that *T. coronaria* extract, which could scavenge the superoxide anion, might inhibit the recruitment of PMNs and thereby reduce inflammation.

REFERENCES

1. Kirtikar, K.R. and Basu, B.D., Indian Medicinal Plants. M/S Periodical Experts, Delhi., 1987, 7, 2176.
2. Henriques, A.T., Melo, A.A and Morreno, P.R.H., **J. Ethnopharmacol.,** 1996, 50, 19.
3. Atta-ur-Rahman, Alvi, K.A. and Muzaffar, A., **Planta Medica.**,1985, 325.
4. Atta-ur-Rahman, Alvi K.A., Muzaffar, A. and Daulatbadi, N., **Phytochemistry.,** 1986 24, 1781.
5. Daniell, B. and Palmisano, G., Eds., In; A. Brossi The alkaloids, Academic press, London, 1986, 27, 130.
6. Kam, T.S., Loh, K.Y., Lim, L.H., Loong, W.L., Chuah, C.H. and Wei, C., **Tetrahedron Letters.**, 1992, 33, 972.
7. Satyanarayana, D., Joshi, A.B., Chandrasekhar, K.S. and Vijayanarayana, K., **Indian Drugs**., 2004, 41, 405.
8. Lollinger, J., Taylor. R and Francis. W., Eds., In; Free radicals and food additives, London, Clarendon Press, 1981, 121.
9. McCord, J.M., Fridovich, I., **J. Biol. Chem**., 1969, 244, 6049.
10. Elizebeth, K. and Rao, M.N.A., **Int . J. Pharm.,** 1990, 58, 237.
11. Ohkawa, H., Ohishi, N., Yagi, K., **Anal. Biochem.,** 1979, 95, 351.
12. Bishayee, S.and Balasubramanian, A.S., **J. Nuero. Chem.,** 1979, 18, 909.
13. Ajith, T.A. and Janardhanan, K.K., **Indian J. Exp. Biol**., 2001, 39, 1166.
14. Marcocci, L., Maguire, J.J., Droy-Lefaix, M.T. and Packer, L., **Biochem. Biophys. Res. Commun.,** 1994, 201, 748.
15. Hemnani, T. and Parihar, M.S., **Indian J. Physiol. Pharmacol.,** 1998, 42, 440.

16. Conner, E.M. and Grisham, M.B., **Nutrition.,** 1996, 12, 274.
17. Winrow, V.R., Winyard, P.G., Morris, C.J., and Blake, D.R., **Brit. Med. Bull.,** 1993, 49, 506.
18. Parke, D.V. and Sapota, A.,.**Int. J. Occup Med. Envirn Health.,**1996, 9, 331.
19. Marietta, M.A., **Trends Biol. Sci.,** 1989, 14, 488.
20. Moncada, A., Palmer, R.M.J. and Higgs, E. A., **Physiol. Patho Physiol. Pharmacol**., 1991, 43, 109.
21. Farrel, A.J., Blake, D.R., Palmer R. M. J. and Moncada, S., **Annals. Rheum Dis.,** 1992, 51, 1219.
22. Larsen, G.L. and Hensson, P.M., **Annu. Rev. Immuno.,** 1983, 1, 335.
23. Brooks, P.M. and Day, R.O., **N. Engl. J. Med.**, 1991, 324, 1716.
24. Lo, T.N., Almeida, A. P. and Beaven, M.A., **J. Pharmacol. Exp. Ther**., 1982, 221, 261.
25. Greenwald, R.A., **Meth. Find. Clin. Pharmacol**., 1991, 13, 75.
26. Wheeler-Aceto, H. and Cowan. A., **Agents Actions**., 1991, 34, 264.
27. Vinegar, R., Truase, J.F., Selph, J.L, Johnston, P.R.,Venable, A.L and Mckenzie, K.K., **Fed. Proc.,** 1987, 46, 126.

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